Unveiling the Phytopharmacological Potentials of *Mesua nagassarium* (Burm. f.): *In vitro* and *In silico* Approaches

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ABSTRACT

This study focuses on the chemical, biological and computational studies of stem bark of *Mesua nagassarium* (Burm. f.). Using extensive chromatographic analyses, five compounds were isolated from the carbon tetrachloride soluble fraction of methanol extract of the plant. The isolated compounds were characterized as taraxerol **(1)**, a mixture of β-amyrin **(2)** and α-amyrin **(3)**, β-stigmasterol acetate **(4)** and kaempferol **(5)** by analysis of their spectral data, notably NMR. This is the first report of isolation of taraxerol and β-stigmasterol acetate from *M. nagassarium*. The *n*-hexane soluble materials of bark of *M. nagassarium* exhibited promising antioxidant (IC_{50} = 6.7 µg/ml) and cytotoxic (IC_{50} = 14.68 µg/ml) properties. In addition, the chloroform fraction showed mild thrombolytic activity with 26.27% clot lysis. The phytoconstituents were evaluated by molecular docking against superoxide dismutase, vascular endothelial growth factor receptor 2 and tissue plasminogen activator. Each compound had various degrees of binding affinity to the receptors. The results revealed strong cytotoxic and antioxidant effects of the extractives, underscoring the necessity for future research for isolating the bioactive phytochemicals against a variety of cellular targets.

Key words: Phytochemistry, pharmacology, taraxerol, kaempferol, *in-silico*.

INTRODUCTION

The medicinal plant and its diverse set of bioactive compounds have long been a part of traditional medicinal uses across the world.[1-3] Indigenous population in India, Pakistan, Indochina, Malaysia, and Thailand use the medicinal plant *Mesua nagassarium* for its traditional therapeutic benefits.[4] Previous studies shown that this plant has antibacterial, antioxidant, hepatoprotective, analgesic, antivenom, anticancer, antiulcer, anti-inflammatory and antiasthmatic effects.[5-7] The fixed oil of this plant is used to treat skin infections, wounds, and rheumatism. It is an important component in Ayurvedic formulations such as Brahma Rasayana and Chyavanprash, which are believed to improve the immunity of human being.[5]

The purpose of these chemico-pharmacological investigations of this plant is to identify the presence of secondary metabolites and evaluate their *in vitro* pharmacological activities, such as antioxidant, cytotoxic and thrombolytic activities. Molecular docking was also employed to predict and understand the biological consequences of the isolated compounds.

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MATERIALS AND METHODS

General experimental procedure. ^{1}H NMR spectra at 400 MHz were obtained using a Bruker AMX-400 spectrometer, and deuterated solvents such as $CDCl_3$ and/or CD_3OD were utilized as the solvents to dissolve the sample. Gallic acid, ascorbic acid and Folin-Ciocalteau reagent were obtained from Merck Germany, while sephadex LH-20 and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma-Aldrich, USA. Vincristine was given by Beacon Pharma Ltd., whereas streptokinase was purchased from Incepta Pharmaceuticals Bangladesh Ltd.

Collection of plant materials. The bark of *M. nagassarium* was collected from University of Dhaka campus, Dhaka, Bangladesh. A voucher specimen was submitted to Bangladesh National Herbarium in Dhaka where a specialist validated the plant's identification and gave an authorization for the specimen (DACB-35158). The bark samples were then chopped into smaller pieces.

Extraction of the plant material. The plant parts underwent a preliminary cleaning process to eliminate dust and visible contaminants before undergoing a two-week shade-drying period with optimal airflow. After the drying process, the bark was ground (500 gm) and subjected to maceration in 2.5 liters of distilled methanol for 15 days with periodic shaking. The resulting mixture was filtered, and concentrated under low pressure at approximately 40^{o}C using a rotary evaporator.[8]

Fractioning of the extract. Modified Kupchan partitioning protocol was used to fractionate 5 gm bark extract (MNME) into four fractions based on their increasing order of polarity such as n-hexane (HESF), carbon tetrachloride (CTCSF), chloroform (CFSF) and water soluble fractions.[9] Each of the fractions was evaporated to dryness and left in a refrigerator until used.

Isolation of compounds. The CTCSF was subjected to lipophilic Sephadex LH-20 column for further separation. The column was initially eluted with hexane-dichloromethane-methanol (2:5:1) followed by dichloromethane-methanol mixtures. Subsequently, the fractions underwent screening using thin-layer chromatography (TLC).

Compounds **1** and **4** were isolated as pure constituents from the column fractions of CTCSF eluted with a mixture of *n*-hexane, dichloromethane and methanol (2:5:1), whereas compounds **2** and **3** were separated as mixture from the same column chromatography. Similar column chromatographic separation of the CTCSF eluted with a 10% methanol in dichloromethane upon repeated chromatography over silica gel provided compound **5**.

Properties of isolated compounds.

Taraxerol (**1**)**.** Colorless crystals, 7 mg 0.008% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (1H, dd, J = 8.0, 3.2 Hz, H-15), 3.20 (1H, m, H-3), 1.08 (3H, s, H-27), 0.97 (3H, s, H-23), 0.94 (3H, s, H-29), 0.92 (3H, s, H-24), 0.90 (3H, s, H-30), 0.79 (6H, s H-25, H-28).

