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Review Article

Chemical and Pharmacological Potential of *Adiantum philippense* Linn and Further Molecular Simulation Study of Its Compounds Against COX-2: An Unexplored Medicinal Fern

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ABSTRACT

Adiantum philippense Linn (Pteridiaceae), commonly known as "Goyalelata" which has been traditionally used to cure of different diseases including dysentery, ulcers, fevers, cooling and elephantiasis. This study aims to summarize the phytoconstituents reported for *A. philippense* and its pharmacological activities as well as further *in-silico* molecular dynamics studies to identify active compounds against COX-2 enzyme as potent analgesic. The data for this study were collected using online databases such as Google Scholar, PubMed, Scopus and Web of Science. Previous studies have established that a number of phytochemicals have been identified from this plant including phenolic compounds (caffeic acid, chlorogenic acid, phloroglucino, esculetin), flavonoids (rutin, quercetin, luteolin), terpenoids (ursolic acid, botulin, carvone and glycyrrhetinic acid). Literature study demonstrated that *A. philipense* has the potential analgesic, antioxidant, antimicrobial, cytotoxic and hepatoprotective effects in both *in-vivo* and *in-vitro* test systems. The plant can act as suitable candidate for the biosynthesis of nanoparticles and application as a therapeutic purpose. The molecular docking analysis of its reported phytoconstituents with CoX-2 showed that quercetin, and luteolin exhibited the most favorable binding affinity with a value of -8.1 and -8.0 kcal/mol, respectively. Further molecular dynamics study revealed that quercetin was the most promising anti-inflammatory compounds present in *A. philippense* confirmed by the RMSD, RMSF, Rg, SASA and hydrogen bond analysis. In summary, it is proved that *A. philippense* one of the potential traditional medicinal fern that possess bioactive compounds which could be useful in the prevention of pain and inflammation.

Keywords: *Adiantum philippense*, medicinal fern, analgesic activity, molecular simulation

Introduction

Plants have been serving as an inevitable source of food, fuel, shelter and medicine to mankind since the inception of civilization. Various pharmacological and phytochemical properties of different plant flora have been documented, especially on angiosperms.[1] Pteridophytes have been known for their medicinal and bioactive constituents that have been attracting scientist to do research on plant-based novel drug therapy.[2] Fern and Fern allies species of Pteridophytes are highly ignored although they have been possessing different bioactive secondary metabolites that has therapeutic properties.[2, 3] Some recent ethnobotanical studies shed light on the therapeutic uses of ferns practiced by various indigenous people worldwide.[4] As they possesses diffident active secondary metabolites, they can be utilized directly or in extracted form to treat a variety of disorders.[5]

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Adiantum philippense Linn, (syn. *Adiantum lunulatum*) (Figure 1) is a fern of Pteridiaceae family and commonly known as "walking Maidenhair fern". *A. philippense* L. is an evergreen, perennial fern. It is found across south-east Asia including Bangladesh, India and Thailand.[6] In Bangladesh it is known as "Goyali lata" and mainly grows in waste areas, roadsides, farm walls and fallow lands. *A. philippense* grows in a creeping or semi erect position.[7] The plant has been traditionally used in medicine as antibacterial agents[8], in cough, asthma, leprosy, hair falling and fever .[9] The leaves are massaged with water and administered with sugar to treat febrile disorders in children .[10] *A. philippense* showed antidiabetic effect in alloxan induced diabetic rats.[11] This plant is also reported for cytotoxic.[12] antioxidant.[12] antibacterial.[13] and thrombolytic activities.[12] The plant also possesses different phytoconstituents including phenolic, flavonoids and terpenes.[8] However, there is no systematic review to-date covering its different pharmacological effects and chemical constituents reported.

From the very ancient time natural products and its derivatives have been used to treat pain and inflammatory disorders.[14] Medicinal plants have been playing an important role in this regards.[15] *A. philippense* and its conspecifics have been reported to possess analgesic-anti-inflammatory properties.[16-18] *In-silico* molecular dynamic studies are imperative techniques for understanding the physical principles of biological macromolecule structure and function.[19] Molecular docking study is a very useful tools to virtually screen natural products from different database to discover potential lead molecules. Molecular docking of reported phytochemicals from traditional medicinal plants against appropriate drug targets has an importance in natural product based drug discovery research.[20] The plant *A. philippense* has anti-

inflammatory properties that evident in both its traditional use and reported activity. The enzyme COX-2 is a universal target to develop analgesic-antiinflammatory agents.[21] There was no report to-date on molecular docking and dynamics study of reported phytoconstituents of *A. philippenese* against COX-2. Therefore, the aim of this review is to summarize the various reported pharmacological effects and identified phytoconstituents of *A. philippense* that is available in published literature using afore-mentioned databases (Google Scholar, PubMed, Scopus and Web of Science) as well as further docking study of identified constituents to COX-2 enzyme to correlate its ethnopharmacological and reported analgesic and anti-inflammatory activity.

Figure 1: Images of *Adiantum philippense*

Materials and Methods

Literature search strategy

The literature survey on *A. philippense* was carried out by searching on PubMed, Scopus, Google Scholar, Web of Sciences and Science Direct databases, using the keywords "*Adiantum philippense*". Articles that reported (a) *in-vivo*, and b) *in-vitro* studies of the *A. philippense* extract or its isolated compounds were taken under consideration in this review.

The selected articles were assessed and information were gathered about the *A. philippense* and its isolated compounds, test model, pharmacological activity, observation, results, probable mechanism of action and the concentration. The data extraction and the selection criteria are mentioned in Figure 2.

