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Research Article

In Vitro Antioxidant, *in vivo*, and *in silico* Anti-Inflammatory Potential of *Microdesmis puberula* Hook. f. ex Planch. Leaf Extract

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ABSTRACT

Microdesmis puberula has been a plant of interest in ethnomedicine for the treatment of various diseases associated with inflammatory and oxidative stress processes. This study evaluated the phytochemical constituents, antioxidant, and anti-inflammatory properties of M. puberula Hook.f. ex Planch (Moraceae) ethanolic leaf extract. Qualitative and quantitative phytochemical analyses of the plant extract were performed using established methods, while the in vitro antioxidant activity was assessed through the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assays. The in vitro anti-inflammatory activity of the extract was examined using the carrageenan-induced paw oedema model. An in silico study was conducted via molecular docking of ligands identified from M. puberula on MAP kinase p38, 5-lipoxygenase, and cyclooxygenase-2 target proteins involved in inflammation. The extract contained significant phytochemicals such as alkaloids, flavonoids, saponins, and tannins, and demonstrated substantial antioxidant properties across all assays. Both the extract-treated and standard drug groups showed a non-significant ($p < 0.05$) reduction in rat paw volume compared

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to the normal control group in the carrageenan-induced paw oedema test, confirming the plant's anti-inflammatory activity. Molecular docking the plant's anti-inflammatory activity. Molecular docking studies indicated strong ligand and protein (inflammatory mediators) binding, highlighting their interactive mechanisms. These findings suggest that the plant possesses valuable antioxidant and anti-inflammatory compounds that warrant further exploration for potential drug development.

INTRODUCTION

Medicinal plants have been utilized in ethnomedicine for centuries to address various health issues and prevent diseases, including epidemics.[1] The global demand for herbal products has increased significantly as awareness grows of the link between bioactive compounds produced through plant metabolism and the biological effects of different plant species.[2] The body's metabolic functions can generate an excess of reactive oxygen species (ROS), which are associated with numerous health problems such as cardiovascular diseases, diabetes, infertility, Alzheimer's disease, and cancer.[3,4] Free radicals primarily damage biomolecules, including lipids, proteins, carbohydrates, and nucleic acids, leading to detrimental effects, such as inflammation.[5] Inflammatory processes involve the coordinated activation of biochemical and immunological signaling pathways, regulating levels of inflammatory chemical agents in local tissue cells and immune response cells migrating from the blood.[6] During an inflammatory state, immune cells experience elevated ROS levels, leading to oxidative stress.[7,8] Inflammation and oxidative stress demonstrate a profound interrelationship, as reflected in multiple converging pathophysiological processes.[9]

In drug discovery, employing *in silico* methods is crucial to the drug design process. This is primarily because they can significantly reduce costs and time while identifying and discovering new prospective chemical scaffolds, impacting the overall drug development trajectory. Computer-aided drug design (CADD) techniques are critical for decreasing the number of animals used in *in vivo* experiments, supporting the creation of safer pharmaceuticals, and modifying existing pharmaceuticals. CADD approaches help medicinal chemists at every stage of the drug discovery process, including design, discovery, development, and lead optimization[10,11]. Computational methods can be complex, necessitating interdisciplinary research and computer science to develop viable, cost-effective drugs.[11]

Microdesmis puberula Hook.f. ex Planch., is a member of the Moraceae family, commonly known by various native names, such as Idi-apata in Yoruba, Mkpiri and Mbugbo in Igbo, Amama, Erankpata in Esan, and Ntanebit in Efik,[12] and is indigenous to West tropical Africa. *M. puberula* and its related species, *M. keayana*, share several similarities ranging from their ethnobotanical description, distribution, uses, and phytochemical constituents.[13,14] Different parts of the plant, such as the leaves, stems, roots, and fruits, have been traditionally used for medicinal purposes, including infertility, malaria, skin conditions, pain, and diabetes, as well as for laxative effects and to prevent tumour growth. Research on this plant has investigated the antioxidant, analgesic, antibacterial, aphrodisiac, antisickling, and antimalarial properties of extracts from different parts of the plant.[12,14,15,16,17] Given the plant's extensive pharmacological significance, a systematic evaluation of *M. puberula* leaf extract for anti-inflammatory efficacy, integrating animal-based studies with computational modeling, is required to generate empirical data to substantiate its traditional therapeutic use.

MATERIALS AND METHODS

Chemicals and Standards

l-Ascorbic acid (Eurostar, UK), gallic acid (Riedel-deHaën®, Germany), quercetin (Sigma–Aldrich, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Molychem, India), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) (Loba Chemie, India), ammonium molybdate (Molychem, India), trisodium phosphate (Loba Chemie, India), Folin-Ciocalteu reagent (Loba Chemie, India), aluminum chloride (Molychem, India), ferric chloride, $FeCl_3.6H_2O$ (Molychem, India), ferrous sulphate, $FeSO_4.7H_2O$ (GHTECH, China), carrageenan (CDH, India), sodium carbonate (Molychem, India), potassium acetate (Loba Chemie, India), methanol (VWR, USA). All the chemicals are of good quality, primarily analytical grade, and were procured locally.

