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Research Article

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Phytochemical analysis and antioxidant potential of *Pseudolachnostylis maprouneifolia*

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ABSTRACT

Pseudolachnostylis maproneifolia is a popular medicinal plant, particularly revered in the eastern province of Zambia, especially within Petauke District. Despite its widespread application in traditional medicine, the chemical composition of this plant has not been studied so far. Consequently, there was a pressing need to investigate the medicinal potential of its leaf extract. This study was carried out through preliminary phytochemical screening and GC-MS analysis of the ethanolic extract. The phytochemical profile revealed various secondary metabolites endowed with antifungal, anti-inflammatory, anti-tumor, anti-cancer, antibacterial, antiviral, and antioxidative properties. To quantify its medicinal potential, the total flavonoid content (TFC) and total phenolic content (TPC) was determined using a UV-Vis spectrophotometer. The methanolic extract exhibited a TFC of 157.20 mg QE/g and a TPC of 63.75 mg GAE/g, with an IC_{50} value of 45.57 µg/mL, signifying a strong antioxidant capability. Notably, the IC_{50} value of the standard (ascorbic acid) was 23.19 µg/mL. Further analysis involved quantifying the mineral elements crucial for treating anemia, such as iron, copper, zinc, and manganese. AAS based analysis revealed concentrations of these elements to be relatively higher, indicating their suitability for treating anemia. Complementing these findings, *in silico* molecular analysis and ADMET analysis of five selected phytochemicals, along with a reference cancer drug, yielded promising results such as a highest binding energy of -9.01 Kcal/mol, high gastrointestinal absorption and the lowest inhibition constant of 249.88 nM. These outcomes strongly advocate for subsequent *in vitro* and *in vivo* studies on these molecules, supporting their importance as potential drug candidates for cancer drug development.

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INTRODUCTION

The *Pseudolachnostylis maproneifolia* tree, belonging to the Phyllanthaceae family, is exclusively found on the African continent, specifically in the southern, central, and certain parts of the eastern regions. Its visual allure is enhanced when its leaves transition to a striking brick-red hue before shedding in winter. This semi-deciduous tree attains a height of up to 12 meters, characterized by white, smooth branches and a rough trunk adorned with gray-brownish irregular scales.

The leaves of this species are small, oval, and arranged alternately on the twigs. The spherical and green-yellow fruits measure about 2.0 cm in radius and contain three seeds within three sections. Indigenous communities in the Eastern province of Zambia have long utilized this plant for both human and veterinary medicine, employing it to treat various ailments.

The geographic distribution of *P. maproneifolia* spans low-altitude zones across South Africa, Zimbabwe, Namibia, Tanzania, the Democratic Republic of Congo, Burundi, Malawi, and Zambia, encompassing southern, eastern, central, northern, western, and Copperbelt provinces. Thriving in frost-free, predominantly loamy or sandy soils, this tree exemplifies adaptability. Figure 1 illustrates the shaded regions in Africa where this particular plant species is found.

In Africa and some Asian countries, ethno-medical knowledge of plants is traditionally applied to treat many diseases. The therapeutical action of a plant depends on its chemical constituents (Halliwell & Gutteridge, 1999). Natural products are traditionally used as medicine and account for almost one-quarter of all modern medicines. According to an estimate, between 70 and 95% of the population in developing countries use traditional medicine (Lu *et al.*, 2011). In Africa, over 80% of the population uses traditional medicine (Kasilo & Trapsida, 2010). From time immemorial, herbal medicines have been used for the relief of symptoms of disease (Maqsood *et al.*, 2010). The ancient records on the use of medicinal plants in the maintenance of health and treatment of human ailments

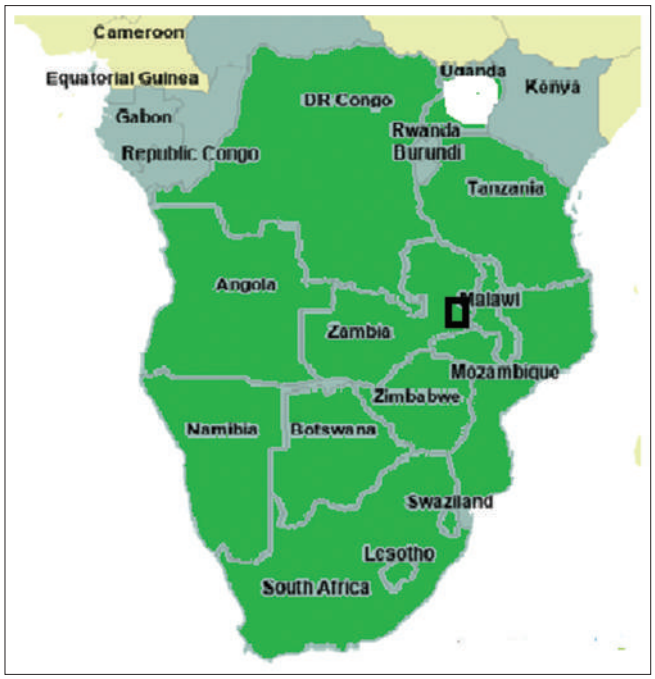


Figure 1: Regions of Africa where *P. maproneifolia* is reported to be growing (sourced from Google)

were found in Nagpur in a Sumerian clay slab, approximately 5000 years old, and from Babylon circa 1770BC (Petrovska, 2012). The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.), which documented oils from Cupressus sempervirens (Cypress) and Commiphora species (myrrh), which are still used today to treat coughs, colds, and inflammation (Cragg & Newman, 2005).

Despite the significant developments observed in modern medicine in recent decades, plants still make an important contribution to health care (Saeed *et al.*, 2012). Medicinal plants have been investigated for their antioxidant properties. Natural antioxidants found in plants are well known for their effectiveness in preventing the destructive processes caused by oxidants. Antioxidants deactivate or stabilize free radicals by donating electrons to them before they attack target molecules in biological cells (Nunes *et al.*, 2012). External sources of free radicals are motor vehicle emissions, ultraviolet radiation, and cigarette smoke. Conversely, oxidative phosphorylation and respiration processes in the mitochondria are the internal sources of free radicals. Free radical reactions cause different diseases, such as diabetes, atherosclerosis, premature aging, degenerative cataracts, cancer, and heart diseases (Kikuzaki *et al.*, 2002). Antioxidants or antidotes of free radicals are required to stop or inhibit free radical reactions to counter the destructive effect of free radicals.

Secondary metabolite compounds derived from plants, especially phenolic compounds, have the potential to be natural antioxidants. Various studies suggest that phenolic compounds such as phenolic acids, lignins, flavonoids, coumarins, cinnamic acids, tannins, and tocopherols have their activities as natural antioxidants (Kähkönen *et al.*, 1999). Antioxidants obtained from plants are highly recommended antidotes because they are safer in the body and can stop oxidative damage through reduction with oxidants by capturing oxygen and forming chelates with catalytic metal compounds (Sayuti & Yenrina, 2015).

To the best of our knowledge, no phytochemical study on *P. maproneifolia* extracts has been conducted. In the present study, total phenolic content (TPC) and total flavonoid content (TFC) was determined along with the free radical scavenging activity of the methanolic extract in vitro model using a UV-visible spectrophotometer. Gas chromatography- mass spectrometric analysis of alcoholic extract was carried out to determine the phytochemical profile of the leaves. Since the plant is also traditionally used to treat anemia, among other diseases, mineral elements of interest such as iron (Fe), copper (Cu), Zinc (Zn), and manganese (Mn) were also determined by using atomic absorption spectrophotometer (AAS).

In recent years, there has been a deliberate effort to explore several small cell-permeable protein kinase inhibitors as potential treatments for various cancers (Jiao *et al.*, 2018). Among the investigated compounds, quinazolines (Wagner *et al.*, 2009) and quinolinecarbonitriles (Tsou *et al.*, 2005) are noteworthy examples of small molecules studied for their kinase inhibitory properties. Additionally, complexes of 3-phenylpyrazolopyrimidine (PhPP) derivatives have also been identified as inhibitors targeting the ATP-binding site of Hemopoietic cell Kinase Hck (Protein Data Bank (PDB) 1QCF) (Zhu *et al.*, 1999). In Silico molecular analysis was also carried out on selected phytocompounds. The present study identified five phytocompounds with anti-cancer activity against the cancer target 1QCF tyrosine kinase based on molecular docking.