Mixture of β-amyrin (**2**) and **α-amyrin** (**3**)**.** White colorless amorphous powder, 7 mg, 0.008% yield;

β-amyrin (2). ^{1}H NMR **(**400MHz, $CDCl_3$) δ: 5.19 (1H, t, *J* = 7.0 Hz, H-12), 3.25 (1H, m, H-3), 1.25 (3H, s, H-27), 1.08 (3H, s, H-28), 0.92 (H-26 s, H-26), 0.90 (3H, s, H-24), 0.86 (3H, s, H-29), 0.80 (3H, s, H-23), 0.79 (3H, s, H-30), 0.72 (3H, s, H-25).

α-amyrin (**3**)**.** ^{1}H NMR (400MHz, $CDCl_3$) δ: 5.09 (1H, t, *J* = 6.8 Hz, H-12), 3.25 (1H, m, H-3), 1.02 (3H, s, H-27), 1.00 (3H, s, H-28), 0.92 (3H, s, H-23), 0.90 (3H, s, H-26), 0.81 (3H, d, *J* = 6.5 Hz, H-29), 0.75 (3H, s, H-24), 0.75 (3H, d, *J* = 6.2 Hz, H-30), 0.72 (3H, s, H-25).

Stigmasterol acetate (**4**)**.** white colorless amorphous powder, 7 mg, 0.008% yield; ^{1}H NMR (400 MHz, CD_3OD) δ 5.36 (1H, d, *J* = 6.5 Hz, H-6), 5.18 (1H, dd, *J* = 15.2, 8.4 Hz, H-22), 5.04 (1H, dd, *J* = 15.2, 8.4 Hz, H-23), 4.65-4.68 (1H, m, H-3), 1.88 (3H, s, CH_3COO-), 1.00 (3H, s, H-19), 0.93 (3H, t, H-24"), 0.87 (3H, d, *J* = 7.0 Hz, H-21), 0.84 (3H, d, H-26), 0.82 (3H, d, *J* = 8.0 Hz, H-27), 0.71 (3H, s, H-18).

Kaempferol (**5**)**.** Yellow crystalline powder, 5 mg, 0.006% yield; ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.89 (2H, d, *J* =

8.5 Hz, H-3', H-5'), 6.38 (1H, br s, H-8), 6.16 (1H, br s, H-6).

Total phenolic content. The total phenolic content in the extractives was determined using the Folin-Ciocalteu reagent.[10] In this study, 2.5 mL of ten-fold diluted Folin-Ciocalteu reagent and 2.5 mL of Na_2CO_3 (7.5% w/v) solutions were combined with 1 mg/mL of bark extractive. The resultant mixture was left in the shade for 30 minutes at room temperature. Subsequently, a UV-Vis spectrophotometer was employed to measure the absorbance at 760 nm. A calibration curve for gallic acid was generated by plotting absorbance against various gallic acid concentrations (250, 125, 62.5, 31.25, 15.625, 7.812, 3.90, 1.95, 0.976, and 0.488 µg/mL). This standard curve's equation was utilized to calculate the total phenolic content of the test samples, expressed in mg of GAE (gallic acid equivalent) per gram of dried extract.

$$y = 0.0037x + 0.073;$$

$$R^2 = 0.9851$$

DPPH free radical scavenging assay. The antioxidant property of the extractives was assessed using the DPPH free radical scavenging method, with ascorbic acid serving as the positive control.[11] Positive control and extractives were prepared at different concentrations (0.97, 1.95, 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, and 500 µg/mL) through serial dilution from mother solutions (1000 µg/mL). Extractives at varying concentrations along with a control (2 mL) were mixed with 3.0 mL of a methanolic DPPH solution (20 µg/mL). Following a 30-minute incubation in the dark, the absorbance was recorded at 517 nm using a UV-visible spectrophotometer (Shimadzu, Japan) at 25°C, with methanol as the blank. The percentage inhibition of DPPH free radicals by the samples was calculated using the following equation.

$$\text{Percent (I\%) inhibition} = 1 - (A_{sample})/A_{blank} \times 100$$

A_{blank} = Absorbance of control (with all reagents without the test material).

A_{sample} = Absorbance of sample.

The IC_{50} value was determined by plotting the percentage inhibition against the extract concentration

Evaluation of cytotoxicity. The cytotoxic properties of the extractives were evaluated using the brine shrimp lethality test technique.[12] Here, vincristine sulfate and dimethyl sulfoxide (DMSO) were used as positive and negative control, respectively.[13] The number of surviving nauplii in each vial was counted using a magnifying glass, and the nauplii mortality rate was estimated using the equation.

$$\% \text{ mortality} = \frac{\text{Number of nauplii taken}}{\text{Number of nauplii death}} \times 100$$

Thrombolytic activity. The thrombolytic activity of the test samples was determined as described by Prasad *et al* (2006).[14] A lyophilized streptokinase vial containing 1,500,000 IU was used as positive control, while distilled water was employed as the negative control.[14] The clot lysis activity was determined using the equation.

$$\text{Percent (\%) of clot lysis} = \frac{\text{Released clot weight}}{\text{clot weight}} \times 100$$