Collection and Identification of Plant Material

Fresh leaves of *M. puberula* were collected from its natural habitat in Ijebu-Ode, Ogun State, Nigeria. The plant was authenticated by a Taxonomist in the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria, and a voucher specimen deposited (FPI 2434) in the same facility.

Preparation of Extract

The leaves were washed with water, air-dried at room temperature, and pulverized to a fine powder. The powdered sample (500 g) was extracted by maceration with 80% ethanol for 72 hours, filtered, and concentrated in a rotary evaporator at 50°C. The concentrate was air-dried and kept in a refrigerator at 4°C until further use.

Preliminary Phytochemical Analysis

The extract was subjected to preliminary qualitative and quantitative phytochemical analysis for the presence of alkaloids, phenolics and tannins, flavonoids, terpenoids, saponins, steroids, glycosides, proteins, and carbohydrates.[18,19]

Determination of Total Phenolic Content

The determination of total phenolic content (TPC) of the extract was carried out following the Folin-Ciocalteu reagent (FCR) method.[20] The calibration curve was prepared using gallic acid. Graded concentrations of gallic acid and 1 mg/ml extract were prepared. Each 1 mL of extract or standard solution was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 10-fold with water) and allowed to stand for 3 minutes. Then, 2 mL of 7.5% sodium carbonate was added to the mixture. The mixture was shaken and left in the dark at room temperature for 30 min; thereafter, the absorbance of the solution was measured at 760 nm against a blank containing all reagents except the standard and the extract. A calibration curve was constructed using gallic acid standards at different concentrations. The total phenolic content of the extract was calculated in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g dry sample). The experiment was carried out in triplicate, and results were presented as mean value and the standard error of the mean (SEM).

Determination of Total Flavonoid Content

The aluminium chloride complex formation assay was used to determine the total flavonoid content (TFC) of the extract. Quercetin, a well-known flavonoid, served as the standard and was prepared in graded concentrations in methanol. A volume of 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate, prepared in methanol, was added to 1 ml of the standard and extract (1 mg/ml). The mixture was thoroughly shaken and left in the dark for 30 minutes to allow the reaction to complete. Afterwards, the absorbance of the solutions was taken at 420 nm using a UV-visible

spectrophotometer. The test was carried out in triplicate, and the total flavonoid content of the extract was estimated as milligrams of quercetin equivalent (QE) per gram of dry sample (mg QE/g dry sample). The values were expressed as the mean ± standard error of the mean (SEM).[21]

In Vitro Antioxidant Assay

DPPH Radical Scavenging Assay

To evaluate the antioxidant activity of the prepared *M. puberula* leaf extract, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was employed.[22] The samples and extract solutions (1 mL) at various concentrations (3.13, 6.25, 12.50, 25.00, 50.00, and 100.00 µg/mL) were combined with a 1 mL portion of the DPPH solution in methanol (0.05 mg/mL). Subsequently, the samples were left in the dark for 30 minutes, after which their absorbances were measured spectrophotometrically at 517 nm, with methanol as the blank. The test was carried out in triplicate, and the percentage inhibition of DPPH radical by the extract and control was calculated as follows:

$$\text{Inhibition of DPPH radical (\%)} \frac{(\text{A control} - \text{A test})}{\text{A control}} x\ 100$$

where '$A_{control}$' is the absorbance of the control (0.05 mg/ml DPPH solution) and 'A_{test}' is the absorbance of the test sample (extract + 0.05 mg/ml DPPH solution).

Ferric Reducing Antioxidant Power (Frap)

The antioxidant activity of the extract was also assessed using the ferric reducing antioxidant power protocol.[23] A mixture of 100 µL of the extract and 3 mL of the FRAP solution was prepared. The reaction mixture was subsequently incubated at 37°C in a dark cupboard for 30 minutes. The absorbance at 593 nm was measured with a UV-visible spectrophotometer (Biobase BK-D560) against a blank (2 mL FRAP + 1 mL H_2O). A standard curve for FRAP was established by combining varying concentrations of $FeSO_4.7H_2O$ (0, 100, 200, 400, 600, 800, and 1000 µM) with FRAP reagent, mirroring the procedure carried out with the extract. Based on the linear calibration curve, the antioxidant capacity of the sample was calculated by its ability to reduce ferric ions and is presented as millimole (mmol) of $FeSO_4$ equivalents per gram dry weight of the sample.

Total Antioxidant Assay (Tac)

The total antioxidant capacity of MPLE was assessed by the phosphomolybdenum method.[24] A 0.3 mL (1 mg/mL) of extract and graded concentrations of ascorbic acid were mixed with 3 mL of phosphomolybdenum reagent solution, which consisted of 0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was then incubated in the dark at 95°C for 90 minutes. Following the incubation period, the tubes were cooled to room temperature, and the absorbance of the reaction mixture was measured at 695 nm via a blank (0.3 mL of phosphomolybdenum reagent + 3 mL of methanol). Ascorbic acid (AA) served as the positive reference standard and was used to establish the standard calibration curve to estimate the antioxidant capacity of the extract. The experiment was conducted in triplicate, and values were expressed as ascorbic acid equivalents in mg per gram of extract.