Molecular docking and dynamics simulations

The three-dimensional structures of compounds found in our literature survey from *A. philippense* along with celecoxib which was used as a control drug were obtained from PubChem. Our target receptor human cyclooxygenase-2 (COX-2) (PDB ID: 5IKT), was obtained from the Protein Data Bank. Non-Protein atoms were removed from the protein structure and polar hydrogens were added using BIOVIA Discovery Studio Version 4.5.0.15071. A redocking experiment was used to validate the docking techniques before starting molecular docking experiments. The docked and native co-crystal locations had a root mean square deviation (RMSD) of less than 2, indicating that the docking techniques and settings utilized in this investigation could consistently predict the compounds' native conformations. For molecular docking, Autodock Vina based virtual screening software Pyrx (Ver. 0.8, 2010) was used for this study.[22] Initial docking grid was set to (26×26×26; 1 Å) enclose the entire active site and the grid center was set at dimensions x=157.553, y=182.637, z=195.147. Ligplot was used to visualize the protein-ligand interactions.[23]

For molecular dynamics (MD) simulations, GROMACS 2021 was used with charmm36 force field.[24, 25] CHARMM General FF (CGenFF) (cgnff.umaryland.edu) server was used to generate ligand parameters files.[26] Protein–ligand complexes were solvated in a decahedron box with TIP3P water model. To neutralize the system, Na^+ and Cl^- ions were used. Following energy minimization, the NVT and NPT ensemble of GROMACS were used to run the equilibration step by controlling the temperature and pressure at 310 K and 1 bar, respectively. Finally, the MD run was carried out for 200 ns to assess the stability of protein-ligand complex. To analyze the trajectories, gmx rmsd, gmx rmsf, gmx sasa, and gmx hbond were utilized to determine root mean square deviation, root mean square fluctuations, solvent-accessible surface area, and number of hydrogen bonds.

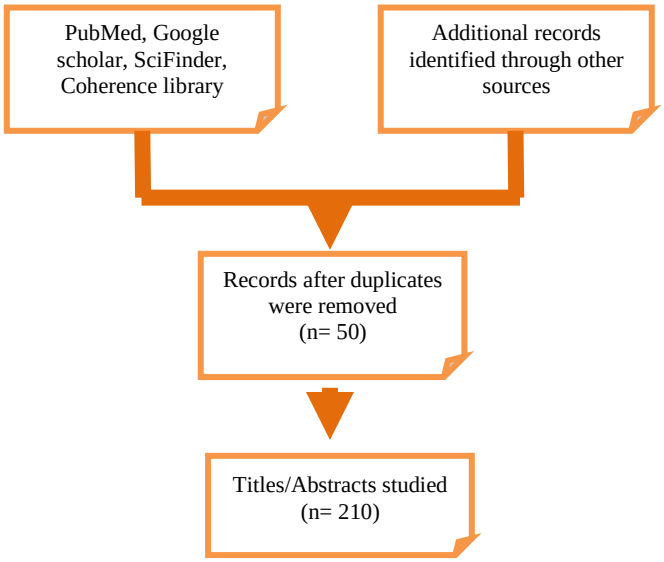


Figure 2: Flow chart of Data extraction

Results and Discussion

Major phytoconstituents in *A. philippense*

The major bioactive compounds found in *A. philippense* were phenolic compounds (caffeic acid, kaempferol, coumarin, chlorogenic acid, esculetin and phloroglucinol), flavonoids (quercitrin, rutin, luteolin, and orientin), and terpenoids (ursolic acid, betulin, 18-β-glycyrrhetinic acid, and carvone). The structures of the isolated compounds along with their reported biological functions are given in Table 1.

Anti-bacterial activity

A. philippense exhibited a broad spectrum of Gram (-) and Gram (+) bacterial inhibition.[13] *A. philippense* is known to contain numerous bioactive phytochemicals, including alkaloids, carbohydrates, flavonoids, glycosides, tannins, terpenoids, and saponins.[12,27] The flavonoids of *A. philippense* show potential antibacterial activity through different mechanisms particularly by damaging cell-membrane, inhibiting the various biosynthesis such as the bacterial respiratory chain, nucleic acid synthesis, and the cell envelope synthesis.

The flavonoids part act not only on the target specific synthases, but also act on the cell-membrane bilayer and the respiratory chain by nonspecifically to kill bacteria. The interaction of hydrophilic flavonoids with the heads of the phospholipids and the interaction of hydrophobic flavonoids with the lipophilic interior part of the lipids bilayer are two critical mechanisms for killing bacteria.[28] Nonspecific interactions between flavonoids and phospholipids can alter membrane properties.[29] Flavonoids with greater lipophilicity are more active due to the increased membrane affinity of their long acyl chains.[30] In case of the Gram-positive bacteria, the fluidity and integrity of cellular membrane can be decreased by lipophilic flavonoids causing bacterial inhibition.[31] Adnan et al. (2020) reported that *A. philippense* crude extract showed antibacterial effect against common food pathogenic bacterial stains such as *S. aureus, E. coli, P. aeruginosa* and *S. flexneri* (Table 2).

Analgesic effect

Pain is actually a complex phenomenon involving peripheral and central sensitization.[32] Analgesic agents generally reduce pain experience by decreasing the passage of nociceptive impulses along primary afferents or by modifying pain perception.[33] The processing of the nociceptive signal, as well as the initiation and maintenance of

central sensitization, are all actively influenced by the cyclo-oxygenase COX-2 isoenzyme.[34] That's why the most plausible mode of action for analgesia is COX-2 inhibition.[35] In this study, we found no reported activity directly against the COX-2 enzyme. However, we found two studies on analgesic activity. The *A. philippense* extract has been shown to have a dose-dependent analgesic response against chemical (formalin test) and thermal (hot plate method) stimuli, showing antinociceptive activity that may involve pain inhibition via peripheral and central mechanisms.[17] *A. lunulatum* was also found to have analgesic properties that helped to fully ease the pain of a fracture and made the healing process bearable.[18] (Table 2).