In drug development, in silico models related to ADMET (absorption, distribution, metabolism, excretion, and toxicity) are frequently employed for a swift and initial assessment of ADMET properties. This preliminary screening aids in prioritizing compounds for further *in vitro* investigations (Tao *et al.*, 2015; Tian *et al.*, 2015; Wang *et al.*, 2016, 2017). This study analyzed five selected phytocompounds and one reference drug for ADMET properties. The ADMET results are interesting enough for these molecules to be considered for *in vitro* and *in vivo* analysis.

MATERIALS AND METHODS

Chemicals

The chemicals such as quercetin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and Gallic acid, Folin- Ciocalteu reagent, aluminium chloride, sodium hydroxide, methanol, sodium nitrite, and sodium carbonate (all were of analytical grade) were procured from Pallav Chemicals & Solvents Pvt. Ltd, Maharashtra, India. Other solvents and chemicals used in this study were also of analytical grade and purchased locally from within Zambia.

Collection of Plant Material

The leaves from *Pseudolachnostylis maproneifolia* were collected in March 2021, in Petauke District of Eastern Province of

Zambia. The leaves were rinsed in water to remove dust particles, dried in the shade for 15 days, ground to powder, and stored in a well-secured container for subjection to study further. Kitwe District Herbarium of the forest department of the Ministry of Environment and Natural Resources of Zambia authenticated the plant as *P. maproneifolia*.

Qualitative Phytochemical Screening

The powdered leaf sample (5 g) was added to 50 mL of double distilled and deionized water and was boiled at 60 °C for 10 minutes. The boiled extract was filtered using Whatman 42 filter paper. The obtained filtrate was boiled till the formation of chocolate color developed. This plant extract was subjected to preliminary phytochemical screening. Following the previously reported methods, the absence or presence of phytochemicals was qualitatively determined.

The preliminary screening of phytocompounds was carried out to qualitatively identify the presence of various phytochemical classes in the sample. Standard chemical tests such as Hager's test for alkaloids, Braymer's test for tannins, Foam test for saponins, and Salkowski test for steroids were used. Further, Libermann's test for phenolics, Shinoda's test for flavonoids, Molisch's test for carbohydrates, Liebermann's test for glycosides, and Borntrager's test for anthraquinones were used for their identification. These tests have been reported in the literature (Ismail *et al.*, 2017; Widjajakusuma *et al.*, 2018; Dada *et al.*, 2019; Patle *et al.*, 2020). Coumarins were also tested using a previously reported method (María *et al.*, 2017).

GC-MS Analysis

The powdered sample was extracted using Soxhlet extraction method. The major advantage of this method is that it is an automatic continuous method requiring less time and solvent consumption (Bitwell *et al.*, 2023). The extraction efficiency of the Soxhlet apparatus was enhanced by operating it at a constant temperature of 75 °C. Ethanol was chosen as the solvent of extraction. After the extraction process was completed, solvent recovery was done by using a rotary evaporator in order to obtain the sample concentrate for GC-MS analysis.

The GC-MS analysis of the solvent extract was done by using a model instrument Thermo-GC Trace Ultra Version 5.0 (THERMO MS DSQ II) attached with a capillary column DB-5 (0.2 µm film × 0.25 mm) I. D × 30 m length. Analysis was conducted by injecting 1 µL of the sample with a split ratio 20:1. The carrier gas used was Helium gas (99.9%) at a 1.0 mL/minute flow rate. The analysis was performed in EI (electron impact) mode with 70 ev (electron volt) of ionization energy. The temperature of the injector was kept constant at 260 °C, while the temperature of the column oven was initially set at 70 °C for 3 minutes and was adjusted to 260 °C at a rate of 6 °C/minute. The final temperature was maintained at 260 °C for 15 minutes, and compound identification was made by comparing the spectral configurations which were obtained with the available mass spectral database (NIST Library).

Mineral Element Analysis

Sample preparation by dry ashing method

A 5.0 g of leaf powder was placed in a porcelain crucible, and another crucible was used as a lid to cover the sample. The powder was heated at 480 °C for 8 hours in a muffle furnace and cooled, then dissolved in 10.0 mL of 20% hydrochloric acid (HCl) and gently warmed to dissolve the residue. The solution was filtered through the acid washed filter paper into 50mL volumetric flask and was diluted to the volume with de-ionized water (Boline & Schrenk, 1977; Paul *et al.*, 2017). The prepared sample's mineral quantification was done by atomic absorption spectrophotometer (AAS).

Mineral element determination by Atomic Absorption Spectrophotometer (AAS)

The extracts were analyzed to determine the concentrations of Manganese (Mn), copper (Cu), iron (Fe), and Zinc (Zn). The content of these mineral elements in *P. maproneifolia* leaves was determined by atomic absorption spectroscopy (AAS) using Perkin-Elmer PinAAcle 500 spectrophotometers at the Copperbelt University.

Sample Preparation for Estimation of Flavonoid Content, Phenolic Content and Antioxidant Power Levels

For this estimation, maceration method of extraction was used (Bitwell *et al.*, 2023). 20 g of the powder was macerated with 300 mL of methanol for three days with occasional shaking. The mixture was filtered using Whatman number 42 (2.5 µm; pore size for slow flow) filter paper. The extract was stored in an amber-colored bottle in a refrigerator in readiness for spectroscopic analysis using a UV-Visible spectrophotometer.

Preparation of standard solution of Quercetin

To 100 mL of 80% methanol, 100 mg of Quercetin was added. The solution was diluted serially in 5 mL of methanol to make 6 different concentrations of 20, 40, 60, 80, 100 and 120 µg/mL as shown in Table 1.

One mL of diluted solutions was taken from each of the six aliquots of different concentrations and was added to 6 different test tubes already with 4 mL of distilled water. At the same time, 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added to the test tubes and 0.3 mL 10% aluminium chloride ($AlCl_3$) was added after 5 minutes. After that, 2 mL of 1 M

Table 1: Dilution for Quercetin

Quercetin (mL)	methanol (mL)	Concentration (µg/mL)
0.1	5	20
0.2	5	40
0.3	5	60
0.4	5	80
0.5	5	100
0.6	5	120

sodium hydroxide (NaOH) was added to the mixtures in the test tubes after 6 minutes. The volume of each mixture in the test tube was thus made to 10 mL by adding exactly 4.4 mL of distilled water. Each mixture in the test tube was run on a UV/Vis spectrophotometer at the wavelength of 510 nm to obtain the absorbance as shown in Figure 2.

Determination of Total Flavonoid Content (TFC)

One mL of the extract was pipetted and placed in 3 separate test tubes, each already containing 4 mL of distilled water. 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added to the test tubes and 0.3 mL 10% aluminum chloride ($AlCl_3$) was added after 5 minutes. Thereafter, 2 mL of 1 M NaOH was added to the mixtures in the test tubes after 6 minutes. The volume of each mixture in the test tube was increased to 10 mL by adding 2.4 mL of distilled water. The samples in the test tubes were left to stand for 15 minutes on a working bench to give an allowance for the completion of the reaction (Zhishen *et al.*, 1999). The samples were run on UV/V is spectrophotometer at the wavelength of 510 nm against the blank (which was methanol) and the obtained absorbance was recorded in Table 2.

Total flavonoid content (TFC) was determined by using the formula below.

$$\text{Total Flavonoid Content (mg QE/g of sample)} = \frac{C \times V \times DF}{m}$$

Where V is the volume of the sample solution (L), DF is the dilution factor, and m is the mass of the sample (g), C is the concentration of Quercetin determined from the calibration curve (mg/L).

$$\text{Total Flavonoid Content (mg QE/g of sample)} = \frac{C \times V \times DF}{m}$$

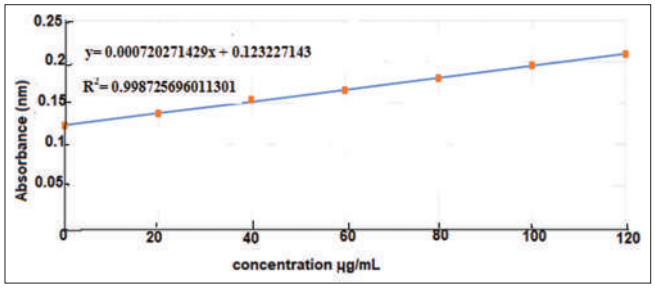


Figure 2: Calibration curve for Quercetin

Table 2: Concentration of Flavonoid content in methanolic extract of *P. maproneifolia* leaf

P. maproneifolia leaf extracts	Concentration (mg/L)	Mean	SD	%RSD	Absorbance
T1	105.1	0.1988	0.000934	0.46730	0.1999
T2	104.7				0.1984
T3	104.6				0.1982
Mean conc.	104.8				

$$= \frac{104.8 \text{ mg/L X } 0.3 \text{ L X } 100}{20 \text{ g}}$$

$$= 157.2 \text{ mg QE/g}$$

Preparation of Standard Solution of Gallic acid for Calibration

Polyphenols present in samples are reduced by Folin-Ciocalteu reagent (FCR) by producing a resulting blue colored complex (Singleton *et al.*, 1999). The standard solution was prepared by dissolving 100 mg of Gallic acid into 100 mL of 80% methanol. The dissolved stock solution was serially diluted to different concentrations of 20, 40, 60, 80, 100, and 120 µg/mL as shown in Table 3.