In silico study. Molecular docking techniques were employed to forecast potential interaction pathways and affinities between the target protein and plant metabolites.[15] A selection of four phytochemicals for the molecular docking study was determined based on literature findings and results from biological tests. The identified compounds include, taraxerol (PubChem ID: 92097), kaempferol (PubChem ID: 5280863), α-amyrin (PubChem ID: 73170), and β-amyrin (PubChem ID: 73145). The 3D conformers of these chosen compounds were obtained in SDF file format from the PubChem database and subsequently converted to PDF format using the Open Babel program integrated into PyRx.[16]

Protein preparation. The targets selected for docking studies included the macromolecules, superoxide dismutase (SOD-1) (PDB ID: 4MCM), tissue plasminogen activator (tPA) (PDB ID: 1A5H) and vascular endothelial growth factor receptor 2

(VEGFR-2) (PDB ID: 4ASD). This decision was based on their well-established roles as influential mediators and regulators in their respective biological functions. The specific active sites on these macromolecules were identified through a review of existing literature. To prepare the protein structures, 'Swiss PDB Viewer 4.1.0' and 'PyMol.' were utilized, involving the removal of water molecules, heteroatoms, and any non-essential ligands or co-factors. Following this, energy minimization and refinement were carried out using the GROMOS96 force-field in vacuo within the latter software suite, aiming to rectify potential structural irregularities and optimize protein conformations.[17-20]

Molecular docking analysis. The exploration of molecular docking was conducted using the Autodock Vina module, a crucial element of the 'PyRx' software suite.[21] AutoGrid was employed to ascertain the positioning of the ligand within the protein binding site, specifying grid coordinates along the three dimensions (X, Y, and Z axes). Subsequent to these docking experiments, the outcomes were scrutinized and visualized utilizing both 'PyMol' and 'Discovery Studio Visualizer 2020'.[22]

ADME/T and toxicological analysis of ligands. Predictions of the isolated compounds' absorption, distribution, metabolism, and excretion (ADME) characteristics were generated utilizing the Swiss ADME computational server. Furthermore, the ProTox-II web server was used to predict the toxicity status of these substances.[23-25]

RESULTS AND DISCUSSION

Phytochemical studies. A total of five compounds (**1-5**) were isolated from the CTCSF. The molecular structures of these phyto-constituents were determined by analyzing their ^{1}H NMR spectra and comparing with the corresponding ^{1}H NMR data published previously.

The ^{1}H NMR spectrum (400 Hz, $CDCl_3$) of compound **1** displayed eight three-proton singlets at δ 0.97, 0.92, 0.79, 0.90, 1.08, 0.94, and 0.90 for eight tertiary methyl groups H-23 to H-30. The double doublet (J = 8.0, 3.2 Hz) at δ 5.53 in the spectrum suggested the presence of olefinic proton at C-15. The spectrum revealed an oxymethine proton resonance at δ 3.20 for H-3. The eight methyl singlets, the oxymethine and olefinic protons revealed that the molecule was a pentacyclic triterpenoid. These ^{1}H NMR spectral data of compound **1** matched well with previously published values of taraxerol.[26,27] Thus, compound **1** was identified as taraxerol. This is the first report of its occurrence from *M. nagassarium*.

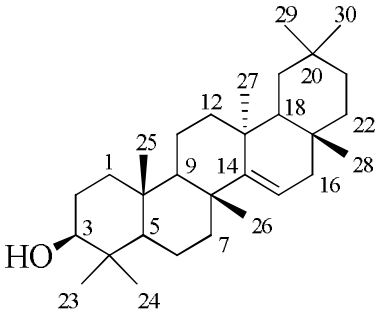
Compounds **2** and **3** were isolated as a mixture from CTCSF. However, careful analysis of the ^{1}H NMR spectral data allowed to identify these as a mixture of β-amyrin (**2**) and α-amyrin (**3**). The ^{1}H NMR spectrum of the mixture displayed a double doublet (J = 10.5 and 5.8 Hz) at δ 3.17 for the oxymethine proton, H-3. The typical olefinic proton (H-12) of β-amyrin nucleus was observed as a triplet at 5.09. In addition, the spectrum displayed eight methyl singlets at δ 1.25, 1.08, 0.92, 0.90, 0.86, 0.79, 0.80, and 0.72. Thus, compound **2** was characterized as β-amyrin. Its identity was further confirmed by comparison of its ^{1}H NMR spectral data with reported values.[29,30]

Another component of the mixture α-amyrin (**3**) was identified by matching the ^{1}H NMR signals that were common to both compounds **2** and **3** and then carefully analyzing the extra signals which were assigned to α-amyrin. Among these, the three protons doublets at δ 0.87 (d, *J* = 6.5) and 0.82 (d, *J* = 6.2) were attributed to the methyl group at C-29 and C -30. The remaining ^{1}H NMR signals were common to both β-amyrin and α-amyrin. On this basis, the identity of compounds **2** and **3** was established as β-amyrin and α-amyrin, respectively.[29,30]

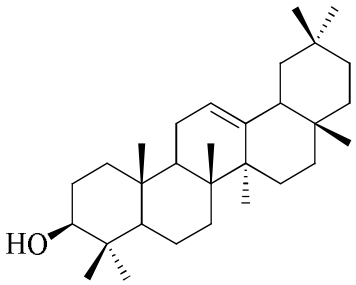
Compound **4** was separated as an amorphous powder from the CTCSF applying 2% methanol in chloroform as the solvent system (R_f value = 0.65). The ^{1}H NMR spectrum showed six methyl proton signals at δ 0.71, 0.82, 0.84, 0.87, 0.93 and 1.00, a pair of double doublets between δ 5.04 and 5.18 and a broad singlet at δ 5.34. These findings indicated that compound **4** was a steroidal compound. The ^{1}H

NMR spectrum further showed an acetylated methyl group at δ 1.88 and a downfield oxymethine proton at δ 4.65. Thus, compound **4** was characterized as Stigmasterol acetate[31] and its identity was substantiated by co-TLC with an authentic sample.