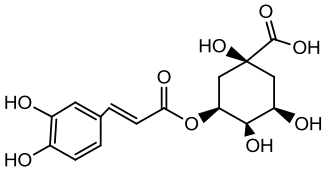
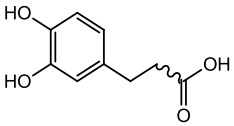
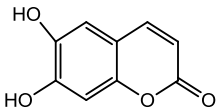
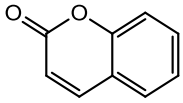
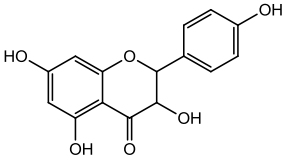
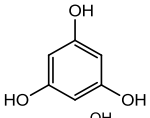
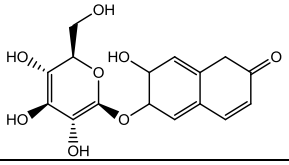
Anti-oxidant activity

Antioxidants are substances that prevent free radicals. The concept of antioxidant activity and antioxidant efficacy are needed to be distinguished in order to understand the antioxidant compound's mechanism. *A. philippense* has proved to be useful traditionally as its content significant amount of antioxidant activity that can prevent a number of diseases (Table 2). Ali *et al*. (2013) reported antioxidant activity of methanolic extract of *A. philippense* using DPPH radical scavenging, total phenolic and flavonoid content assays.[12] The results revealed. that the DPPH radical scavenging activity of *A. philippense* extract increased with the concentration (IC_{50} < 200 µg/mL).

Anti-microbial activity

Plants containing flavonoids have been used by physicians to treat human diseases for a long time.[36]. These compounds show their effect by inhibiting the DNA gyrase of the parasites, cytoplasmic membrane function and energy metabolism.[37] Plant terpenoids have also been proposed as very promising source of new antimicrobial agent, showing activity against viruses, bacteria, fungi and protozoa.[38] Endophytes provide benefits to their hosts while also contributing to various structural features with biological potential.[39] Ramesha *et al*. (2020) showed that the leaf section contained antimicrobial constituents, phomalactone, in fungal endophytes (*Nigrospora sphaerica*) from *A. philippense* using TLC-bioautography and hyphenated spectroscopic techniques.[39] Phomalactone showed the highest antibacterial effect against *E. coli* when tested by disc diffusion assay (Table 2).

Table 1: List of phytoconstituents and reported activity of identified compounds from *A. philippense*

Compound name	Chemical Structure	Activity	Ref
Phenolic Compounds			
Chlorogenic acid		Antioxidant, Antimicrobial, Anti-inflammatory, hepatoprotective, neuroprotective, cardiovascular protective	71, 72
Caffeic acid		Antimicrobial, antioxidant, hepatoprotective, antihypertensive	73-76
Esculetin		Bone health promoter, anti-inflammatory, anti-hepatitis, neuroprotective, anti-diabetic	77-81
Curcumin		Wound healing, anti-cancer, anti-diabetic, neuroprotective, anti-inflammatory, skin protective, anti-microbial, CNS protective	82-89
Kaempferol		Osteoprotective, cardioprotective, antifungal, hepatoprotective, anti-allergic	90-95
Phloroglucinol		Anti-inflammatory, hepatoprotective, anti-oxidant, neuroprotective	96-99
Esculin		Gastroprotective, anti-inflammatory, anti-oxidant, anti-bacterial, anti-viral, anti- apoptotic	100-105
Flavonoids			

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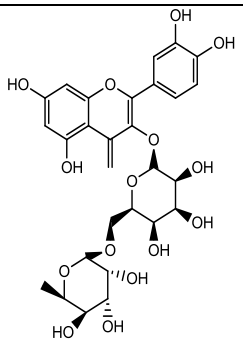
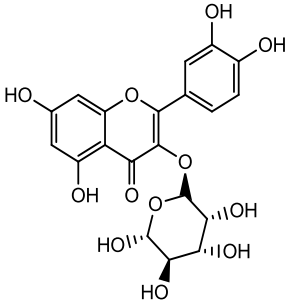
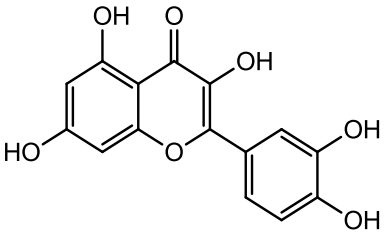
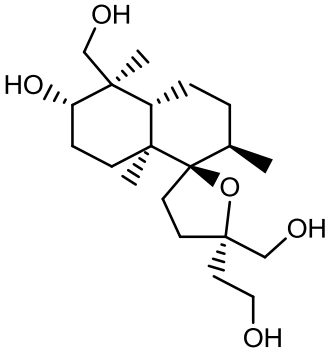
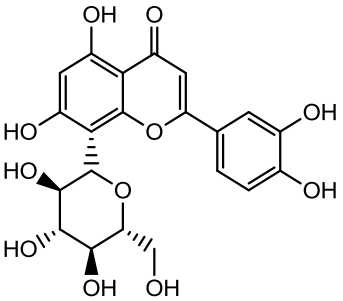
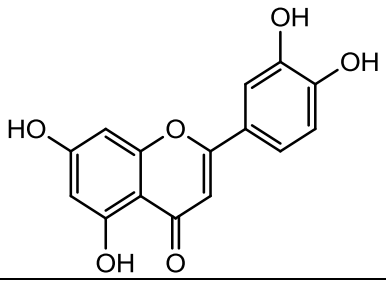
Rutin		Anti-microbial, anti-inflammatory, anti-tumor, anti-asthma, anti-oxidant, hepatoprotective	106-110
Quercitrin		Anti-viral, hypolipemic activity, induce apoptosis, antibacterial, antibiofilm	111-114
Quercetin		Anti-obesity, antimicrobial, anti-lipase, antioxidant, anti-cancer, prevents cytotoxicity, anti-aging	115-119
Lagochilin		Anti-fungal, anti-bacterial, anti-cholinesterase, weak hemostatic	120-122
Orientin		Antibacterial, antioxidant, antithrombotic, antiplatelet, vasodilation, anti-inflammatory	123-126
Luteolin		Anti-inflammatory, antiviral, anti-tumor, anticancer, increases insulin sensitivity	127-130