Animals and Ethical Approval

The male albino rats, weighing 150–220 g, were obtained from the Animal Research Centre, Afe Babalola University, Ado-Ekiti. The plants were kept under standard conditions, with a temperature of approximately 25°C, a 12-hour natural light, and a daylight cycle for 14 days. They were given

a standard diet (Topfeed finisher pellet) and had access to water. The experimental protocols followed Ethical Guidelines for the Care and Well-being of Research Animals (NIH, 1992). Ethics approval was applied for and granted by the Ethics Committee of the College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Nigeria (ABUADHREC/05/10/2023/217).

Acute Oral Toxicity

Acute oral toxicity of the extract was carried out according to test number 423 of the Organization for Economic Cooperation and Development (OECD) guidelines (OECD, 2002). In the experiment, ten (10) male Wistar rats fasted overnight were assigned equally to both the experimental and control groups. A limit test was performed using a single oral dose of 2000 mg/kg. Before dosing, the rats were deprived of food for 3–4 hours but were allowed free access to water. The rats were carefully observed for the initial 30 minutes post-dosing and then for 4 hours. Following the survival of the treated rats, four additional rats were given the same dose under the same conditions. The same procedure was repeated for the control group, which received 10 mL/kg body weight of distilled water. Clinical observation was performed daily for any signs of death or treatment-related side effects. The animals were then monitored for 14 days to document any potential delayed deaths or adverse effects.[25,26]

Anti-Inflammatory Activity

The carrageenan-induced paw oedema protocol was employed to investigate the anti-inflammatory activity of MPLE.[27] Employing the G-power sample size calculation tool, thirty male Wistar rats were distributed into 5 groups, with each group having 6 rats. Group 1 (negative control) was administered 1 mL/kg of normal saline (0.9%). Group 2 received the standard drug diclofenac at a dosage of 10 mg/kg body weight. Groups 3, 4, and 5 were given MPLE at doses of 100, 200, and 400 mg/kg body weight, respectively. Subsequently, a 0.1 mL carrageenan suspension (1% w/v in 0.9% normal saline) was injected into each rat's sub plantar region of the right hind paw. The paw diameter was measured using a Vernier caliper before the injection and at 1, 2, 3, 4, and 5 hours after. The percentage inhibition of edema was calculated based on the formula provided and compared with that of the control group, which was given the vehicle.

Percentage inhibition =

$$\frac{\text{Change in control} - \text{change in treatment}}{\text{Change in control}} \times 100$$

The change in paw thickness values (mm) was calculated from the difference between the left and right paw volumes.

Molecular Docking

Ligand Library Generation and Preparation

The phytochemicals described for *M. puberula,* as shown in Figure 1, were obtained from the literature.[12,14,16]. The 2D and 3D structures of the compounds were not available in the online chemical libraries; thus, the 2D structure was constructed via the MarvinSketch suite, Build 22.12.0-1538, ChemAxon (https://www.chemaxon.com). The 2D structures were transformed into 3D structures with the aid of the ligprep tool of Schrödinger Suite software, Maestro 11.5.[28]

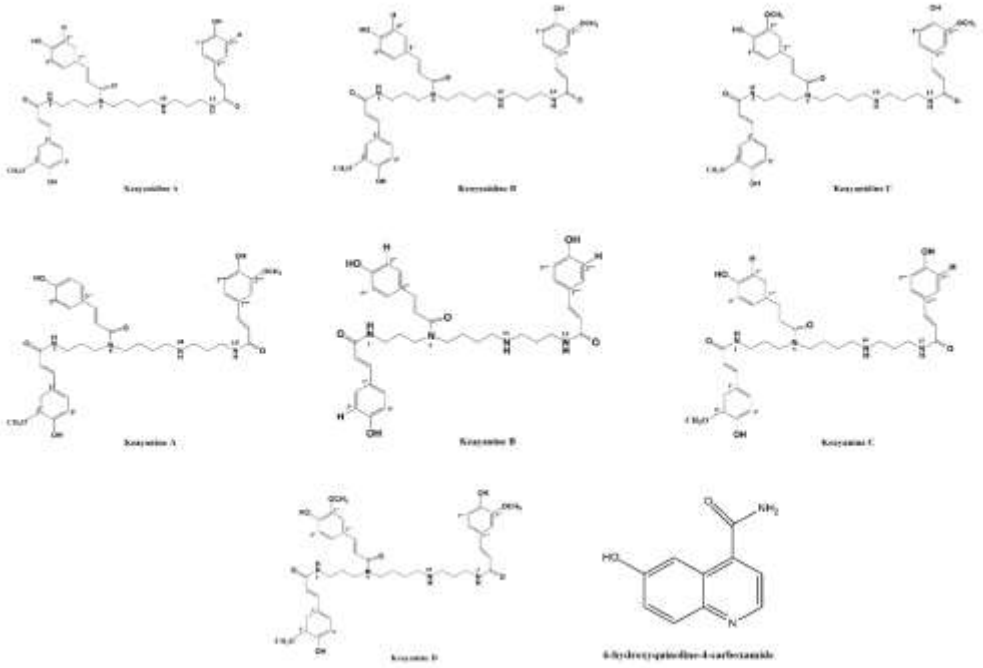


Figure 1. The 2D Structure of phytochemicals isolated from M. puberula and M. keayana plants used for the molecular docking study.[12]

