Asian Journal of Chemistry; Vol. 38, No. 4 (2026), 1087-1094
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2026.35289

Isolation, Characterisation and *in silico* Studies of Bioactive Terpenoid and Flavonoid from the Seeds of *Mucuna pruriens*

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Received: 18 November 2025 *Accepted*: 23 March 2026 *Published online*: 8 April 2026 AJC-22332

In this work, new bioactive terpenoids (**1-2**) and flavonoid (**3**) have been isolated from the chloroform extract of the seeds of *Mucuna pruriens*. The structures of the isolated compounds were established by spectroscopic analysis. *In vitro* antibacterial and antifungal activities of these compounds have been studied. Compound **2** showed the highest inhibition value (16.0 ± 0.5 mm) against *S. aureus* and (19.3 ± 0.6 mm) against *B. subtilis*. Compound **2** also exhibited significant antifungal activities with inhibition value (16.0 ± 0.6) mm and (6.0 ± 1.0) mm against *T. harzianum* and *A. niger*, respectively. Molecular docking studies and ADME prediction of the compounds were performed. Compound **2** showed good binding affinity ranging between -7.5 to -7.6 kcal/mol with targeted receptors.

Keywords: *Mucuna pruriens*, Triterpenoid, Flavonoid, Bioactivity, Molecular docking.

INTRODUCTION

Mucuna pruriens is popularly known as Alkushi in Bangladesh [1] and belongs to the family Fabaceae and genus *Mucuna*. It is an annual twinning, climbing shrub with long vines that can reach over 15 m in length and distributed throughout India and Bangladesh. Flowers are white to dark purple in colour and hang in long clusters. The seeds are black in colour with pale brown specks uniform in shape 9 to 12 mm long. *M. pruriens*, an important species in this genus, has been utilised as a food source since its seeds contain high protein content. Therefore, populations, in local areas, consume both the green pods and the mature beans of *M. pruriens* by cooking or boiling them.

Furthermore, the roasted and ground seeds of *M. pruriens* have been produced as a coffee substitute for at least several decades in Bangladesh. It has a wide variety of medicinal applications against severe ailments [2-4]. Previous biological studies have reported that *M. pruriens* possesses anti-Parkinson [5], antidiabetic [6], antioxidant [7,8] antibacterial activities [9], antimicrobial activity [10] and antivenom activity [11] as well as male fertility and aphrodisiac effects [12,13]. The different classes of compounds such as glycosides, alkaloids, steroids, flavonoids and amino acids were reported [14,15]. In addition, the seeds of *M. pruriens* contain various kinds of phytochemical, such as carbohydrates and amino acids, as well as antinutrient substances such as polyphenols, tannins, saponins [16,17] and alkaloids [18]. As a part of our ongoing research for bioactive natural products, the current phytochemical investigation on the seeds of *M. pruriens* has led to the isolation of three new natural products (**1-3**). The present paper describes the structure determination, bioactivity and molecular docking studies of the isolated compounds.

EXPERIMENTAL

All the reagents were purchased from Merck and Sigma-Aldrich and were used directly without any purification. IR spectra were recorded on a Shimadzu FT-IR 20 spectrophotometer, Japan. NMR experiments (^{1}H, ^{13}C, COSY, HSQC and HMBC) were carried out on a Bruker AVANCE 400 spectrometer (Bruker AG, Karlsruhe, Germany). Mass spectra were measured on a MAT 95XL Finnigan instrument (Thermo Quest Finnigan, Germany) for electrospray ionisation (ESI).

Plant material: Matured seeds of *Mucuna pruriens* were collected from the hilly region of the district of Chattogram situated in the southeastern region of Bangladesh during the month of August 2025. The plant was identified by Prof. A. Rahman, Department of Botany, University of Chittagong and a voucher specimen was deposited at the Department of Botany, University of Chittagong.