To each 1 mL of the above concentrations, 5 mL of 10% Folin-Ciocalteu reagent was added, followed by 4 mL of 7.5% Na_2CO_3, making a final volume of 10 mL. The obtained blue-colored mixture was shaken gently and incubated for 90 minutes at room temperature to complete the reaction. The absorbance was measured at a wavelength of 765 nm against the blank (methanol) as shown in Figure 3.

Determination of Total Phenolic Content (TPC)

The extract's Total phenolic content was evaluated using the Folin-Ciocalteu method (Singleton *et al.*, 1999). 1 mL of the extract was placed into three different test tubes using a micro pipette, and 5 mL of 10% Folin-Ciocalteu reagent was added, followed by 4 mL of 7.5% Na_2CO_3, making a final volume of 10 mL. The obtained blue-colored mixture was shaken gently and incubated for 90 minutes at room temperature to complete the reaction. The absorbance was measured at a wavelength of 765 nm against the blank (methanol) and was recorded in Table 4.

Total phenolic content (TPC) was determined by using the formula below:

Table 3: Dilution of Gallic acid

Gallic acid (mL)	Methanol (mL)	Concentration (µg/mL)
0.1	5	20
0.2	5	40
0.3	5	60
0.4	5	80
0.5	5	100
0.6	5	120

Table 4: Concentration of phenolic content in Methanolic leaf extracts of *P. maproneifolia*

Sample	S. No.	Concentration (mg/L)	Mean	Absorbance
P. maproneifolia	T1	42.0	0.7332	0.7269
leaf	T2	42.6		0.7354
extract	T3	42.8		0.7374
	Mean conc.	42.5		

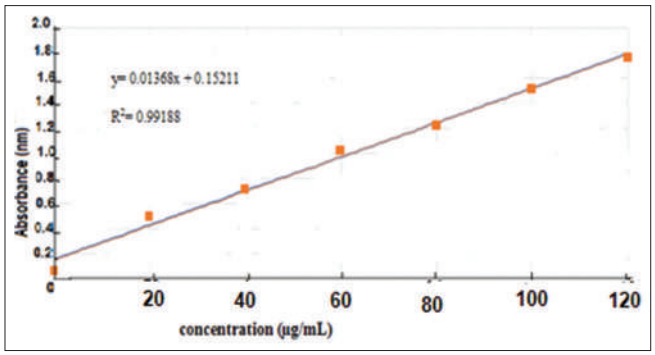


Figure 3: Standard calibration curve for Gallic acid

$$\text{Total Phenolic Content (mg GAE/g of sample)} = \frac{C \times V \times DF}{m}$$

Where; V is the volume of the sample solution (L), DF is the dilution factor and 'm' is the mass of the sample (g), C is the concentration of Gallic acid in the extract determined from the calibration curve (mg/L).

$$\text{Total Phenolic Content (mg GAE/g of sample)} = \frac{C \times V \times DF}{m}$$

$$= \frac{42.5 \text{ mg/L X } 0.3 \text{ L X } 100}{20\text{g}}$$

= 63.75 mg GAE/g

Preparation of DPPH solution

Four mg of 1, 1-Diphenyl, 2-picryl-hydrazyl (DPPH) was weighed on a watch glass and was dissolved in 100 mL of 99% methanol in a dark room. After completely wrapping aluminum foil around the bottle, the solution was stored in a cool and dark place. A blank solution of methanol was prepared and placed in a cuvette. A control solution was prepared by mixing 1 mL of DPPH solution with 1 mL of methanol. An appropriate volume of the control solution was transferred to the cuvette, and the absorbance of the solution was measured at the wavelength of 517 nm.

Preparation of standard solution of Ascorbic acid for calibration

Ascorbic acid of 100 mg was weighed on a watch glass and dissolved in 100 mL of distilled water. The solution serially diluted to different concentrations of 20, 40, 60, 80 and 100 µg/mL using distilled water as shown in Table 5.

In 5 test tubes, 1 mL of 20, 40, 60, 80 and 100 µg/mL was pipetted out. In each of the 5 test tubes containing 5 different concentrations, 3 mL of DPPH was added and gently mixed

Table 5: Dilution for ascorbic acid

Ascorbic acid solution (mL)	Distilled water (mL)	Concentration (µg/mL)
0.2	10	20
0.4	10	40
0.6	10	60
0.8	10	80
1.0	10	100

(Brand-Williams *et al.*, 1995). All the volumes were increased to 10 mL by adding distilled water. The solution was incubated at room temperature for 30 minutes. The change in color from dark violet to different degrees of discoloration based on concentrations was observed. Absorbance of the solutions was measured at a wavelength of 517 nm using UV/V is spectrophotometer against the blank. The obtained absorbance was used to determine inhibition percentage using the formula below.

$$I\% = \frac{AC - AO}{AC} \times 100$$

where, AC = absorbance of the control,
AO = absorbance of the sample solution, and
I% = percentage of inhibition.

Percentage inhibition versus concentration was plotted on graph where, IC_{50} (half-maximal inhibition concentration) value was determined by using Graph pad prism Software version 9.5.1 (733) as shown in Figure 4 below.

Preparation of extract solution

Volumes (aliquots) of 1, 2, 3, 4 and 5 mL of the extracts were pipetted in 5 volumetric flasks respectively and then filled to 10 mL with methanol. 1 mL was taken from each volumetric flask and put into 5 separate ones. Subsequently, 3 mL of DPPH was added and thoroughly mixed. The volume of each volumetric flask was increased to 10 mL by adding 99% methanol. The solution was incubated for 30 minutes at room temperature. The colour variations were observed from dark violet to various levels of discoloration based on concentration. Absorbance of the solutions was measured in triplicates at a wavelength of 517 nm using UV/V is spectrophotometer against the blank. The percentage inhibition was determined by using the formula below.

$$I\% = \frac{AC - AO}{AC} \times 100$$

Percentage inhibition versus concentration was plotted on graph where, IC_{50} (half-maximal inhibition concentration) value was determined by using Graph pad prism Software version 9.5.1 (733) as shown in Figure 5.

Statistical Analysis

The data from each experiment was recorded from three replications (n=3) and the results obtained were represented as mean ± S.D (standard deviation). Linear regression coefficient R^2 for flavonoid and phenolic content with antioxidant activity was analyzed by Graph pad prism Software version 9.5.1 (733).

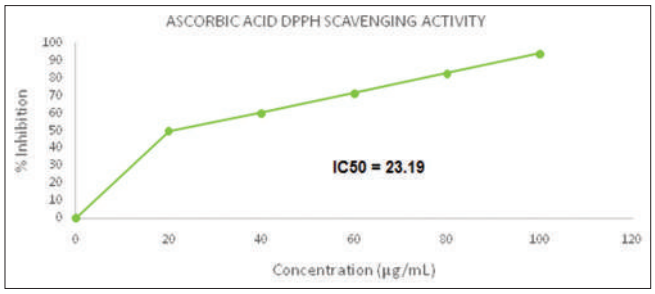


Figure 4: DPPH scavenging activity of ascorbic acid

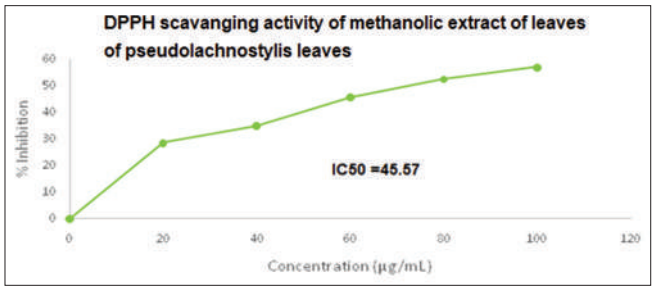


Figure 5: DPPH scavenging activity of *P. maproneifolia* leaf extract

Molecular Docking

Preparation of ligands

The SDF file of 3D structures of selected molecules such as 2,5-Diphenyl-1,3-oxazole (L1), (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (L2), 18-(1,4,7,10,13,16-hexaoxacyclononadec-18-yl)-1,4,7,10,13,16-hexaoxacyclononadecane (L3), 15-(1,4,7,10,13-pentaoxacyclohexadec-15-yl)-1,4,7,10,13-pentaoxacyclohexadecane (L4), [[7-(benzoyloxyamino)-7-oxoheptanoyl]amino] benzoate (L5), identified in the study plant sample and reference cancer drug molecule Imatinib, 4-[(4-methylpiperazin-1-yl)methyl]-N-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]phenyl]benzamide (R) were downloaded from PubChem. They were prepared using Avogadro 1.2.0 software by adding hydrogens and optimizing the geometry to lead to mol2 files which were subsequently converted to a pdbqt file by choosing torsions and selecting active bonds using AutoDock 4.2 tools (Morris *et al.*, 2009).