Target Retrieval and Preparation

The X-ray crystal structures of the selected targets, MAP kinase p-38 (PDB ID: 1A9U),[29] 5-lipoxygenase (PDB ID: 6N2W),[30] and cyclooxygenase-2 (PDB ID: 3LN1). They were retrieved from the Protein Data Bank (https://www.rcsb.org) and their corresponding bound ligands (Figure 2). Protein visualization was performed with the PyMOL Molecular Graphics System (Version 2.5, Schrödinger, LLC). The proteins were prepared before docking, following standard procedures.[28]

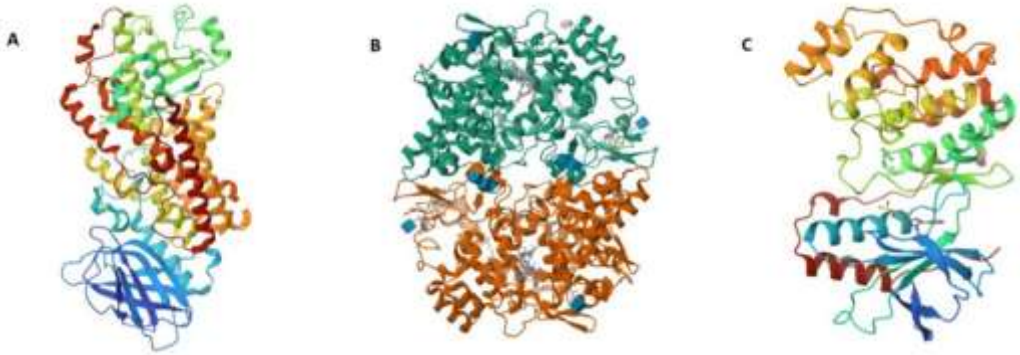


Figure 2. 5-Lipooxygenase bound to Nordihydroguaiaretic acid (NDGA) (A); Cyclooxygenase-2 (COX-2) in complex with celecoxib, a COX-2 selective inhibitor, (B); Map kinase p-38 complex structure with SB 203580 (C) (https://www.rcsb.org/).

Virtual Screening and Docking Platform

The ligand library of isolated phytochemicals from *M. puberula* in 3D format was docked to the active sites of the selected targets to predict the compounds with the best inhibitory potential in preventing inflammation. The Schrödinger Suite Software Maestro 11.5 was used for the docking study via the standard molecular docking principle.[31]

Statistical Analysis

The data were subjected to one-way ANOVA followed by Dunnett's *post hoc test*. The results are presented as the mean ± standard error of the mean (SEM), and the significance level was set at $p < 0.05$. All the statistical analyses were performed using GraphPad Prism Software Version 9.0 (GraphPad Software Inc., United States).

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Phytochemical analysis of *M. puberula* leaf extract (MPLE), as shown in Tabe 1, revealed the presence of alkaloids, flavonoids, polyphenols, and saponins. These phytochemicals could be responsible or contribute to the plant's medicinal properties. Polyphenols such as flavonoids, phenolic acids, tannins, coumarins, lignans, and lignins are the most abundant antioxidant compounds found in natural products.[32,33,34], most of their biological properties attributed to their redox properties which enables them to function as reducing agents, donate hydrogen, quench singlet oxygen, bind to metal ions, and reduce ferryl haemoglobin.[35]

Table 1. Preliminary phytochemical analysis of MPLE

Phytochemical	*M. puberula*
Alkaloid	+
Flavonoid	+
Phenolics and Tannins	+
Saponin	+
Anthraquinones	-
Cardiac glycosides	+
Steroids	-
Phlobatannins	-
Carbohydrates	-
Amino acids	-

Keywords: (+) means present, and (-) means absent

Total Phenolic and Flavonoid Contents

The TPC and TFC of MPLE were estimated from the gallic acid and quercetin standard calibration curves, and expressed as mg gallic acid equivalent (GAE)/g of extract and mg quercetin equivalent (QE)/g of extract. MPLE was found to have a total phenolic and flavonoid content of 1.604±0.20 mgGAE/g dry extract and 26.55±1.78 mgQE/g dry extract, respectively (Table 2). The presence of polyphenols and flavonoids in MPLE strongly substantiates their antioxidant properties and ability to quench the action of reactive oxygen species such as hydroxyl radicals, superoxide anion radicals, and lipid peroxy radicals. *M. puberula* is commonly used in traditional medicine to treat various ailments,[12] primarily caused by oxidative stress and lipid peroxidation from reactive oxygen species (ROS). One established process in which lipid peroxidation occurs is through free radical chain reactions. Substances that can scavenge radicals may directly react with peroxide radicals, thus stopping peroxidation chain reactions, which play crucial roles in the development of various disease conditions.[5,36,37] This study has validated that *M. puberula* is rich in antioxidant phytochemicals, as demonstrated in Table 2, making it a potential source of lead compounds for the development of therapeutic agents that are effective against ROS-dependent or potentiating ailments.