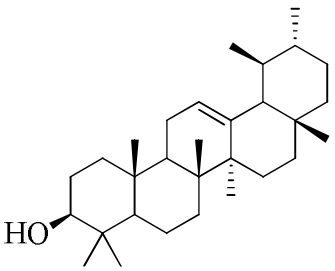
The ^{1}H NMR spectrum of compound **5** showed two broad singlets at δ 6.38 and 6.16, which indicated the presence of H-8 and H-6 of a the flavonol moiety. Furthermore, two doublets (J = 8.5 Hz) reach of two proton intensity at δ 8.05 and 6.89 clearly indicated the presence of para di-substituted benzene ring of the flavone nucleus. These two protons were assigned to at H-2'/H-6' and H-3'/H-5', respectively. The high field resonance of the H-3'/H-5' suggested that C-4' was oxygenated. On this basis, compound **5** was characterized as kaempferol, the identity of which was further confirmed by comparison of its spectral data with published values.[28]



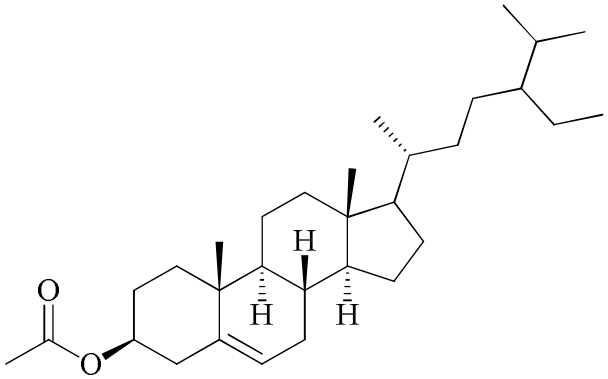
Taraxerol (**1**)



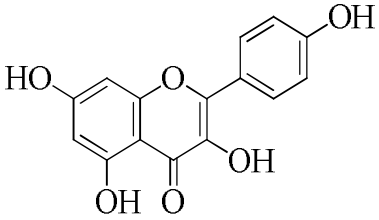
β amyrin (**2**)



α amyrin (**3**)



Stigmasterol acetate (**4**)



Kaempferol (**5**)

Figure 1. Compounds isolated from the stem bark of *M. nagassarium.*

Total phenolic content. Among the extractives, the MNME showed the highest phenolic content (50.81 mg of GAE/g) followed by HESF (36.75 mg of GAE/g) and CTCSF (29.45 mg of GAE/g), while the lowest phenolic content was detected in CFSF (16.75 mg of GAE/g) (Table 1).

DPPH free radical scavenging activity. Comparisons were made between the antioxidant potential of the bark extractives and standard ascorbic acid (IC_{50} = 4.65 µg/ml) (Table 1). The most significant scavenging activity against free radicals was exhibited by HESF (IC_{50} = 6.7 µg/ml), followed by MNME (IC_{50} = 10.13 µg/ml). Noteworthy DPPH free radicals scavenging activity was also shown by CFSF (IC_{50} = 18.57 µg/mL). In contrast, CTCSF demonstrated the lowest free radical scavenging activity (IC_{50} = 20.41 µg/mL) (Table 1) as compared to the standard ascorbic acid (IC_{50} = 4.65 µg/ml).

Brine shrimp lethality test. The cytotoxic efficacy of all the extractives was compared with the

standard vincristine sulfate (LC_{50} = 0.75 µg/ml). The extractives exhibited significant cytotoxicity, with HESF demonstrating the highest lethality (LC_{50} = 14.68 µg/ml), followed by MNME (LC_{50} = 23.79 µg/ml) and CTCSF (LC_{50} = 32.96 µg/ml) (Table 1). Among the fractions, CFSF displayed the lowest lethality (LC_{50} = 68.43 µg/ml).

Evaluation of thrombolytic activity. Among the fractions, only CFSF displayed potential thrombolytic activity (68.43%) whereas MNME (8.43%), HESF (5.43%) and CTCSF (4.56%) showed negligible thrombolytic activity (Table 1) compared to the standard streptokinase (71.28%) and negative control (4.48%) (Table 1).

Table 1. Pharmacological activities of methanolic extract of bark of *M. nagassarium* and its Kupchan partitionates.

Test groups	Total phenolic content (mg of GAE/g extractives)	DPPH free radical scavenging test IC_{50} (µg/ml)	Brine shrimp lethality test LC_{50} (µg/ml)	Thrombolytic activity % of clot lysis
Standard	N/A	4.65 (Ascorbic acid)	0.75 (Vrincristine sulphate)	71.28 (Streptokinase)
Negative control	N/A	N/A	N/A	4.48
MNME	50.81	10.13	23.79	8.43
HESF	36.75	6.7	14.68	5.43
CTCSF	29.45	20.41	32.96	4.56
CFSF	16.75	18.57	68.43	26.27

Table 2. Macromolecule target receptors.