Receptor preparation

Recent advancements have highlighted the involvement of tyrosine kinases in cancer pathophysiology. Tyrosine kinase inhibitors present a promising avenue for novel cancer therapy. In our study, we focused on the receptor 1QCF. The crystal structure of the protein complex utilized in our study was obtained from the Protein Data Bank (www.rcsb.org/pdb.pdb). The processed protein, acquired through ChimeraX software, underwent various modifications, including removing nonstandard atoms and bonds from the selected chain and residue. Subsequently, using autodock 4.2 tools, water molecules were eliminated, hydrogens added, nonpolar hydrogens merged, Collman charges introduced, and AD4 type atoms were assigned to generate the pdbqt file using AutoDock 4.2 tools (Morris *et al.*, 2009).

Docking procedure

The grid parameters were set by modifying the dimensions of X, Y, and Z to 126. Gpf files were generated from ligand and protein pdbqt files using AutoDock 4.2 tools for autogrid run. Dpf files were created from ligand and protein pdbqt files using AutoDock 4.2 by setting genetic algorithms to 70 runs to achieve desired conformations, accepting default docking parameters, and selecting Lamarckian genetic algorithm with 2500000 energy evaluations. In order to get the desired conformations, 70 runs were carried out. Further, autogrid and autodock runs were executed. The docking results were retrieved from glg files (Morris *et al.*, 2009).

ADMET Studies

ADMET analysis plays a vital role in assessing the pharmacodynamic characteristics of a molecule. The SWISSADME web-based server (www.swissadme.ch/accessed on 8 December 2023) was used to evaluate these properties for both phytocompounds and known drugs. This online tool facilitated the determination of ADMET properties by loading ligand or drug smiles, sourced from PubChem (Laskowski *et al.*, 1993; Diana *et al.*, 2017).

RESULTS

Preliminary Qualitative Phytochemical Screening Results

The preliminary phytochemical screening revealed the presence of various classes of compounds such as alkaloids, glycosides, saponins, flavonoids, tannins, triterpenoids and steroids, quinones, and coumarins, as given in Table 6.

GC-MS Results

The chromatogram of *P. maproneifolia* leaf extract is depicted in Figure 6. The phytochemical GC screening using NIST library revealed 67 annotated molecules based on retention time, matched peaks and several other criteria. The molecules are depicted in Table 7.

Compounds Identified using GPNS Library

Some compounds identified from GNPS (Global Product's Social Molecular Networking) library in the Leaf extract from the analysis of GC-MS raw data are given in Table 8. The raw data was converted into mzML using MS convert. mzML file was uploaded on GNPS platform. The molecules depicted in Table 8 are accessible at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=209c5998b7f740de98493188a87fa878. Although a total of 40 molecules were annotated, only five with greater than 70% cosine similarity and more than 6 shared peaks have been considered.

Table 6: The results of the phytochemical screening of aqueous leaf extract of *P. maproneifolia*

Test	Present (+)
Alkaloids	+
Glycosides	+
Saponins	+
Flavonoids	+
Tannins	+
Triterpenoids and steroids	+
Quinones	+
Coumarins	+

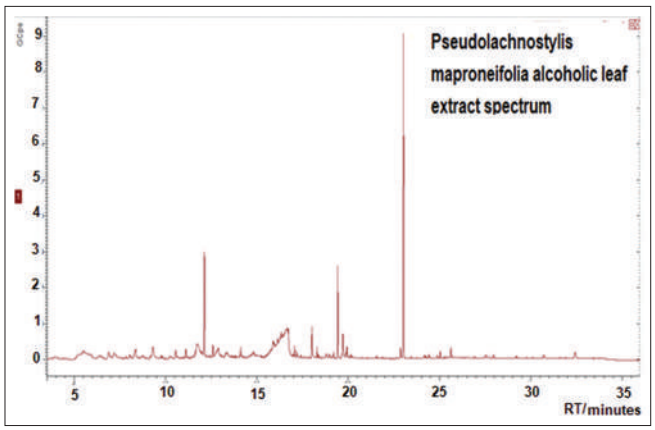


Figure 6: GC-MS spectrum of alcoholic leaf extract of *P. maproneifolia*

Mineral Elements Concentration

The concentrations of the selected elements are presented in Table 9. Iron has the highest concentration of 0.927 mg/kg and copper has the lowest concentration of 0.394 mg/kg. Mn and Zn has 0.713 mg/kg and 0.496 mg/kg respectively.

Flavonoid Content and Total Phenolic Content

Total phenolic and flavonoid content is shown in Table 10.

Free Radical Scavenging Activity

Free radical scavenging potential is stipulated in Table 11. The IC_{50} value for ascorbic acid was found to be 23.19 µg/mL and for *P. maproneifolia* it was 45.57 µg/mL.

Molecular Docking Results

Docking analysis enables the determination of interactions between protein and ligand. Bioactive compounds with the lowest binding energy represent the most significant interactions. In the present study, phytocompounds of *P. maproneifolia* (L1, L2, L3, L4, and L5) and a control cancer drug imatinib 4-[(4-methylpiperazin-1-yl) methyl]-N-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino] phenyl] benzamide, and a tyrosine kinase inhibitor were docked to receptor 1 QCF, and results are presented Table 12. In the present study, L2 demonstrated the highest binding affinity score of -9.01 kcal/mol, and L5 showed the lowest binding affinity score of -6.15 kcal/mol. The binding energy of the reference cancer drug (R) has a binding affinity score of -9.22, comparable to that of L2. The inhibition constant of L2 (249.88 nM) is much lower than the reference drug (666.57 µM). Ligand efficiencies vary from -0.25 to -0.41. Ligand efficiency (LE) is the binding energy per heavy atoms (HA) and is interpreted as a measurement of goodness of interaction between a given compound and its target protein (Hopkins *et al.*, 2004).

The interactions between phytocompounds and receptor 1QCF are depicted in Table 13. This table provides a visual representation of binding sites between the target protein and the selected phytocompounds, highlighting the specific residues that are capable of forming molecular interactions.

In the present study, docking of 1QCF with reference drug (R) generated hydrogen bonds (MET A:341, ALA A:390), carbon hydrogen bond (GLU A:339), Pi-Cation (LYS A:295), Pi-Anion (ASP A:404), Pi-Sigma (LEU A:393), and Pi-Alkyl (ALA A:293, LEU A:273, VAL A:281, ALA A:403). The docking of L1 with 1 QCF formed conventional hydrogen bond (MET A:341), carbon hydrogen bond (GLU A:339), Pi-Sigma (THR A:338, LEU A:273, LEU A:393), and Pi-Alkyl (LYS A:295, VAL A:281, ALA A:293). The docking of L2 with 1QCF created carbon hydrogen bond (SER A:114), Pi-Alkyl (LYS A:257), and Alkyl (LYS (A:324), HIS A:326, TRP A:118). The docking of L3 with 1QCF formed carbon hydrogen bonds (CYS A:245, LEU A:89, SER A:248). Further, docking of L4 with 1QCF generated conventional hydrogen bond (LYS A:104), and carbon hydrogen bonds (LEU A:89, TYR A:90, GLU A:147). Similarly, docking of L5 with 1QCF created conventional hydrogen bond (PHE A:340, HIS A:326, THR A:290, LYS A:291, HIS A:289), Pi-Sigma (ILE A:95), and Pi-Cation (LYS A:252).

ADMET Analysis Results

The ADMET analysis results are given in the Table 14.