Table 2. Total phenolic, flavonoid contents and *in vitro* antioxidant activity of MPLE

Sample	Part used	TPC (mgGAE/g dry extract)	TFC (mgQE/g dry extract)	DPPH (IC_{50}) (µg/ml)	FRAP (mmol $FeSO_4$ equivalent/g extract)	TAC (mg ascorbic acid equivalent/g dry plant extract)
MPLE	Leaf	1.604±0.20	26.55±1.78s	211.10±2.51	651.38±14.87	295.11±5.22

Key: TPC – Total phenolic content; TFC- Total flavonoid content; DPPH - 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay; FRAP - ferric reducing antioxidant power; TAC – Total antioxidant capacity; IC50 – Half-maximal inhibitory concentration.

Free Radical Scavenging Activities

The antioxidant properties of the MPLE were assessed using the DPPH scavenging assay, the Ferric reducing antioxidant power (FRAP) assay (expressed as mmol $FeSO_4$ per g of dry extract), and the total antioxidant capacity (expressed as mg of ascorbic acid equivalents per g of dry extract). The extract showed a concentration-dependent DPPH radical scavenging activity. At 500 µg/mL, the extract and ascorbic acid achieved the highest percentage inhibition of 71.81 ± 1.14% and 95.50 ± 0.69%, respectively (Figure 3A). The IC_{50} of MPLE was determined to be 211.10 µg/mL (Table 2). The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging antioxidant assay measures the extract's ability to neutralize the DPPH radical, a stable free radical with a strong absorption band at 517 nm. When an antioxidant donates an electron or hydrogen atom to DPPH, the radical is reduced to a more stable molecule, resulting in a purple colour in the solution.[38,39,40] The ability of MPLE to reduce Fe^{3+} to Fe^{2+} through electron donation was evaluated by the FRAP assay, another measure of antioxidant activity. The ferric reducing antioxidant power of MPLE was found to be 653.28 ± 14.87 mmol $FeSO_4$ equivalent/g dry extract, based on the standard curve shown in Figure 3B. The extract demonstrated notable ferric reducing antioxidant power compared with standard gallic acid. Following reduction by antioxidants in the sample, the formation of Perl's blue at 695 nm was measured to determine the amount of Fe^{2+} complex. An increase in absorbance generally indicates greater reductive capacity.[41] Additionally, the total antioxidant capacity of the extract was evaluated via the phosphomolybdenum assay, which reduces Mo^{VI} to Mo^{V} and creates a green phosphate/Mo^{V} complex at an acidic pH. The results in Table 2 showed that MPLE possesses a TAC value of 294.99 ± 5.22 mg ascorbic acid equivalents per g of dry extract.

The findings from the DPPH, FRAP, and TAC assays validated the antioxidant activity of MPLE as detailed in Table 2. Research on the stem bark extracts of *M. puberula* have demonstrated the plant's strong DPPH radical scavenging properties, with IC_{50} values of 1.1 µg/mL and 1.2 µg/mL for methanol and petroleum ether extracts, respectively.[42] Furthermore, the antioxidant effects of *M. keayana* aqueous root extract (a species closely related to *M. puberula*) against superoxide anion, hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and hydroxyl radical (HO•) were observed to be substantially dose-dependent in both cellular and non-cellular systems against superoxide radical anions.[16,43] An investigation into some spermidines isolated from a methanolic root extract of *M. keayana*, showed these compounds possessed potent antioxidant effects against DPPH.[43] This study's results align

with prior findings on the antioxidant activity of MPLE and its close relative, *M. keayana*, indicating that the plant is an effective scavenger of free radicals capable of preventing lipid autoxidation, thus offering significant benefits in the treatment of diseases associated with oxidative stress and lipid peroxidation.