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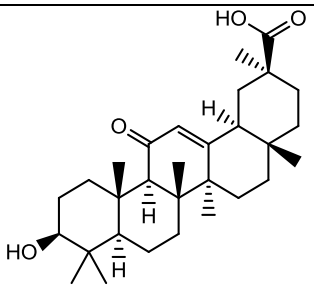
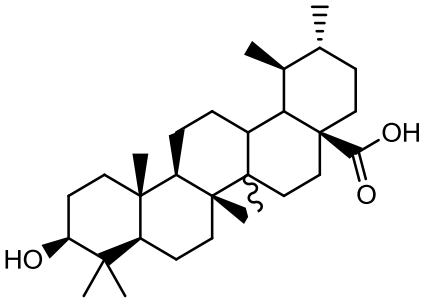
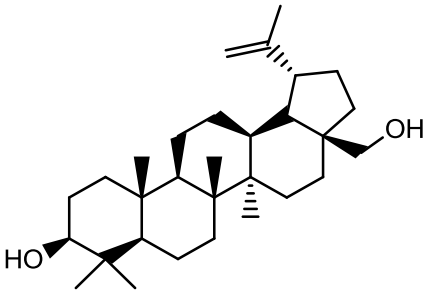
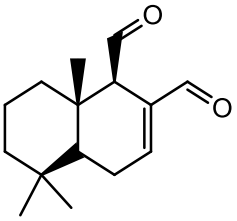
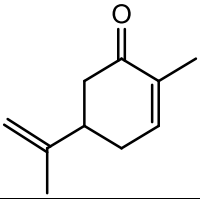
Terpenoids			
18-*β*-glycyrrhetinic acid		Hepatoprotective, antiviral (against rotavirus), anti-tumor	131-133
Ursolic acid		Anti-tumor, anti-angiogenic, antibacterial, antioxidant, hepatoprotective, anti-sickling	134-138
Botulin		Used in anal fissure, treats nonrelaxing puborectalis syndrome	139, 140
Carvone		Immunomodulatory, antifungal, anticancer, carvone, antimicrobial, cytotoxic and chemoprotective	141-145
Polygodial		Antibacterial, antifungal, antifeedant, prevents blue mussels	146-149

Antidiabetic and anti-adipogenesis effect

Diabetes promotes oxidative stress, inhibits pancreas cell to glucose uptake and worsens differentiation of adipocyte that has catastrophic impact on normal cells.[40] *A. phillippense* possesses antihyperglycemic activity by reducing some of the aforementioned effects of diabetes. Paul et al. (2012) showed that the ethanolic extract of *A. philippense* possesses protective effect against H_2O_2-induced cytotoxicity and also showed insulinotropic action in an isolated mouse pancreatic glucose uptake experiment. It also prevented the maturation of pre-adipocytes into adipocytes in 3T3-L1 cell line.[11] (Table 2).

Hepatoprotective activity

A. philippense and its related species have been traditionally used to cure a variety of diseases. The hepatoprotective efficacy of *A. philippense* against ethanol-induced hepatotoxicity was investigated in rats.[41] Ethanol (2 g/kg) administration in experimental animals resulted in considerable damae in the liver of the animals. Pretreatment with the ethanolic extract of *A. philippense* protect the liver ddamae and considerably reduced the level of serum glutamic-pyruvic transaminase (ALT), serum glutamic-oxaloacetic transaminase (AST), alkaline phosphatase (ALP), total protein, and total bilirubin at 250 and 500 mg/kg doses, orally (Table 2).

Cytotoxic activity

A. philippense showed concentration dependent cytotoxic effect in brine shrimp (*Artemia salina)* lethality bioassay.[12] The methanolic extract of *A. philippense* leaves was tested at various concentration of 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL and the results showed that the percentage of cytotoxicity increased with the increase in concentration.[12] The LC_{50} of the extract was found 106.4 µg/mL

whereas for standard vincristine sulphate, the value was 08.50 µg/mL. The brine shrimp lethality bioassay is a prognostic of cytotoxicity bioassay that helps to detect cytotoxic compounds presents in crude extracts against simple zoological organism *Artemia salina*. A number of constituents have been reported from the plant *A. philippense* including phenolics and flavonoids and it is reported that phenolics and flavonoids possess cytotoxic activity.[42, 43] (Table 2).

Table 2: Reported pharmacological activity with possible mechanism of action of *A. philippense*

Dose	Type of activity	Type of assay	Mechanism of action	Ref
***In vitro* study**				
50-500 µg/ml	Anti-bacterial	Agar cup/well diffusion	Broad spectrum antimicrobial against gram (+) and gram (-)	13
140 µg/ml	Anti-oxidant	DPPH radical scavenging	Scavenging free radical	12
6 - 400 µg/ml	Cytotoxic	Brine shrimp lethality bioassay	NA	12
12.86 ± 1.02 µg/ml	Thrombolytic	Clot disruption	Selectively bind to platelet thrombi	12
150 µg/ml	Antimicrobial	Disc diffusion	Broad spectrum activity against human and plant pathogen	39
56.50 ± 2.50	Anti-oxidant	DPPH	Free radical scavenging	150
0.50	Cytotoxic	Brine shrimp lethality bioassay	NA	150
250 and 500 mg/kg	Hepatoprotective and antioxidant activities	Experimental animals (rat).	By reducing the elevated levels of serum AST, ALT, ALP, total protein, bilirubin	41
***In vivo* study**				
500 and 250 mg/kg	Anti-hyperglycemic	By estimating blood glucose level by GOD/ POD	NA	11
100 - 1000 µg/ml	Anti-diabetic	Glucose uptake aasay	NA	151
	Anti-adipogenesis	Adipocyte differentiation assay	Inhibiting activity of peroxisome proliferator-activated receptor gamma (PPAR)	

Thrombolytic activity

Ali et al., 2013 reported that the methanol extract of *A. philippense* showed moderate thrombolytic activity compared to that of streptokinase which was used as a standard.[12] It is reported that plant flavonoids (quercetin and rutin) has anti-thrombotic activity through inhibition of non-enzymatic lipid peroxidation and free-radical generation during binding of activated platelets to vascular endothelium.[44] Adherence of activated platelets to vascular endothelium inhibit the production and function of endothelial prostacyclin and destroy endothelial-derived relaxing factor (EDRF) (Table 2). [44]