Macromolecules	PDB ID	Control drug	Vina Search Space		Reference
			Center	Dimensions (Angstrom)	
Superoxide Dismutase1 (SOD-1)	4MCM	L-Ascorbate	X=19.8072 Y=124.4892 Z=7.2520	X=17.6406 Y=25.0 Z=14.4886	[32]
			Y=193.3815 Z=203.7955	Y=25.0 Z=21.94	
Tissue Plasminogen Activator (tPA)	1A5H	Streptokinase	X=8.5013 Y=40.3782 Z=53.1544	X=25.2893 Y=28.9224 Z=21.2496	[33]
Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2)	4ASD	Sorafenib	X=-24.3385 Y=0.8553 Z=-9.3748	X=20.1746 Y=21.0296 Z=20.7792	[34]

Molecular docking studies. Among the isolated compounds, compounds **1-3** and **5** were selected based on their structure for molecular docking studies. Docking experiments were carried out to evaluate the diverse bioactivity of the selected compounds against multiple important macromolecules: SOD-1, tPA and VEGFR-2. Table 2 presents details regarding the target receptors, their binding sites and the associated control compounds.

In the molecular docking analysis of four compounds against SOD-1, all molecules exhibited promising interaction with the enzyme (Table 3). Compounds **1** and **5** displayed the strongest binding affinity, reflected by excellent binding scores of -6.2 kcal/mol surpassing the standard L-ascorbate, which achieved a score of -5.1 kcal/mol (Figure 2). These findings suggested that these compounds as potential candidates for further investigation as SOD-1 modulators.

Table 3. Interactions of the compounds 1-3 and 5, and L-ascorbate (control) with SOD-1.

Ligands	Binding affinity (kcal/mol)	Ligand-macromolecule interactions	
L-Ascorbate (Control)	-5.1	A:HIS63, A:ASN65, A:ARG69, A:HIS80	Hydrogen Bond
Taraxerol (**1**)	-6.2	A:ASN65	Hydrogen Bond
		A:PRO62, A:LYS136, A:HIS80*	Hydrophobic
β-amyrin (**2**)	-5.0	A:PRO62, A:LYS136	Hydrophobic
α-amyrin (**3**)	-6.0	A:PRO62, A:LYS136	Hydrophobic
Kaempferol (**5**)	-6.2	A:HIS63*, A:ARG69*	Hydrogen Bond
		A:GLU133	Electrostatic
		A:LYS136, A:HIS80*, A:PRO62	Hydrophobic