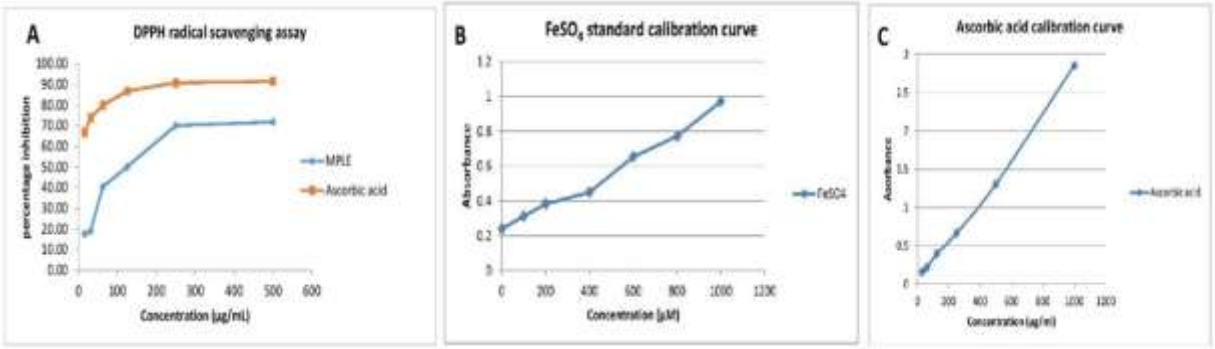


Figure 3. DPPH radical scavenging assay (A); $FeSO_4$ calibration curve for FRAP (B); Ascorbic acid calibration curve for TAC (C). The data are presented as the means ± standard deviations (SDs) (n = 3).

Acute Oral Toxicity

M. puberula leaf extract did not cause any deaths within 24 hours in the acute oral toxicity test, and there were no observable changes in behaviour within two weeks after the administration of 2000 mg/kg, which supports other previous findings by Okany et al. (2012) and Akpanyung et al. (2013),[17,44] who reported a wide safety margin for the extract. Therefore, based on the OECD guidelines for testing chemicals, MPLE is considered safe and falls under approximately category 5 of substances with acute oral toxicity above 5000 mg/kg.[26]

In Vivo Anti-Inflammatory Activity

To evaluate the potential anti-inflammatory effects of MPLE, the carrageenan-induced inflammation assay was used, a common method for assessing the anti-inflammatory activity of drugs in animal models.[45,46] After carrageenan injection, there was a notable increase in the thickness of the rats' paws. The extract significantly reduced paw inflammation in a dose-dependent manner ($P < 0.05$). At doses of 100, 200, and 400 mg/kg MPLE, the inhibition of rat paw oedema was most prominent at the 5th hour, with levels reaching 59.01%, 63.29%, and 66.37%, respectively, compared to 74.77% in the standard drug group. Importantly, the extract showed anti-inflammatory effects from the first hour of measurement at all tested doses (Table 3). These findings corroborate with preceding studies,[47] that *M. puberula* possesses anti-inflammatory activities, making it a potential source of lead compounds for the development of therapeutic agents that are effective against ROS-dependent or potentiating ailments.

Research indicates that acute inflammation caused by carrageenan involves a two-phase response, with the initial phase of about one hour involving the release and effects of histamine, bradykinin, serotonin, and substance P, which contribute to vascular permeability. The later phase (after one hour) is mainly driven by lysosomal enzymes, proteases, and prostaglandins, resulting in the abnormal release of inflammatory fluids and oedema at the site of inflammation.[48] Infiltration of the inflammation site by polymorphonuclear (PMN) cells triggers the excessive release of proinflammatory mediators such as nitric oxide, prostaglandins, and cytokines, prolonging this phase.[49] Our study demonstrated that MPLE has an anti-inflammatory effect on the initial paw

thickness measurement, indicating that the extract can inhibit both early and later stages of inflammation. The plant extract contains phytochemicals like flavonoids and polyphenolic acids, which significantly contribute to its peripheral anti-inflammatory properties.[50] Research has also demonstrated that phytochemicals such as flavonoids enhance anti-inflammatory activity in plants by inhibiting prostaglandin synthetase, particularly endoperoxidase.[51,52]

Table 3. Effect of MPLE on carrageenan-induced paw edema in rats

Groups	Dose (mg/kg)	Change in paw thickness mm (hours post-treatment)				
		1st	2nd	3rd	4th	5th
Control (saline)	-	1.90±0.07	2.36±0.14	2.82±0.20	2.74±0.14	2.50±0.07
Diclofenac	10 mg/kg	1.08±0.05* (43.06)	1.52±0.21* (36.56)	1.28±0.22* (52.91)	1.00±0.13* (62.58)	0.62±0.08* (74.77)
MPLE	100	1.12±0.06* (34.61)	1.36±0.08* (40.53)	1.58±0.12* (41.89)	1.30±0.07* (51.60)	1.02±0.05* (59.01)#
	200	1.44±0.11* (24.44)	1.32±0.27* (44.47)	1.48±0.12* (46.07)	1.48±0.18* (51.60)	0.92±0.13* (63.29)
	400	1.30±0.14* (31.28)	1.08±0.15* (54.17)	1.44±0.14* (47.56)	1.00±0.09* (63.35)	0.84±0.07* (66.37)

Data represent the mean ± SEM (n = 5); values with '*' are significantly different from those of the normal group ($p < 0.05$).

In Silico Screening of *M. puberula* against Several Inflammatory Proteins

In drug design, *in silico* approaches have been explored to predict, generate, and confirm ligands as possible drug candidates. Figure 4 below shows the glide scores and MM/GBSA values of the phytochemicals from *M. puberula* against MAP kinase p-38, 5-LOX, and cyclooxygenase 2 (COX-2). The 2D interactions of the amino acids at the active sites of MAPK p-38, 5-LOX, and COX-2 with the phytochemicals from *M. puberula* and the co-crystallized ligands/synthetic inhibitors are shown in Figure 5.