Extraction and isolation: Seeds of *M. pruriens* (3.5 kg) were dried powdered and extracted successively with petroleum ether (3 × 72 h), chloroform (3 × 72 h) and methanol (3 × 72 h). The sample was then filtered through Whatman No. 1 filter paper in Buchner funnel. The filtered solution was evaporated under vacuum in a Rotary evaporator at 40 ºC to a constant weight. Evaporation to dryness petroleum ether extract gave solid mass 32 g, chloroform extract gave 65 g and methanol extract gave 62 g. The concentrated extracts were stored in airtight container in the refrigerator below 10 ºC. The chloroform extracts (5 g) were chromatographed on a silica gel column and eluted with *n*-hexane-EtOAc (4:1) to give compound **1** (177 mg), compound **2** (210 mg) and a mixture of 720 g. The solid mass 720 mg was rechromatographed on a silica gel column and eluted with *n*-hexane-EtOAc (2:1) to give compound **3** (270 mg). Three compounds (**1-3**) were purified by repeated crystallisation from solvent system *n*-hexane-EtOAc (3:1).

Test for steroid

Salkowski test: About 2-3 drops of conc. H_2SO_4 was added to chloroform solution of compound **1** and compound **2**, shaken and allowed to stand, appearance of red colour in lower layer indicates the presence of sterol [19,20].

Liebermann-Burchard test: Compounds **1** and **2** were mixed with the chloroform and few drops of acetic anhydride and mixed well. Conc. H_2SO_4 was added from the sides of the test tube slowly until the ring appears; appearance of reddish-brown ring indicates the presence of steroid [19,20].

Test for flavonoid

Shinoda test: To a compound **3**, a few small pieces of magnesium ribbon and 3-4 drops of conc. HVl were added. Appearance of red to pink colour after few minutes indicates the presence of flavonoid [20].

Lead acetate test: To a compound **3** added few drops of aqueous basic lead acetate solution. Formation of yellow precipitate indicates presence of flavonoid [20].

Alkaline reagent test/NaOH test: Few drops of NaOH solution were added to extract. Intense yellow colour disappeared after adding dilute HCl which indicates the presence of flavonoids [20].

Compound 1: White amorphous solid, m.p.: 130-131 ºC, soluble in $CHCl_3$, R_f: 0.66 (*n*-hexane-EtOAc, 1:5); IR (KBr, ν_{max}, cm^{-1}): 3412 (O-H), 1734 (>C=O, ester). ^1H and ^{13}C NMR (400 and 100 MHz, $CDCl_3$, Table-1). EI-MS: *m/z* 687.4824 $[M+1]^+$ ($C_{41}H_{66}O_8$), 363, 323, 201, 163, 123.

Compound 2: Brown amorphous solid, m.p.: 145-146 ºC, soluble in $CHCl_3$, R_f: 0.62 (*n*-hexane-EtOAc, 1:5); IR (KBr, ν_{max}, cm^{-1}): 3420 (O-H), 1736 (>C=O, ester), 1704 (>C=O, ketone). ^1H and ^{13}C NMR data (400 and 100 MHz, $CDCl_3$, Table-2). EI-MS: *m/z* 711.4857 $[M+1]^+$ ($C_{43}H_{66}O_8$), 375, 337, 213, 163, 125.

Compound 3: White amorphous solid, m.p.: 172-173 ºC, soluble in $CHCl_3$, R_f: 0.48 (*n*-hexane-EtOAc, 1:2); IR (KBr, ν_{max}, cm^{-1}): 3388 (O-H), 1695 (>C=O, α,β-unsaturated ketone). ^1H and ^{13}C NMR data (400 and 100 MHz, $CDCl_3$, Table-3). EI-MS *m/z* 663.3864 $[M+1]^+$ ($C_{40}H_{54}O_8$), 445, 221, 155, 137.