In this study, physicochemical, lipophilicity, solubility, drug likeness, pharmacokinetic and medicinal properties of phytocompounds (L1, L2, L3, L4, L5) from *P. maproneifolia* leaf and reference cancer drug were analysed. Phytocompounds L1, L4, and L5 and reference cancer drug had no violations, while L2 (MLOGP>4.15) had one, and L3 had two violations (MW>500, NorO>10). Gastrointestinal absorption (GI) is a key property determining the efficacy of an oral drug. In this study, all the molecules except L2 had high GI values. Five prominent isozymes (CYP1A 2, CYP2C 19, CYP2C 9, CYP2D 6, and CYP3A 4) show all the NO in the case of L2, L3, and L4 and do not act as inhibitors, while two isozymes in L1, one in L5 and four in reference drug act as inhibitor during the metabolism of these candidates. Synthetic accessibility demonstrates the ease of synthesis of the drug. This model assigns numerical values from 1(easiest to synthesize) to 10 (most difficult to make). The values L1 (2.85), L2 (6.30), L3 (5.65), L4 (5.07), L5 (2.92) and reference drug (3.75) are quite favourable.

Table 7: Compounds identified in the Leaf extract by GC-MS analysis using NIST library

S. No.	RT	Match	R. Match	Prob	Name	CAS No	MW	Formula
1	5.926	760	760	54.70	Propanoic acid, 2-mercaptomethyl ester	53907-46-3	120	$C_4H_8O_2S$
2	8.370	904	924	30.72	Heptenediamide, N, N-d-benzoyloxy-	None	398	$C_{21}H_{22}N_2O_6$
3	9.167	706	764	33.06	Benzofuran, 2,3-dihydro	496-16-2	120	C_8H_8O
4	9.308	787	806	81.96	5-Hydromethylfurfural	67-47-0	126	$C_6H_6O_3$
5	9.766	708	756	23.83	Nonanoic acid	112-05-0	158	$C_9H_{18}O_2$
6	10.554	824	906	50.56	2-Methoxy-4-vinylphenol	7786-61-0	150	$C_9H_{10}O_2$
7	11.115	818	849	22.44	Eugenol	97.53-0	164	$C_{10}H_{12}O_2$
8	11.217	708	709	28.98	2-Mystristynoyl pantetheine	None	484	$C_{25}H_{44}N_2O_5S$
9	11.487	741	762	9.22	Ylangene	14912-44-8	204	$C_{15}H_{24}$
10	11.642	777	798	12.35	Nonadecane	629-92-5	268	$C_{19}H_{40}$
11	11.736	839	863	82.84	1,2,3-Benzenetriol	87.66-1	126	$C_6H_6O_3$
12	12.126	913	915	52.87	Caryophyllene	87.44-5	204	$C_{15}H_{24}$
13	12.488	706	710	14.25	Cis-5,8,11,14,17-Eicosapentaenoic acid	10417.94-4	302	$C_{20}H_{30}O_2$
14	12.592	847	862	48.74	Humulene	6753.98-6	204	$C_{15}H_{24}$
15	13.315	705	728	36.15	1,2,3,4,5-Cyclopentanepentol	56772-25-9	150	$C_5H_{10}O_5$
16	14.117	809	821	13.51	Heptadecane	629.78-7	240	$C_{17}H_{36}$
17	14.202	754	754	27.84	Z, Z, Z-4,6,9-Nonadecatriene	None	262	$C_{19}H_{34}$
18	14.533	705	708	43.26	Melezitose	597.12-6	504	$C_{18}H_{32}O_{16}$
19	15.016	708	714	22.66	Melezitose	597.12-6	504	$C_{18}H_{32}O_{16}$
20	15.097	707	711	16.13	Desulphosinigrin	5115.81-1	279	$C_{10}H_{17}NO_6S$
21	15.563	714	732	24.92	1,2,3,4,5-Cyclopentanepentol	56772.25-9	150	$C_5H_{10}O_5$
22	16.342	705	716	15.77	3-O-Methyl-d-glucose	None	194	$C_7H_{14}O_6$
23	16.432	732	751	31.15	Myo-inostol, 4-methyl	472-95-7	194	$C_7H_{14}O_6$
24	17.064	847	866	14.31	1,2-Benzenedicarboxylic acid, bis (2-methylproyl) ester	84.69-5	278	$C_{16}H_{22}O_4$
25	17.175	775	850	20.16	Neophytadiene	504.96-1	278	$C_{20}H_{38}$
26	18.017	816	816	53.19	n-Hexadecanoic acid	57.10-3	256	$C_{16}H_{32}O_2$
27	18.296	825	825	27.78	Eicosanoic acid, ethyl ester	18281.05-5	340	$C_{22}H_{44}O_2$
28	18.360	727	806	9.32	Tetracontane, 3,5,24-trimethyl-	55162.61-3	604	$C_{43}H_{88}$
29	18.454	710	747	33.73	Cis-Sinapyl alcohol	104330.63-4	210	$C_{11}H_{14}O_4$
30	18.556	702	719	17.24	Oleic acid	112.80-1	282	$C_{18}H_{34}O_2$
31	18.646	711	714	27.79	Melezitose	597.12-6	504	$C_{18}H_{32}O_{16}$
32	18.976	759	873	65.25	Oxazole, 2,5-diphenyl-	92.71-7	221	$C_{15}H_{11}NO$
33	19.079	742	749	24.29	15,15′-Bi-1,4,7,10,13-pentaoxacylohexadecane	109773.67-3	466	$C_{22}H_{42}O_{10}$
34	19.199	827	851	7.10	1-Hexadecanol	36653.82-4	242	$C_{16}H_{34}O$
35	19.427	899	899	82.21	Phytol	150.86-7	296	$C_{20}H_{40}O$
36	19.711	809	809	10.17	9,12-Octadecadienoyl chloride (z, z)-	7459.33-8	296	$C_{18}H_{31}ClO$
37	19.926	811	811	8.69	E-11-Hexadecenoic acid, ethyl ester	None	298	$C_{18}H_{34}O_2$
38	19.983	790	794	13.22	Oleic acid	112-80-1	282	$C_{18}H_{34}O_2$
39	20.146	752	777	20.23	10-Bromodecanoic acid ethyl ester	55099-31-5	278	$C_{12}H_{23}BrO_2$
40	20.580	730	744	33.56	Oleic acid	112-80-1	282	$C_{18}H_{34}O_2$
41	20.709	785	788	7.75	9,12-Octadecadeienoyl chloride (Z, Z)-	7459-33-8	298	$C_{18}H_{31}ClO$
42	70.769	765	765	10.72	[1,1-Bicylopropyl] 2-Octanoic acid-2-Hexylmethyl ester	56687-68-4	322	$C_{21}H_{38}O_2$
43	21.061	733	747	20.13	Oleic acid	112-80-1	282	$C_{18}H_{34}O_2$
44	21.555	728	747	31.72	18,18′Bi-1,4,7,10,13,16-hexaoxacyclononadecane	109773-68-4	554	$C_{26}H_{50}O_{12}$
45	21.844	740	756	25.68	Oleic acid	112-80-1	282	$C_{18}H_{34}O_2$
46	22.675	709	785	6.38	7-Hexadecenal, (Z)-	56797-40-1	238	$C_{16}H_{30}O$
47	22.869	776	776	44.99	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	23470-00-0	330	$C_{19}H_{38}O_4$
48	22.929	722	740	22.82	Oleic acid	112-80-1	282	$C_{18}H_{34}O_2$
49	23.022	930	930	74.43	Bis (2-ethylhexyl) phthalate	117-81-7	390	$C_{24}H_{38}O_4$
50	23.444	744	814	10.37	Tetracontane, 3,5,24-trimethyl	55162-61-3	604	$C_{43}H_{88}$
51	23.766	722	737	16.18	15,15′Bi-1,4,7,10,13-pentaoxacyclohexadecane	109773-67-3	465	$C_{22}H_{42}O_{10}$
52	24.183	829	850	17.75	Hentriacontane	630-04-6	436	$C_{31}H_{64}$
53	24.243	760	798	8.51	2-Methyl-Z, Z-3,13-Octadecadienol	None	280	$C_{19}H_{36}O$
54	24.416	722	746	15.74	Octadecanoic acid, 3,3-dihydroxypropyl ester	123-94-4	358	$C_{21}H_{42}O_4$
55	24.879	751	813	4.49	Hexacosyl acetate	822-32-2	424	$C_{28}H_{56}O_2$
56	25.034	868	875	47.64	Squalene	111-02-4	410	$C_{30}H_{50}$
57	25.218	724	731	12.75	[1,1-Bicyclopropyl]-2-octanoic acid, 2-hexyl-methyl ester	56687-68-4	322	$C_{21}H_{38}O_2$
58	25.626	858	858	16.37	Hentnacontane	630-04-4	436	$C_{31}H_{64}$
59	25.682	715	721	6.78	2-Hexadecanol	14852-31-4	242	$C_{16}H_{34}O$
60	26.017	712	721	18.88	1,1-Bicyclopropyl]-2-octanoic acid, 2-hexyl-methyl ester	56687-68-4	322	$C_{21}H_{38}O_2$
61	27.539	741	810	4.34	Hexacosyl acetate	822-32-2	424	$C_{28}H_{56}O_2$
62	27.961	775	776	33.84	Vitamin E	59-02-9	430	$C_{29}H_{50}O_2$
63	29.222	741	842	6.32	Oxirane, tetradecyl	7320-37-8	240	$C_{16}H_{32}O$
64	30.710	749	764	12.48	Beta-Sitosterol	83-46-5	414	$C_{29}H_{50}O$
65	31.592	714	736	27.76	Methyl-3-cis, 12-cis-octadecatrienoate	None	292	$C_{19}H_{32}O_2$
66	31.888	712	740	13.83	Tricyclo[20,8, (7,16)]tricontane, 1 (22),7 (16)-diepoxy-	None	444	$C_{30}H_{52}O_2$
67	32,421	805	812	10.35	Methyl-3-cis, 9-cis, 12-cis-octadecatrienoate	None	292	$C_{19}H_{32}O_2$