MAP kinases play crucial roles in the regulation of cell processes. p38 MAPKs are involved in inflammatory processes via the production of inflammatory mediators, including cyclooxygenase-2 (COX-2) and tumour necrosis factor-α (TNF-α).[53] COX-2 and 5-lipoxygenase (5-LOX) play key roles in prostaglandin and leukotriene synthesis, and the need for the discovery of a dual anti-inflammatory agent that blocks both enzymes has been identified.[54,55,56] Thus, the blockade of these enzymes will help prevent inflammation. An effective means of predicting the binding affinity of chemical compounds/phytochemicals is MM/GBSA. Previously, Prime MM/GBSA was used and described as an accurate tool for post-docking analysis of docked complexes.[57] A lower score indicates better binding and stability of the docked complex, whereas a higher value indicates poor binding and stability. Figure 4 shows that the phytochemicals from our plant have similar binding energies and, by implication, affinities and stabilities with the reference molecules. For COX-2, keayanine A has good binding affinity, with a glide score of -10.63, whereas keayanine B has the best interaction with MAPK p-38, with a glide score of -8.51, which is better than that of the co-crystallized ligand (-7.31). Compared with the co-crystallized ligand SB203580 (-8.15, -44.35), keayanine B has a strong and superior glide score and MMGBSA (-9.84, -71.45).

The spermine alkaloids from our plant show optimum interactions with key amino acids at the active site of these receptors, similar to those observed for synthetic inhibitors. For p38, the synthetic co-crystallized ligand (SB203580) shows pie-pie stacking with TYR^{35} and H-bonding with LYS^{53} and MET^{109}. A similar interaction was observed for Keayanine B and Keayanine D. Similar to SB203580 and ATP, Keayanine D accepts hydrogen from MET^{109} while forming an H-bond with LYS^{53}. These interactions were also observed for Keayanine B. These interactions have been described as key for MAPK p38 activity and selectivity.[29] Concerning 5-LOX, PHE^{359} and TRP^{599} interact with the co-crystallized inhibitor. It also forms a polar H-bond with ASN^{407}, ARG^{596}, HIE^{600}, and ILE^{673}. A similar interaction was observed for keayanine D, which has the same π-π interaction and H-bond with GLN^{363}, $ARG^{596,}$ and HIE^{600}. Keayanine C interacts similarly to keayanine D, with an additional H-bond with GLU^{417}. For COX-2, celecoxib shows a pie-cation interaction with ARG^{106} and H-bonds with GLN^{178}, LEU^{338}, SER^{339}, ARG^{499}, and PHE^{504}. H-bonds with LEU^{338} at the active site are associated with celecoxib activity against COX-2.[58,59] Keayanine A, C, and D interact with COX-2 in a similar pattern as celecoxib, showing H-bond interactions with LEU^{338}, as shown in Figure 5. The results show the potential of these spermine alkaloids as anti-inflammatory compounds.

These findings suggest the potential of our plant and its phytochemicals as anti-inflammatory agents, which may exert their activity by inhibiting the 5-LOX, COX-2, and MAP kinase signaling pathways.