*Interactions common with the control

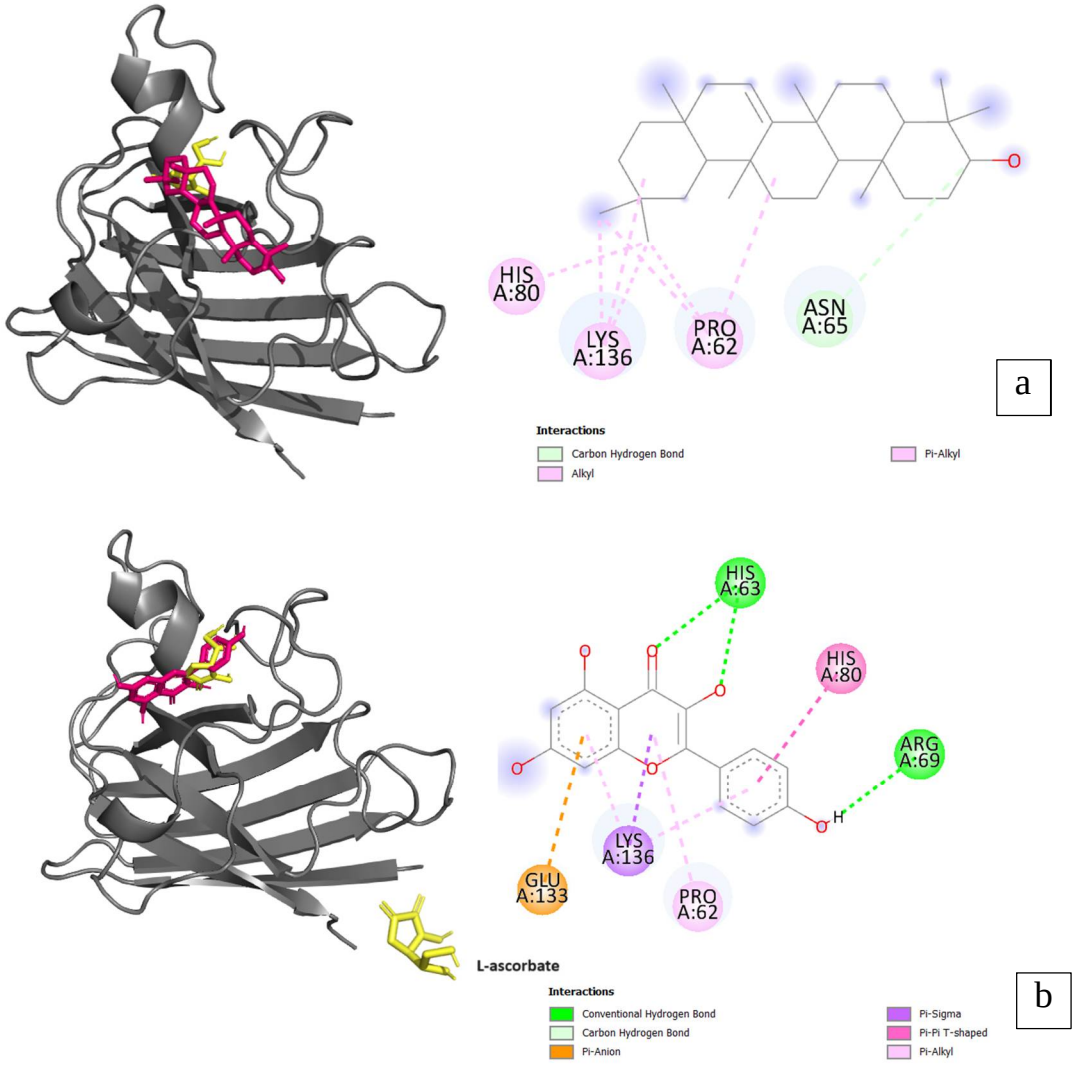


Figure 2. Interactions of (a) Taraxerol (**1**) and (b) Kaempferol (**5**) with SOD-1.

In the docking studies targeting VEGFR-2, compound **5** emerged as the sole compound with promising affinity with binding score of -8.3 kcal/mol which revealed favorable interaction potential with the receptor when compared to the standard sorafenib with -10.6 kcal/mol (Table 4) (Figure 3). The remaining compounds exhibited negligible binding affinity towards VEGFR-2.

Compound **3** exhibited the strongest binding affinity against tPA, registering a value of -9.5 kcal/mol, while Compound **1** displayed the second-highest affinity at -8.1 kcal/mol (Figure 4) and the standard Streptokinase had the binding score of -6.5 kcal/mol. The interactions involved in these bindings were exclusively comprised of either hydrogen bonds or hydrophobic interactions (Table 5).

Table 4. Interactions of the compounds 1-3 and 5, and Sorafenib (control) with VEGFR-2.

Ligands	Binding affinity (kcal/mol)	Ligand-macromolecule interactions	
Sorafenib (Control)	-10.6	A:GLU885, A:GLY922, A:GLU917	Hydrogen Bond
		A:VAL848, A:LEU889, A:PHE1047, A:LEU1019, A:LEU840, A:PHE918, A:ALA866, A:LYS868, A:VAL916, A:CYS1045	Hydrophobic
		A:ILE1044	Halogen
Taraxerol (**1**)	-0.6	A:LEU889*, A:ILE892, A:VAL898, A:VAL899, A:LEU1019, A:ILE1044	Hydrophobic
β-amyrin (**2**)	-3.8	A:ILE892, A:VAL898, A:VAL899, A:LEU1019*, A:LEU889*, A:ILE888, A:ILE1044*	Hydrophobic
α-amyrin (**3**)	-1.2	A:ILE892, A:VAL898, A:VAL899, A:LEU1019*, A:LEU889*, A:ILE1044*	Hydrophobic
Kaempferol (**5**)	-8.3	A:CYS919, A:ASP1046	Hydrogen Bond
		A:LEU1035, A:CYS1045*, A:LEU840*, A:VAL848*, A:ALA866*, A:VAL916*	Hydrophobic
		A:CYS1045*	Other (Pi-Sulfur)

*Interactions common with the control

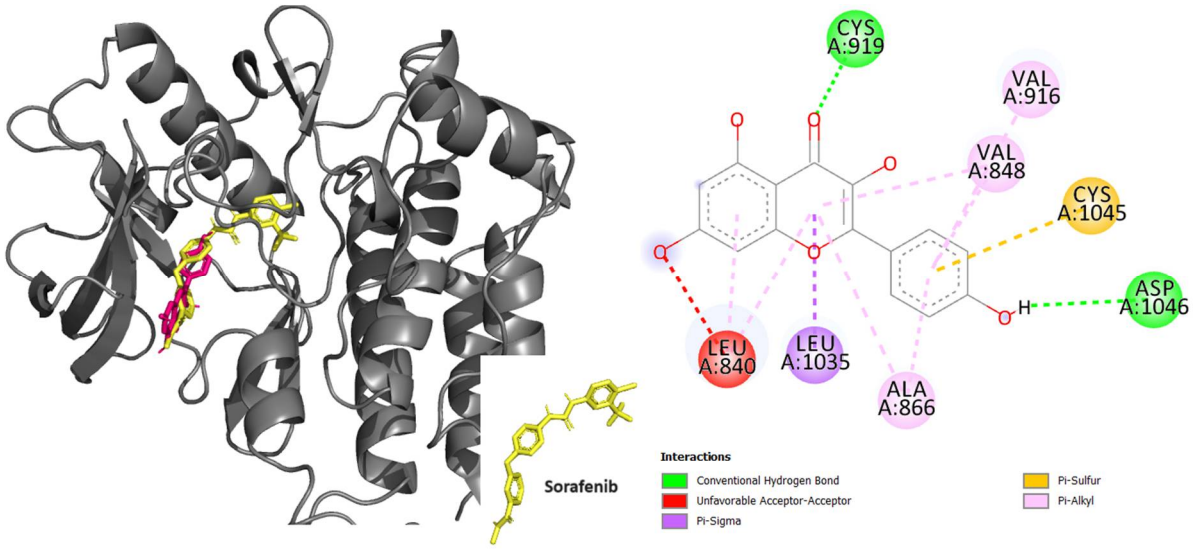


Figure 3. Interactions of Kaempferol (**5**) with VEGFR-2.