Synthesis of nanoparticles

An important development in nanotechnology is the creation of an efficient, dependable, and quick method for synthesizing nanoparticles using biological systems. The study of materials which have at least one dimension between 1 and 100 nm are known as nanotechnology. Au, Ag, Pt, and Pb are widely investigated noble metal nanoparticles.[45, 46] Although physical and chemical approaches are common for synthesis of nanoparticles with distinct size and shape, they are costly and possibly hazardous to the environment. As a result, it is necessary to develop clean, nontoxic, cost-effective, and ecologically friendly technologies for synthesizing nanoparticles. Researchers have developed biological approaches for the creation of nanoparticles in response to these concerns. Gold nanoparticles (AuNPs) are being investigated for use in a variety of purposes including earl cancer diagnosis and treatment, targeted delivery of drug, as biomedicine, imaging and different electrochemistry uses.[47] Whereas, silver is considered as safest next generation antimicrobial agent.[48]. Silver nanoparticle (AgNPs) can be applied in expensive angles concerning bio-labelling, antibiotics, antibacterial, antifouling and antiparasitic properties, and drug delivery mechanisms.[49,50,51,52,53,54,55] It has also ability to reduce dye and useful in radiation therapy.[56,57]

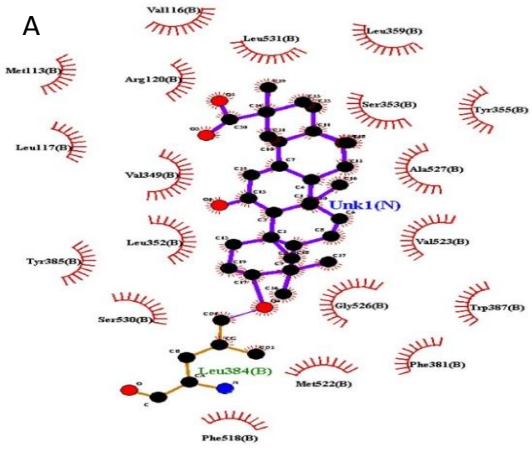
Literature review revealed that the presence of terpenoids or alkaloids in geranium extract causes metal ion reduction and the stabilization of AuNPs or AgNPs.[58] *A. philippense* possesses different bioactive constituents including flavonoids, polyphenols, terpenes and saponins.[12,27] These polyphenolic compounds have role as antioxidant and play an important role in the bio-production of gold and silver salts.[59 60] The results of transmission electron microscopy (TEM) and energy dispersive x-ray spectroscopy (EDS) analysis revealed that nanoparticles were successfully generated by employing *A. philippense* extract.[61] The synthesis of AgNPs and AuNPs is quick and environmentally favorable. The synthesis of silver and gold nanoparticles were confirmed by UV–Vis spectrophotometer surface plasmon spectra where the absorbance maxima was recorded at 452 and 546 nm.[62] The optimum condition for AuNPs synthesis was at pH

11 with 1:1 *A. philippense* extract and 5 mM of tetrachloroauric acid whereas the optimum condition for AgNPs synthesis was 1:1 extract and 9 mM of silver nitrate at pH 12. The size of monocrystalline AuNPs and polycrystalline AgNPs ranged from 10 - 18 nm. Elemental analysis of the complexes was conducted and Au and Ag were confirmed by EDS and XRD studies.[60]

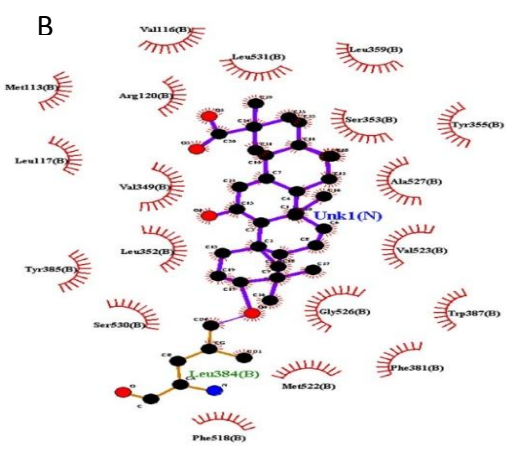
The medicinal properties of ferns may make them a good choice for the production of nanoparticles. The use of such medicinally significant plants has a promising future in nanotechnology for medication delivery and therapeutic applications. The use of synthesized capped nanoparticles will undoubtedly aid in understanding the nature of the capping agent and taking advantage of its therapeutic and biological applications. These nanoparticles also shown cytotoxicity by triggering apoptosis or cell cycle arrest.[61]

In Silico molecular docking analysis

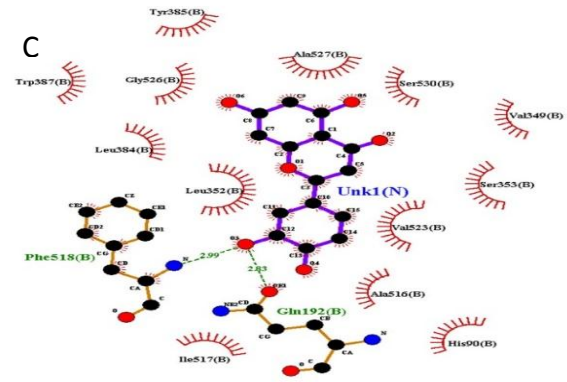
The enzyme COX-2 was selected as a target against the reported bioactive compounds from *A. philippense* that reported to have analgesic properties. However, no report on *A. philippense* extract or its compounds against COX-2 and therefore, the aim of the *in-silico* study was to know further insights about the analgesic properties. The *in-silico* studies is a popular technique to identify bioactive lead structure. From the molecular docking studies, it was revealed that three compounds, celecoxib, quercetin, luteolin, and kaempferol strongly interacted with the protein with a binding affinity of -10.7, -8.1, -8.0, -7.9 kcal/mol, respectively. Figure 3 represents the interacting residues of our top four compounds. Control drug, celecoxib formed one hydrogen bond with Leu384 and interacted hydrophobically with Met113, Leu117, Tyr385, Val116, Arg120, Val349, Leu352, Ser530, Leu531, Leu359, Ser353, Ala527, Val523, Gly526, Met522, Phe518, Tyr355, Trp387, and Phe381 (Figure 3A). Quercetin interacted with Leu384 by forming hydrogen bond similar to celecoxib, whereas hydrophobic interactions were seen with Met113, Leu117, Tyr385, Ser530, Leu352, Val349, Arg120, Val116, Leu531, Leu359, Ser353, Ala527, Val523, Gly526, Met522, Phe518, Phe381, Trp387, and Tyr355 residues (Figure 3B). Luteolin formed hydrophobic bonds with residues Leu352, Leu384, Trp387, Gly526, Tyr385, Ala527, Ser530, Val349, Ser353, Val523, Ala516, and His90 and two hydrogen bonds with Phe518, and Gln192 residues (Figure 3C). Kaempferol showed two hydrogen bonds with Tyr385, and His90 and ten hydrophobic interactions with Val349, Ala527, Tyr355, Ser530, Val523, Leu352, Phe518, Ser353, Ile517, and Ala516 (Figure 3D). Apart from the top four compounds, quercitrin also demonstrated similar binding energy compared to kaempferol, interacted hydrophobically with Leu531, Leu359, Val116, Ala527, Arg120, Val349, Gly526, Tyr385, Ser530, Leu352, Val523, Gln192, Ala516, Ser353, Ile517, and Tyr355 and via hydrogen bonds with His90, and Phe518. The lowest binding affinities were observed for compounds such as 18-beta-glycyrrhetinic acid, fern-9(11)-en-25-oic acid, fern-9(11)-ene, betulin, adiantone, rutin, and lagochiline, binding energy within -4.9 kcal/mol to -1.9 kcal/mol. The docking results of our investigated compounds are shown in Table 3.