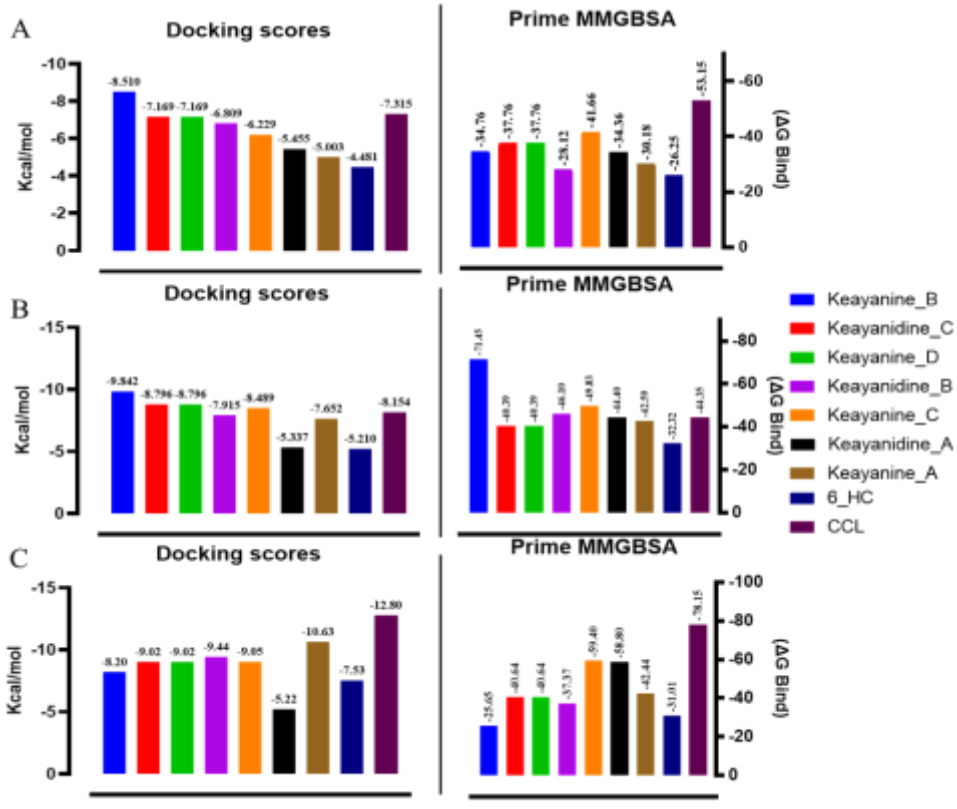


Figure 4. Glide scores and MM/GBSA of the phytochemicals from *M. puberula* against A- MAPK p-38, B- 5-LOX, and C- COX-2. 6_HC = 6-hydroxyquinoline-4-carboxamide, CCL = cocrystallized ligand.

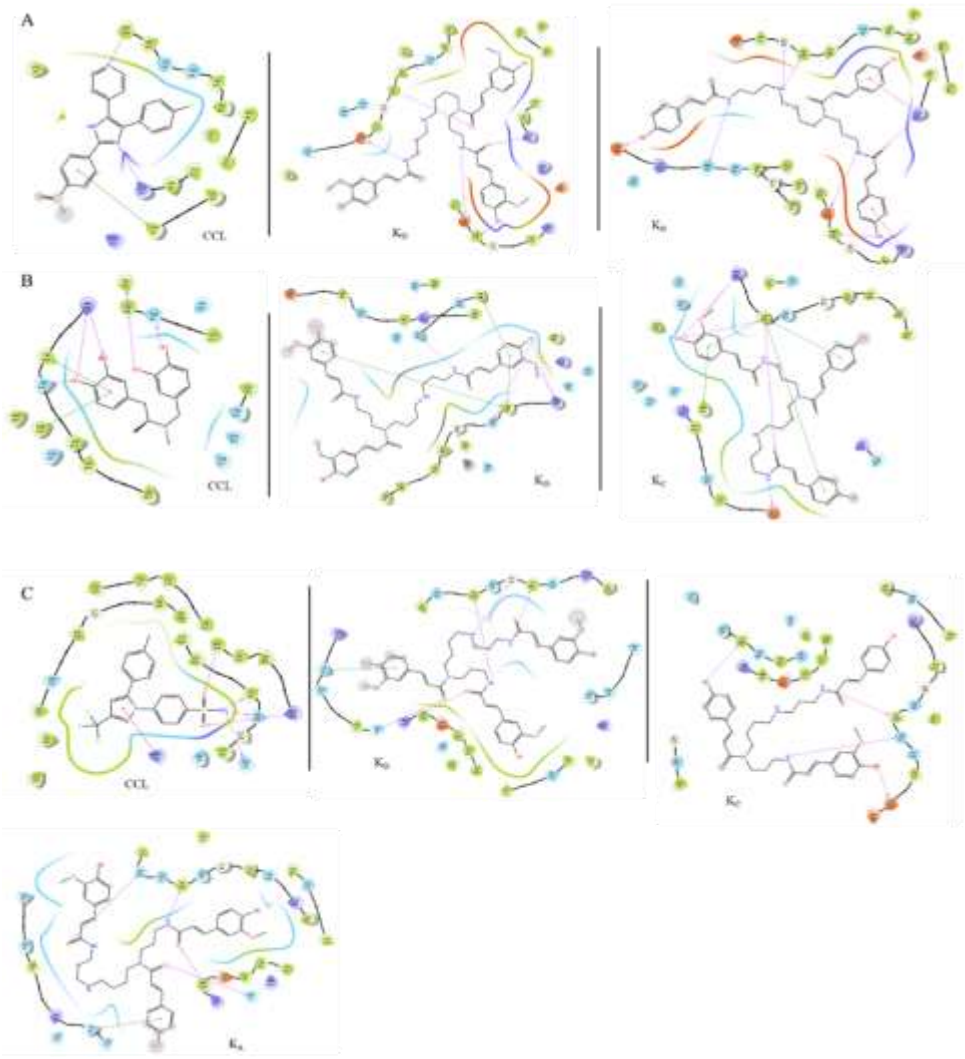


Figure 5. 2D-Molecular interactions of amino acid residues of A- MAPK p-38, B- 5-LOX, and C- COX-2 with spermine alkaloids from *M. puberula*. K_A- Keayanine A, K_B- Keayanine B, K_C- Keayanine C, K_D- Keayanine D, and CCL- Cocrystallized ligand/synthetic inhibitor.

CONCLUSION

Leaves of *M. puberula* were found to contain diverse phytochemicals, and the extract demonstrated significant antioxidant and anti-inflammatory properties. Such pharmacological activities substantiate its traditional use in managing inflammatory conditions. Moreover, the results underscore the importance of continued research to identify and characterize bioactive molecules that could be developed as novel drug candidates for inflammation and ROS-associated pathologies.

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