Table 5. Interactions of the compounds 1-3 and 5, and Streptokinase (control) with tPA.

Ligands	Binding affinity (kcal/mol)	Ligand-macromolecule interactions	
Streptokinase (Control)	-6.5	A:THR98, A:THR175	Hydrogen Bond
		A:TYR99, A:TRP215	Hydrophobic
Taraxerol (**1**)	-8.1	A:TRP215*, A:LEU217, A:HIS57, A:TYR99*	Hydrophobic
β-amyrin (**2**)	-7.7	A:ARG174, A:TYR99*, A:TRP215*	Hydrophobic
α-amyrin (**3**)	-9.5	A:TYR99*, A:ARG174, A:HIS57, A:TRP215*	Hydrophobic
Kaempferol (**5**)	-7.4	A:TYR99*, A:ALA190, A:GLY219, A:GLN192	Hydrogen Bond
		A:HIS57, A:CYS191; GLN192, A:TRP215; GLY216, A:GLY216; LEU217, A:ALA190	Hydrophobic

*Interactions common with the control

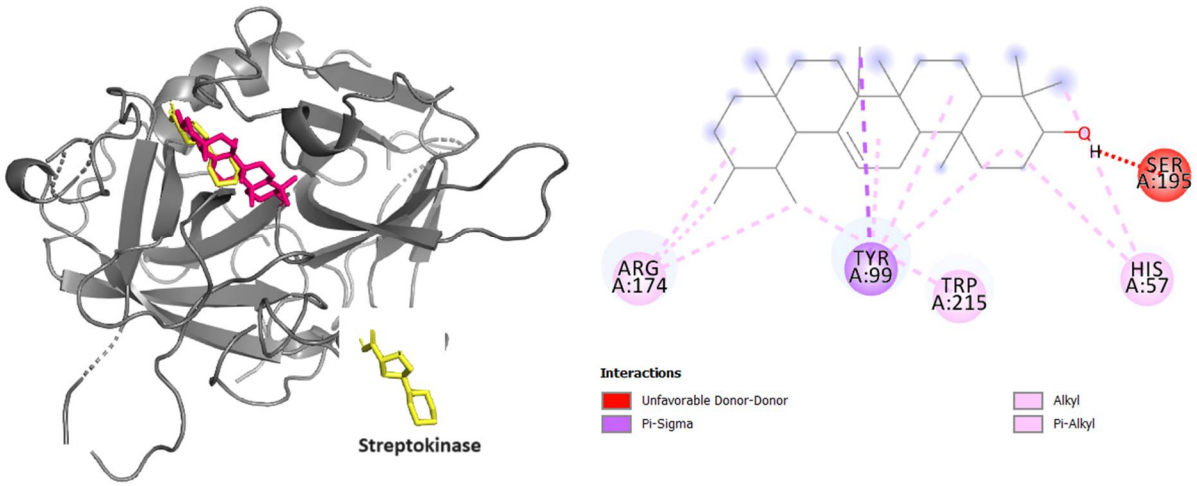


Figure 4. Interactions of α-amyrin (**3**) with tPA.

Prediction of pharmacokinetic (ADME) and toxicological properties. Compound **5** stands out among the selected compounds due to its favorable pharmacokinetic properties as predicted by the SwissADME tool. Specifically, it showed strong absorption in the gastrointestinal tract, and the ability to inhibit two types of cytochrome-P450 enzymes (CYP-inhibitors), although it doesn't meet the criteria for BBB permeant and Pgp substrate. Moreover, it met essential drug-likeness criteria, including Lipinski, Ghose, Veber, Egan, and Muegge rules, with no violations. Conversely, the other compounds did not exhibit promising pharmacokinetic predictions according to this tool (Table 6). Regarding toxicity analysis, all compounds showed high probability of immunotoxicity, except compound **5**. Compounds **1**, **2**, and **3** fall into toxicity class VI, suggesting they are non-toxic when swallowed, while compound **5** falls into class V indicating it might cause toxic manifestations if ingested (Table 7).

Compounds sourced from plants are being used to treat a wide range of health problems. Natural treatments based on plants and herbs have grown popularity due to their efficacy, purity, and cost-efficiency in treating a variety of ailments.[35,36] Our current study has unveiled the isolation and characterization of taraxerol (**1**), mixtures of β-amyrin (**2**) and α-amyrin (**3**), stigmasterol acetate (**4**) and kaempferol (**5**) from the bark of *M. nagassarium*. The structures of the isolated compounds were determined by extensive spectroscopic studies. Notably, this study marks the first instance of identifying taraxerol and sitgmasterol acetate from *M.*

nagassarium, as per extensive literature searches. The current investigation also sheds light on the potential *in vitro* capabilities, such as cytotoxic, thrombolytic, and antioxidant properties, of crude extracts and its kapchan fractions derived from *M. nagassarium*.

Table 6. ADME properties of the isolated compounds.