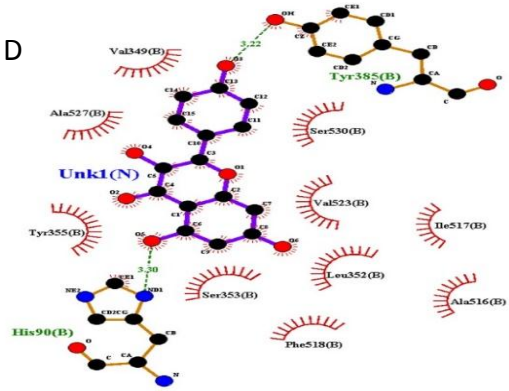
Celecoxib



Quercetin



Luteolin



Kaempferol

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Figure 3: Molecular docking result visualization of top 4 ligands on Ligplot+

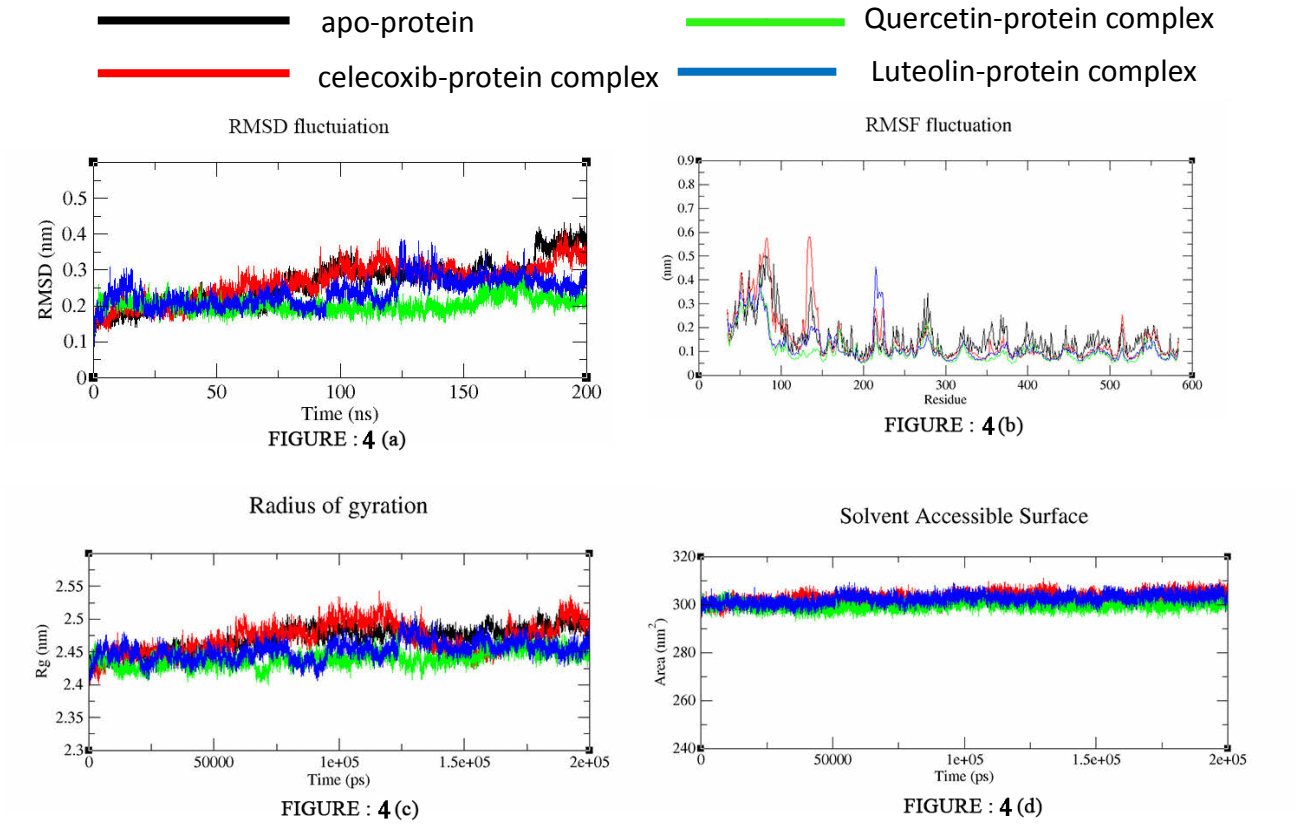

Figure 4: a) Plot of RMSD of backbone atoms vs. time (in nano seconds), b) RMSF of backbone atoms versus residue number, c) Radius of gyration (Rg) versus time (in pico seconds), d) solvent accessible surface area (SASA) versus time (in pico seconds) for apo-protein (black), celecoxib-protein complex (red), Quercetin-protein complex (green), Luteolin-protein complex (blue).