Table 8: Phytocompounds identified using GNPS libraries in the Leaf extract of Pseudolachnostylis maprouneifolia by GC-MS

Number	GNPS Libraries Derived Compound Name	Scan Number	Library Class	Cosine	Shared Peaks	IUPAC Name
1	Hexadecane	46	Gold	0.91	6	Hexadecane
2	Fumaric acid, 2 -ethylhexyl 2,2,3,3,4,4,5,5 -octafluoropentyl ester	111	Gold	0.76	7	1-O-(2-ethylhexyl) 4-O-(2,2,3,3,4,4,5,5-octafluoropentyl) (E)-but-2-enedioate
3	2,5,5,11- TETRAMETHYL-8- ISOPROPYL-4,7,9-TRIOXA- 1,11-DODECADIENE	22	Gold	0.74	6	2-methyl-1-[2-methyl-2-(2-methylprop-2-enoxy) propoxy]-1-(2-methylprop-2-enoxy) propane
4	2,5-DIPHENYL-OXAZOLE	94	Gold	0.73	7	2,5-diphenyl-1,3-oxazole
5	Icosanoic acid	88	Gold	0.72	9	Icosanoic acid

Table 9: Mineral elements revealed by AAS

Sample	Extractive mass (g)	Mineral	Dilution factor	Extracting solution	Dilution solution	Concentration (mg/L)	Concentration (mg/kg)
Leaf Extract	4.0	Zinc (Zn)	20	20% HCl	Distilled water	0.496	0.496
		Manganese (Mn)				0.713	0.713
		Copper (Cu)				0.394	0.394
		Iron (Fe)				0.927	0.927

Table 10: Total Flavonoid content and Total Phenolic content

Sample	Phytochemical	Content
Methanolic leaf extract	flavonoids	157.2 mgQE/g
	phenolic compounds	63.75 mgGAE/g

Table 11: Free radical scavenging activity of *P. maproneifolia* and standard ascorbic acid

Sample	IC_{50} value	Intensity
Ascorbic acid	23.19 µg/mL	Highly active
P. maproneifolia leaf extract	45.57 µg/mL	Highly active

DISCUSSION

Qualitative Phytochemical Screening

The qualitative phytochemical analysis of the leaf extract of *P. maproneifolia* revealed a diverse array of phytochemicals such as quinones, coumarins, flavonoids, alkaloids, steroids, glycosides, saponins, tannins, and terpenes. The presence of these compounds is influenced by factors like the quantity of plant material, cultivation conditions, collection season, analytical methods, and plant-to-plant variability in medicinal content (Harborne, 1973).

Tannins identified in the extract demonstrate versatile properties, including anti-HIV activity, astringency, antioxidant, anti-inflammatory, diuretic, antimicrobial, antitumor, and skin-regeneration effects. Steroids, also present, exhibit anti-inflammatory properties, contributing to their medicinal significance (Chaddha & Mittal, 2016). Saponins, found in the extract, are known for their anti-carcinogenic and anti-diabetic properties, reducing cholesterol levels and inhibiting platelet aggregation and thrombosis. They have been successfully employed as cough suppressants, tonic sedatives, and in treating liver inflammation. Glycosides of flavonoids, particularly flavonoid c-glycosides, play a crucial role in the diet, showcasing diverse bioactivity such as antioxidant, anticancer, antitumor, hepatoprotective, antidiabetic, anti-inflammatory, antiviral, and antifungal activities (Xiao, 2016).

According to epidemiological and experimental studies, monoterpenes have been promising in preventing and treating various cancers, including mammary, skin, lung, forestomach, colon, pancreatic, and prostate carcinomas (Kris-Etherton *et al.*, 2002). Coumarins exhibit physiological activities such as anti-HIV, antibacterial, anticancer, antioxidant, anti-coagulant, and immune modulation properties. Quinones, also present, display antifungal, anti-malarial, analgesic, cardiotonic, and anti-convulsant activity (Marella *et al.*, 2013). Notably, the anthraquinone antitumor drug mitoxantrone, derived from 9, 10-anthraquinone, is utilized in the treatment of different cancer types, while derivatives of 9, 10-anthraquinone have been researched and applied as antibacterial and antiviral agents (Evison *et al.*, 2016; Frecentese *et al.*, 2016).

Compounds Revealed by GC-MS Profiling

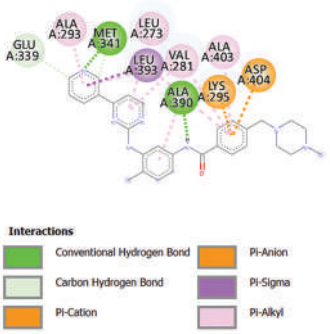
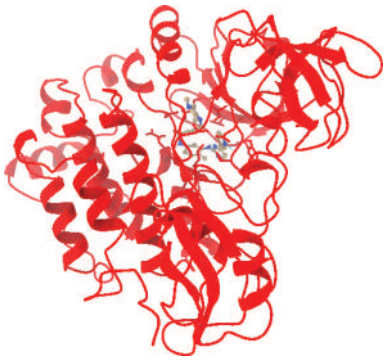
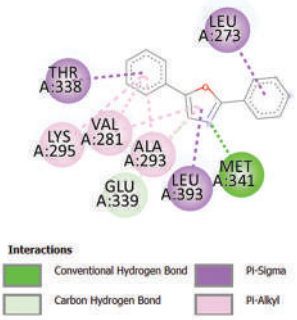
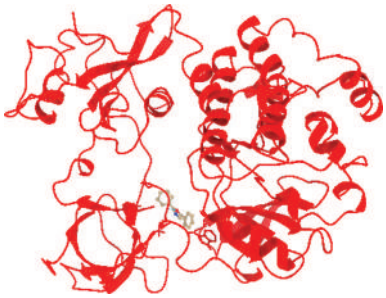
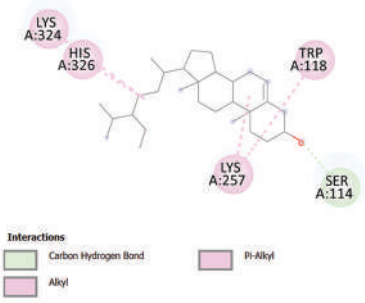
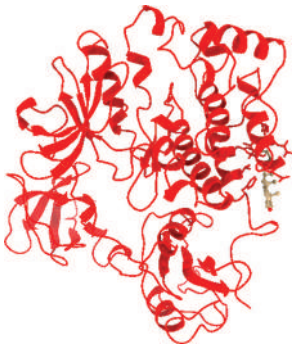
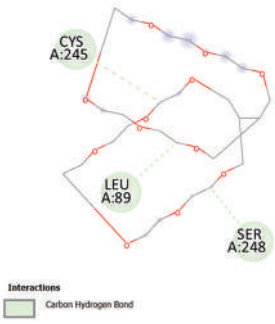
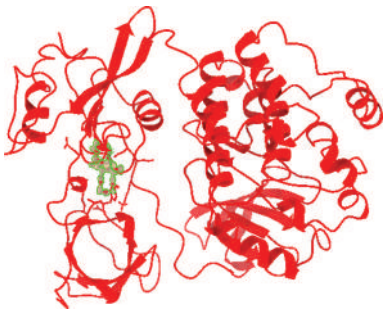
Compounds of interest revealed by GC-MS profiling were; terpenes such as, humulene (α-caryophyllene), β- caryophyllene, phytol, squalene and Limonene. Organic acids including hexadecanoic acid, lauric acid, propanoic acid and oleic acids. Heterocyclic compounds such as, oxirane- tetradecyl, oxazole 1, 5-diphenyl and benzofuran,2,3-diphenyl, N-(3- aminopropyl)-morpholine, steroids (progesterone) and vitamin E (Tocopherol).