Compound	GI absorption	BBB permeant	Pgp substrate	CYP Inhibition (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4)	Lipinski violations	Ghose violations	Veber violations	Egan violations	Muegge violations	Bioavailability Score
Taraxerol (**1**)	Low	No	No	0/5	1	3	0	1	2	0.55
β-amyrin (**2**)	Low	No	No	0/5	1	3	0	1	2	0.55
α-amyrin (**3**)	Low	No	No	0/5	1	3	0	1	2	0.55
Kaempferol (**5**)	High	No	No	3/5	0	0	0	0	0	0.55

Table 7. Toxicity profile of the phytoconstituents of interest (Toxicity class I: Fatal if swallowed, class II: Fatal if swallowed, class III: Toxic if swallowed, class IV: Harmful if swallowed, class V: May be harmful if swallowed, class VI: Non-toxic).

Compound	Predicted Toxicity Class	Predicted LD_{50} mg/kg	Toxicity Predicted
Taraxerol (**1**)	6	70000	Immunotoxicity**
β-amyrin (**2**)	6	70000	Immunotoxicity**
α-amyrin (**3**)	6	70000	Immunotoxicity**
Kaempferol (**5**)	5	3919	None

Low probability of toxicity*

High probability of toxicity**

Various pharmacological effects of plant extracts were investigated to explore novel medicinal alternatives and validate their traditional use. In the pursuit of reliable and effective antioxidant options from natural sources, the extract of *M. nagassarium* was scrutinized. The antioxidant capacity of this plant was assessed through TPC and the DPPH radical quenching method. Though the results of the TPC exerted considerable outcomes, free radical scavenging test through DPPH assay of different fractions demonstrated promising effectiveness of the extractives. The current study revealed the same antioxidant potential of *M. nagassarium* that previous investigation reported. For instance, Teh *et al* [37] showed significant antioxidant activity of *M. nagassarium* with low EC_{50} values of 9.77 µg/mL compared to the standard ascorbic acid (EC_{50} = 5.62 µg/mL). Another study showed that ethyl acetate extract derived from *M. nagassarium* bark had noteworthy scavenging activity against nitric oxide (NO), ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid), DPPH, and NBT (nitroblue tetrazolium) induced free radicals.[38] Phenolic compounds in plants can function as antioxidants through various mechanisms.[15,39-42] Polyphenols' hydroxyl groups absorb reactive free radicals, thereby safeguarding cellular organs against oxidative stress-induced damage from free radicals.[43]

Cancer ranks among the diseases claiming numerous lives annually across the globe. Investigation indicated that in the southern region of Thailand, the ingestion of raw seeds extract exhibits notable therapeutic benefits in cases of esophageal carcinoma.[44] In this current study, nearly all extractives of *M. nagassarium* exhibited strong cytotoxicity, suggesting the presence of potential cytotoxic or anticancer compounds. An earlier investigation similarly demonstrated equivalent levels of cytotoxicity in the brine shrimp lethality bioassay. Both the crude methanol extract and its carbon tetrachloride soluble fraction exhibited substantial cytotoxicity, with LC_{50} values of 2.99 and

1.74 μg/ml, respectively, in comparison to vincristine sulphate (with an LC_{50} value of 0.543 μg/ml).[7] Another study showcased a notable anticancer properties of the seed extract against human neuroblastoma, rat glioblastoma, and human embryonic kidney cell lines.[45] It also demonstrated the effective anticancer properties of on the human liver cancer cell line HepG2, the murine colon cancer cell line CT26, and the murine melanoma cell line B16F1. In HepG2 cell line, kaempferol notably suppressed protein kinase B phosphorylation and triggered the cleavage of caspase-3, caspase-7, caspase-9, and poly-ADP ribose polymerase in HepG2 cells.[46] On the contrary, three distinct pentacyclic triterpenoids, namely taraxerol, α amyrin, and β amyrin, demonstrated exclusively hydrophobic interactions with the receptor but they showed insignificant binding affinity.

In the molecular docking study, all compounds exhibited varying degrees of binding affinities towards the macromolecules. Especially, when interacting with SOD-1, all isolated compounds demonstrated significant binding affinities compared to the control compound, L-ascorbic acid. Furthermore, it's worth mentioning that compounds **1** and **5** shared common binding sites with the control compound when interacting with SOD. Regarding their potential anticancer and thrombolytic properties, only compounds **5** and **3** displayed stable and remarkable binding affinity with VEGFR-2 and tPA, respectively. The primary mode of interaction between the compounds and their respective receptors mainly involved hydrogen bonds and hydrophobic interactions. These results suggest that the biological activity exhibited by the extractives may be mediated through these macromolecular targets. Further exploration in this direction could offer more insights.

CONCLUSION

A total of five compounds were detected from the phytochemical investigation of the methanolic extract of *M. nagassarium*, while taraxerol and stigmasterol acetate are the first time report from this plant. The potent antioxidant and cytotoxic activities were detected from the extractives, compared with the respective standards and previous investigations. Furthermore, in the molecular docking study, the diverse binding affinities observed among the compounds towards various macromolecules, suggested a potential mechanism underlying the biological activity exhibited by the extractives. As a result, the outcomes warrant further investigations for clarification and potential therapeutic application.

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COMPETING INTERESTS:

Authors declare no conflict of interest.

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