Table 3: Result of molecular docking of identified phytoconstituents in *A. philippense* against CoX-2 enzyme

Ligand	Binding Affinity	Interacting amino acids	
		Hydrophobic Interaction	**H bond**
Celecoxib	-10.7	Met113, Leu117, Tyr385, Val116, Arg120, Val349, Leu352, Ser530, Leu531, Leu359, Ser353, Ala527, Val523, Gly526, Met522, Phe518, Tyr355, Trp387, Phe381	Leu384
Quercetin	-8.1	Met113, Leu117, Tyr385, Ser530, Leu352, Val349, Arg120, Val116, Leu531, Leu359, Ser353, Ala527, Val523, Gly526, Met522, Phe518, Phe381, Trp387, Tyr355	Leu384
Luteolin	-8	Leu352, Leu384, Trp387, Gly526, Tyr385, Ala527, Ser530, Val349, Ser353, Val523, Ala516, His90	Phe518, Gln192
Kaempferol	-7.9	Val349, Ala527, Tyr355, Ser530, Val523, Leu352, Phe518, Ser353, Ile517, Ala516	Tyr385, His90
Quercitrin	-7.9	Leu531, Leu359, Val116, Ala527, Arg120, Val349, Gly526, Tyr385, Ser530, Leu352, Val523, Gln192, Ala516, Ser353, Ile517, Tyr355	His90, Phe518
Chlorogenic acid	-7.4	Trp387, Ser530, Leu352, Ala527, Val349, Leu359, Val116, Leu531, Tyr355, Ser353, Met113, Val523, Gly526, Phe518, Leu384	Met522, Tyr385
Coumarin	-7.3	Phe381, Val349, Ser353, Leu352, Gly526, Ser530, Met522, Phe518, Val523	

Esculin	-7.3	Se353, Leu352, Phe518, Ser530, Gly526, Phe381, Leu384, Trp387, Ala527, Met522, Val523	
Orientin	-7.1	Leu531, Val116, Ala527, Val349, Tyr355, Arg120, Ser353, His90, Val523 Phe518, Ile517, Leu352, Leu384, Trp387, Gly526	Ser530, Met522, Tyr385
Carvone	-7	Ser530, Ala527, Ser353, Val523, Gly526, Trp387, Tyr385, Met522, Leu353, Leu384, Phe518	
Caffeic Acid	-6.8	Val349, Ser353, Ala527, Gly526, Val523, Phe518, His90	Leu352, Gln192
Filicenol-B	-5.7	Leu93, Tyr115, Ser119, Arg120, Leu123, Phe470, Glu524, Met471, Leu472, Pro86, Val89, Val116, Ile112	
Ursolic acid	-5.1	Tyr348, Phe518, Tyr385, Leu352, Ser530, Val349, Met113, Leu531, Val116, Tyr355, Leu359, Arg120, Ser353, Gly526, Trp387, Val523	Met522, Ala527
18-beta-glycyrrhetinic acid	-4.9	Met113 , Leu117, Tyr385, Ser530, Leu352, Val349, Arg120, Val116, Leu531, Ser353, Ala527, Val523, Gly526, Met522, Phe518,Phe381, Trp387, Tyr355, Leu359	Leu384
Fern-9(11)-en-25-oic acid	-4.9	Trp100, Ile112, Val89, Val116, Arg120, Leu123, Met471ser119, Tyr115, Leu93	Glu524
Fern-9(11)-ene	-4.9	Ile112, Leu93, Val116, Tyr115, Arg120, Ser119, Leu123, Glu524, Phe470, Leu472, Met471, Pro86, Val89	
Betulin	-3.8	Met113 , Tyr385, Ser530, Leu352, Val349, Arg120, Leu531, Ser353, Ala527, Val523, Gly526, Met522, Phe518,Phe381, Trp387, Tyr355, Leu359, Tyr348, Phe381	Val116
Adiantone	-3.4	Trp100, Ile112, Leu93, Val116, Arg120, Phe470, Glu524, Leu123, Pro86, Ser119, Tyr115, Val89	
Rutin	-2.9	Pro86, Val89, Glu524, Ser119, Leu93, Ile112, Phe357, Tyr355, Ser353, Val523, Leu352, Gly526, Ala527, Leu531, Val349,	Arg120, Val116
Lagochilin	-1.9	Phe381, Ala527, Ser530, Gly526, Val116, Tyr355, Leu531, Val523, Ser353, Val349, Leu384, Leu352, Trp387	Arg120, Met522

The active site of COX-2 consists of Leu117, Arg120, Phe205, Phe209, Val344, Ile345, Tyr348, Val349, Leu352, Ser353, Tyr355, Leu359, Phe381, Leu384, Tyr385, Trp387, Phe518, Val523, Gly526, Ala527, Ser530, Leu531, Gly533, and Leu534 residues. The Tyr385 residue is accountable for removing a arachidonic acid proton and the Ser530 act as acetylation site of aspirin.[63] The crystal structures of human COX-2 in complex with different analgesic (e.g. mefenamic acid, flufenamic acid, meclofenamic acid etc) showed that the carboxylate group of all the inhibitor's interacted with Tyr-385 and Ser-530 side chains .[64]. It can be seen from the Figure 3 that celecoxib, quercetin, luteolin, and kaempferol interact with many of the COX-2 active site lining amino acids, especially with Tyr385 and Ser530 which are functionally important to inhibit the COX-2 enzyme.

Molecular dynamics analysis

Root mean square deviation (RMSD) considers deviations between two three-dimensional structures over time .[65] The RMSD of backbone atoms of apo-protein, celecoxib-protein complex, quercetin-protein complex, and luteolin- protein complex was analyzed over 200 ns to evaluate the stability of all the systems (Figure 4a). In all systems, slight conformational changes followed by structural rearrangements were observed in the first 20ns. Apoprotein exhibited several conformational changes over the whole study period. Quercetin-protein complex showed fluctuations between 0.005 Å -3.2 Å with an average RMSD of 2.04 Å, which is lesser compared to other protein-ligand complexes with no abrupt changes in the RMSD value. Therefore, through binding with the protein, quercetin stabilized the protein. On the other hand, the RMSD of luteolin-protein complex was high during initial 20ns and again showed abrupt fluctuations at 122-125ns within 1.95 Å to 3.86 Å. celecoxib- protein complex depicted a gradual rising trend compared with other protein-ligand complexes. Regarding to overall RMSD for all systems, apo-protein fluctuated within 0.005 Å-4.32 Å with an average RMSD of 2.63 Å whereas the RMSD of quercetin- protein complex, luteolin- protein complex, and celecoxib- protein complex fluctuated from 0.005 Å up to 3.2 Å, 3.86 Å, 0.416 Å respectively. However, our overall RMSD analysis showed that quercetin was superior for stabilizing protein following luteolin and celecoxib.