Humulene is also called α-caryophyllene is a natural occurring monocyclic sesquiterpene. β- caryophyllene has anti-microbial, anti-inflammatory, anti-bacterial, anti-oxidant properties. It is well known for its ability to relieve anxiety and pain. β- caryophyllene reduces blood cholesterol, prevents osteoporosis and treats seizures. Interaction of humulene and β- caryophyllene makes it practical in the treatment of arthritis, bursitis and fibromyalgia. β- caryophyllene increases bone strength while decreasing bone break down which can either be adipogenesis or osteoclatogenesis (Marella *et al.*, 2013).

Table 12: Docking analysis of selected ligands from *P. maproneifolia* 1QCF receptor

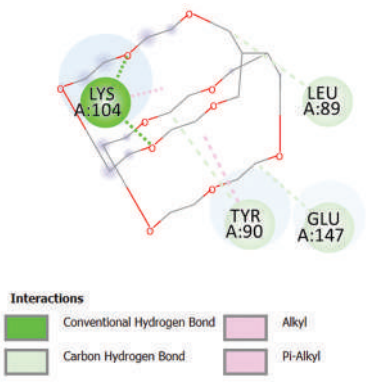
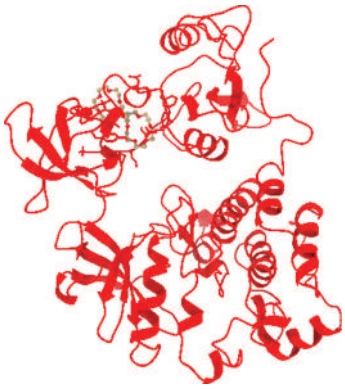
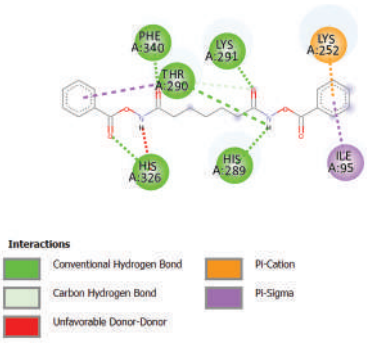
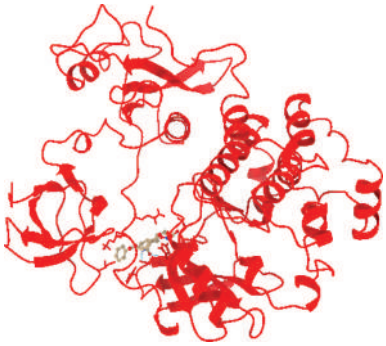
Ligand	Binding energy Kcal/mol	Inhibition constant (Ki)	Total internal energy kcal/mol	Torsional free energy kcal/mol	Unbound energy kcal/mol	Cluster RMSD	Ligand efficiency
L1: CID 7105	-6.95	8.06 μM	-0.44	+0.60	-0.44	0.00	-0.41
L2: CID: 222284	-9.01	249.88 nM	-0.83	+2.09	-0.83	0.00	-0.30
L3: CID: 560657	-6.87	9.19 μM	-0.81	+0.30	-0.81	0.00	-0.18
L4: CID: 560684	-7.83	1.81 μM	-0.46	+0.30	-0.46	0.00	-0.24
L5	-6.15	823.61 μM	-3.39	+3.58	-3.39	0.00	-0.21
R	-9.22	666.57 μM	-2.94	+2.09	-2.94	0.00	-0.25

Table 13: Interactions between plant compounds (ligands) and 1QCF receptor depicted in 2D and 3D

Ligand	Residue	Interactions	2D complexes	3D complexes
R (CID: 5291) 4-[(4-methylpiperazin-1-yl) methyl]-N-[4-methyl-3-[(4-pyridin-3 -ylpyrimidin-2-yl) amino] phenyl] benzamide	MET A :341, ALA A :390 GLU A :339 LYS A: 295 ASP A :404 LEU A :393 ALA A: 293, LEU A: 273, VAL A: 281, ALA A: 403	Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Cation Pi-Anion Pi-Sigma Pi-Alkyl		
L1 (CID: 7105) 2,5-Diphenyl-1,3-oxazole	MET A :341 GLU A :339 THR A :338, LEU A :273, LEU A :393 LYS A :295, VAL A :281, ALA A :293,	Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Sigma Pi-Alkyl		
L2 (CID: 222284) (3S,8S,9S,10R,13R, 14S,17R)-17 -[(2R,5R) -5-ethyl-6-methylheptan-2-yl] -10,13-dimethyl -2,3,4,7,8,9,11, 12,14,15,16,17 -dodecahydro -1H -cyclopenta[a] phenanthren -3-ol	SER A: 114 LYS A: 257 LYS (A: 324), HIS A: 326, TRP A: 118	Carbon Hydrogen Bond Pi-Alkyl Alkyl		
L3 (CID: 560657) 18-(1,4,7,10,13,16-hexaoxacyclononadec-18-yl) -1,4,7,10,13,16-hexaoxacyclononadecane	CYS A: 245, LEU A: 89, SER A: 248	Carbon Hydrogen Bond		

(Contd...)

Table 13: *(Continued)*

Ligand	Residue	Interactions	2D complexes	3D complexes
L4 (CID: 560684) 15-(1,4,7,10,13-pentaoxacyclohexadec-15-yl) -1,4,7,10,13-pentaoxacyclohexadecane	LYS A: 104 LEU A: 89, TYR A: 90, GLU A: 147	Conventional Hydrogen Bond Carbon Hydrogen Bond		
L5 (CID: 569848) [[7-(benzoyloxyamino) -7-oxoheptanoyl] amino] benzoate	PHE A: 340, HIS A: 326, THR A: 290, LYS A: 291, HIS A: 289 ILE A: 95 LYS A: 252	Conventional Hydrogen Bond Pi-Sigma Pi-Cation		

Organic acids are involved in a broad range of basic cellular processes which includes the modification of cellular pH or reduction and oxidation states of different chemical substances. Due to their ability to modify the reduction and oxidation states of different chemical substances, organic acids enhance the absorption of iron which results in their importance in the effective treatment of anemia. Other organic acids such hexadecanoic acid, Gallic acid and Lauric acid exhibit strong anti-microbial and anti-inflammatory activity (Swamy & Srinath, 2015). Oleic acid improves blood circulation by strengthening heart muscles and lowering blood cholesterol. Oleic acid is beneficial to the blood, brain, skin and cells. Oleic acid fights free radical and boosts the body's immunity system. Propanoic acid is used in the synthesis of profen medicines as 2- aryl propanoic acid such as ibuprofen, naproxen, ketoprofen, flurbiprofen and many others (Rostom *et al.*, 2007). The three compounds are terpenes. That is Limonene being a monoterpenes, Phytol a diterpene and Squalene a triterpene. These organic compounds exhibit anti-microbial, anti-oxidant and anti-cancer activities (Kim & Karadeniz, 2012).

Heterocyclic compounds that were detected in the extracts were Oxirane tetra-decyl ($C_{16}H_{32}O$), Oxazole 1, 5-diphenyl ($C_{15}H_{11}NO$) and Benzofuran 2,3-dihydro (C_8H_8O). Oxiranes are also called ethylene oxides (C_2H_4O) which are capable of destroying most viruses, bacteria and fungi. Oxirane reacts with microbial protoplasm, DNA, proteins and deactivates enzymes (Tim & Madhu, 2013). The five membered aromatic oxazole compounds are used as medical drugs including anti-viral, anti-fungal, anti-bacterial, anti-tubercular, anti-cancer, anti-oxidative and neuropathic (Zhang *et al.*, 2018). Numerous studies indicated that benzo-furan compounds have strong biological activities. Natural products containing benzo-furan rings are many sources of some drugs and clinical drug candidates (Parekh *et al.*, 2011). N-(3- aminopropyl)-Morpholine is well-known for three main pharmacological activities which include; (1) receptor modulation during pain and in mood disorder (2) deactivates enzymes and receptors that are responsible for neurodegenerative diseases such as Alzheimer's disease and (3) inhibits activities of enzymes involved in the pathology of central nervous system (CNS) tumours (Lenci *et al.*, 2021). Quinoline has antifungal, anti-malarial, analgesic, cardiotonic, and anti- convulsant activity (Marella *et al.*, 2013). Vitamin E is a fat-soluble nutrient which is an anti-oxidant and needed in immune system and cellular signalling of the body. Tocopherol keeps the body free from wrinkles and lines on the face, therefore, it serves as effective treatment to age related health complications (Layokun *et al.*, 2006).