Root mean square fluctuations (RMSF) is the fluctuations observed in residues or atoms present in a macromolecule .[66] In this study, we analyzed RMSF of backbone residues of apo protein as well as all protein ligand complexes. As shown in Figure 4b, the results of RMSF graphs showed a similar pattern although the regions suffered a greatest fluctuation. Apo protein and all protein-ligand complexes showed high fluctuations (>4 Å) in N- terminal. Regarding to the active site residues (300-500), quercetin-protein complex exhibited the

least fluctuations. Moreover, the other two protein-ligand complexes, luteolin-protein, and celecoxib-protein complexes, also showed lesser fluctuations than apo-protein. In other regions, quercetin-protein complex showed least fluctuations than apo protein and other protein ligand complexes. Luteolin-protein complex manifested high fluctuations within 214-216 residues. On the other hand, celecoxib-protein complex displayed high fluctuations within 133-135 residues. However, the results of RMSF analysis manifested that quercetin-protein complex were more stable than luteolin and celecoxib-protein complex.

Radius of gyration (R_g) is a routinely used parameter to predict the compactness of macromolecules .[67] In this study, we analyzed R_g of apo protein and all protein ligand complexes. Quercetin-protein complex illustrated low fluctuations and higher stability than any other complexes in this study as shown in Figure 4c. This behavior further explains its high compactness resulting in the stability of the protein. Following quercetin, luteolin-protein complex manifested higher compactness than celecoxib-protein complex throughout the study. Between 80-125ns, celecoxib-protein complex exhibited higher R_g compared with luteolin-protein complex.

Solvent-accessible surface area (SASA) is the surface area of a molecule that interact with the solvent molecule.[68] Figure 4d shows the average SASA value for celecoxib-protein complex, quercetin-protein complex and luteolin -protein complex was 303.08 nm2, 299.67 nm2 and 302 nm^2, respectively. The SASA values of all complexes showed that the quercetin-protein complex was less exposed to the water solvent than celecoxib-protein complex and luteolin-protein complex. These findings indicated high stability and compactness of quercetin bound protein.

Hydrogen bonds has an essential role in the stabilizing of a macromolecule. The binding affinity of a compound also depends on hydrophobic interactions .[69,70] Figure 5 shows the number of hydrogen bonds ranging between 0 and 3 for celecoxib-protein complex, 0 and 4 for luteolin-protein complex, 0 and 3 for quercetin-protein complex. Luteolin-protein complex showed highest number of hydrogen bonds at a time while celecoxib-protein complex illustrated highest average number of hydrogen bonds during the simulation period. Celecoxib-protein complex showed 2 hydrogen bonding over most of the time, while luteolin-protein complex and quercetin-protein complex showed 1 hydrogen bond. All hydrogen bonds along with the hydrophobic interactions has impact on the stability of the helical conformation. Thus, the compounds might be able to modify the function of COX-2 through hydrogen bond formations with the COX-2.

Conclusion

The literature study suggested that *A. philippense*, a species of fern, has potential pharmacological effects and possesses several classes of bioactive compounds. This study revealed that the extract or isolated compounds of *A. philippense* has potential analgesic, anti-bacterial, anti-oxidant, and cytotoxic activities. Further molecular docking and dynamics simulations investigations showed that the identified constituents quercetin, and luteolin were the most promising anti-inflammatory compounds present in *A. philippense*. Therefoe, it is evidenced that *A. philippense* one of the potential traditional medicinal ferns that possess bioactive compounds which could be useful in the prevention of different diseases including pain.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

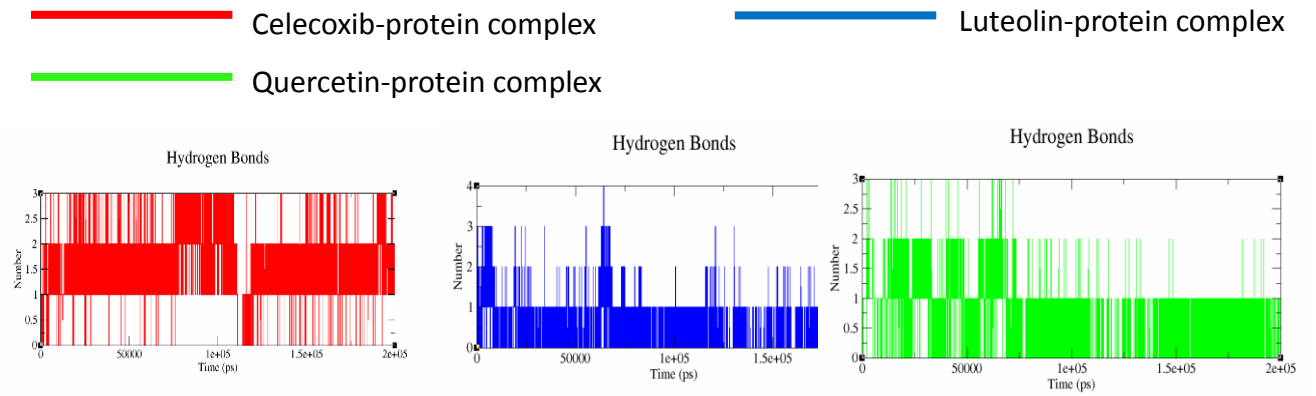

Figure 5: Plot of Number of hydrogen bonds versus time (in pico seconds) for celecoxib-protein complex (red), Quercetin-protein complex (green), Luteolin-protein complex (blue).

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