Mineral Elements Responsible for the Effective Treatment of Anemia in the Extract

Mineral elements that were found in the extract were iron, copper, zinc and manganese. Copper is needed to make red blood cells and for energy production cycle of cells. Copper may be used to treat anemia because it helps the body absorb iron from the intestines. Zinc is a required co- factor for enzymes that synthesize heme portion of haemoglobin and severe deficiency in Zinc in the diet results into anemia (Fukushima *et al.*, 2009). Red blood cells use iron to on hemoglobin to transport oxygen around the body. To make hemoglobin, cells require iron to build up a component called heme. Inadequate amount of iron

Table 14: ADMET analysis of phytocompounds and reference cancer drug

ADMET properties	Phytocompounds					Drug
	L1	L2	L3	L4	L5	R
	CID: 7105	CID: 222284	CID: 560657	CID: 560684	CID: 569848	CID: 5291
Physicochemical properties						
Molecular weight (g/mol)	221.25	414.71	554.67	466.56	398.41	493.60
Topological Surface Area (TPSA) (A°)	26.03	20.23	110.76	92.30	110.80	86.28
Num. H-bond acceptors	2	1	12	10	6	6
Num. H-bond donors	0	1	0	0	2	2
Molar Refractivity	67.38	133.23	135.89	114.49	103.37	154.50
Lipofilicity						
XLOGP3	4.67	9.34	-1.48	-1.19	3.46	3.52
ILOGP	2.84	4.79	4.61	4.15	3.03	4.04
MLOGP	2.76	6.73	-2.67	-1.99	3.41	2.15
WLOGP	4.01	8.02	0.45	0.41	2.71	3.49
Water Solubility						
Log S (ESOL)	-4.76	-7.90	-2.28	-1.92	-3.87	-5.07
Class	Moderately soluble	Poorly soluble	Soluble	Very soluble	Soluble	Moderately soluble
Drug likeness						
Lipinski	0	1	2	0	0	0
Veber	0	0	0	0	1	0
Ghose	0	3	3	1	0	2
Egan	0	1	0	0	0	0
Muegge	0	2	1	0	0	0
Bioavailability score	0.55	0.55	0.17	0.55	0.55	0.55
Pharmacokinetics						
Gastrointestinal (GI) Absorption	High	Low	High	High	High	High
Blood brain barrier (BBB) permeability	Yes	No	No	No	No	No
P-gp substrate	No	No	Yes	Yes	No	Yes
CYP1A 2 inhibitor	Yes	No	No	No	No	No
CYP2C 19 inhibitor	Yes	No	No	No	Yes	Yes
CYP2C 9 inhibitor	No	No	No	No	No	Yes
CYP2D 6 inhibitor	Yes	No	No	No	No	Yes
CYP3A 4 inhibitor	No	No	No	No	No	Yes
Log Kp (skin permeation) cm/s	-4.33	-2.20	-10.73	-9.99	-6.27	-6.81
Medicinal Chemistry						
Pas assay interference compounds (PAINS)	0	0	0	0	0	0
Brenk	0	1	0	0	2	0
Synthetic accessibility	2.85	6.30	5.65	5.07	2.92	3.78
Lead Likeness	2	2	1	1	2	3

in individual's diet results into the body's failure to produce enough red blood cells or haemoglobin. Anaemia is a clinical condition that is characterized by a reduction in the haemoglobin concentration with or without a reduction in red blood cell count (Mogilevski *et al.*, 2016). Manganese has strong anti-oxidant properties and may reduce disease risks. Manganese is part of the anti-oxidant enzyme superoxide dismutase (SOD) which is arguably one of the most important anti-oxidants in the body (Holley *et al.*, 2011). The element concentrations (Table 9) are in the similar range as previously reported study (Ansari *et al.*, 2004). However, appreciable iron content of 0.927 mg/kg together with reasonable amounts of Cu, Mn and Zn may be responsible for the plant leaves being so effective to treat anemia.

Total Phenolic Content (TPC), Flavonoid Content (TFC) and Radical Scavenging Activity

The phenolic and flavonoid content were measured at 63.75 mg GAE/g and 157.2 mg QE/g, respectively (Table 10). Notably, the phenolic content in plants directly correlates with their antioxidant capacity. Phenolic compounds function as hydrogen donors, effectively reducing agents to neutralize free radicals (Zeb, 2020). Flavonoids, containing hydroxyl groups with radical scavenging capabilities, play a crucial role in the plant's antioxidative activity. These compounds are known to contribute significantly to the antioxidative potential. In human diets, the daily intake of flavonoids, ranging from a few hundred milligrams to one gram, particularly from fruits and vegetables, has demonstrated inhibitory effects on carcinogenesis and mutagenesis (Gulcin, 2020). The IC_{50} value for ascorbic acid was determined to be 23.19 µg/mL while that of the plant extract was 45.57 µg/mL, classifying it as highly active. A compound with IC_{50} value lower than 50 is considered as highly active antioxidant. The robust antioxidant properties of *P. maproneifolia* leaf extracts can be attributed to their elevated polyphenol levels. Notably, polyphenols with unsubstituted hydroxyl functionalities have been identified as potent free radical quenchers, effective against degenerative diseases (Singh *et al.*, 2021).

In Silico Molecular Analysis

The in silico molecular analysis was carried out using AutoDock 4.2, focused on binding energy scores as the primary indicator

of interaction affinity between selected phytocompounds of *P. maproneifolia* and the receptor, producing promising results. Notably, the binding energy scores for L2 (-9.01) and the cancer drug (-9.22) were found to be comparable (Table 12). The inhibition constant for L2 (249.88 nM) demonstrated a significantly lower value compared to the reference cancer drug imatinib (666.57 μM) (Table 12). It's important to highlight that the inhibition constant (Ki) is inversely proportional to the drug's potential as an inhibitor.

ADMET parameters play pivotal roles in drug discovery and development. An ideal drug candidate should exhibit ample efficacy against the therapeutic target and demonstrate appropriate ADMET properties at a therapeutic dose (Guan *et al.*, 2019). The ADMET variables for the phytocompounds from *P. maproneifolia* align with those of the cancer drug imatinib. Notably, gastrointestinal (GI) absorption, a critical criterion, is high for all phytocompounds except L2. Further, the key isozymes critically used during the metabolism process of the selected phytochemicals act as substrates rather than inhibitors for four of them, except L1. Consequently, the in-silico analysis strongly supports the potential of the selected phytochemicals from *P. maproneifolia* as viable candidates for drug development for cancer treatment. Thus, these compounds merit to be considered for further *in vitro* and *in vivo* studies.

CONCLUSION

The phytochemical profile results of leaf extract of *P. maproneifolia* obtained through both qualitative screening and GC-MS analysis, which was conducted in this study, revealed the presence of tannins, saponins, triterpenoids, flavonoids, alkaloids, cardiac-glycosides, coumarins, quinones and steroids, promising classes of compounds with medicinal properties. These secondary metabolites possess anti-fungal, anti-diarrhea, anticancer, antitumor, antibacterial, antiviral, anti- malarial, anti-HIV, anti-inflammatory, anti-tubercular, neuromodulatory, haemolytic, hepatoprotective and antioxidant properties. The presence of mineral elements such as iron, copper and zinc in the extract confirmed the plant's probable scientific basis to treat anemia. The obtained values of the total flavonoid and total phenolic content along with the determined value of antioxidant activity by DPPH method, shows that *P. maproneifolia*'s medicinal application is not only limited to the treatment of anemia, but can also be used for broad-spectrum prevention and treatment of various diseases. In silico analysis of selected phytochemicals from *P. maproneifolia* strongly support these molecules as potential drug candidate for in vitro and in vivo analysis focusing on cancer treatment.

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