Antimicrobial assay: The isolated compounds (**1-3**) were evaluated for *in vitro* antibacterial and antifungal activity using the agar disc diffusion method [21]. Mueller Hinton Agar (Himedia, India) and Potato Dextrose Agar (Himedia, India) served as the basal media for bacterial and fungal strains, respectively. Sterile, uncontaminated plates were inoculated with test organisms using a cotton swab. After preparation the basal media were incubated for 24 h with continuous monitoring. Only non-contaminated dishes were preferred for this assay. Inoculation process of test organism on media was performed by a sterile cotton bar. The discs containing sample were smoothly placed on pre-inoculated agar plates. The plates were than incubated aerobically at 37 ºC for 24 h and at 26 ºC for 48 h in case of antibacterial and antifungal assays, respectively. DMSO (dimethyl sulfoxide) was used as control for both experiments. Cefixime and amphotericin B were used as positive controls in antibacterial and antifungal assays, respectively. Each disc was charged with 25 µL of sample solution in DMSO which contain 300 µg of synthesised compounds. In addition, 10 µL of cefixime amphotericin B solution in DMSO was used to charge per disc which contains 50 µg of standard cefixime or amphotericin B as positive control. The dishes were then incubated for 24 h and the diameter of inhibition zones (mm) were measured in millimeters using a measuring scale. The experiment was performed in triplicate. The study tested two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*) and two fungal strains (*Trichoderma harzianum*, *Aspergillus niger*).

Molecular docking analysis: Molecular docking analyses of compound **2** was executed using the software programs Gaussian 09, PyRx 0.8 and PyMol [22]. The structural optimisation of the compound was conducted with Gaussian 09, utilizing the B3LYP/6-31+G (d,p) basis set within the DFT approach.

Protein preparation: The 3D crystal structures of the target proteins were obtained from the Protein Data Bank (PDB) database (https://www.rcsb.org) [23]. To remove non-protein elements, ligands and water molecules, the Discovery Studio program was utilized, assigning polar hydrogen atoms and Kollman charges to the receptor protein. The energy calculations and the creation of a 3D grid were performed using Auto Dock Vina [24], while PyRx software was employed to analyze ligand-protein interactions. All docked complexes were then evaluated for their lowest binding energy values (Kcal/mol) and were examined for hydrogen and hydrophobic interactions using Discovery Studio.

***In silico* ADMET prediction:** The pharmacokinetics analysis of the isolated compounds **1-3** was observed with the Lipinski's rule of five and Veber's rule [25]. The percentage of absorption (% ABS) was calculated using eqn. 1:

$$ABS\ (\%) = 109 - (0.3459 \times TPSA) \quad (1)$$

where TPSA stands for topological polar surface area.

RESULTS AND DISCUSSION

Three new natural compounds **1-3** were isolated and characterised from the chloroform extract of *Mucuna pruriens* seeds. The structures of all compounds **1-3** were established by IR, ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and mass spectroscopic analysis.

Compound **1** (Fig. 1a), gave Salkowski and Liebermann-Burchard reaction for steroid and terpenoid [19,20]. The IR spectrum of compound **1** showed a broad absorption for O-H stretching at $\nu_{max.}$ 3412 cm^{-1}. It also showed sharp absorptions at ν_{max} 1734 cm^{-1} for >C=O function of an ester. ^{1}H NMR spectrum showed five olefinic protons appeared at δ 5.26 (1H, t, *J* = 4.8 Hz, H-6), 5.27 (1H, t, *J* = 4.8 Hz, H-8′), 5.32 (1H, t, *J* = 4.8 Hz, H-9′), 5.36 (1H, t, *J* = 4.8 Hz, H-8″) and 5.34 (1H, t, *J* = 4.8 Hz, H-9″). Four down fielded absorptions for four methane protons appeared at δ 4.30 (1H, m, H-3), 4.31 (1H, m, H-17), 4.31 (1H, m, H-2′), 4.28 (1H, d, *J* = 4.4 Hz, H-2″) and down fielded two sets of methylene protons appeared at δ 4.12 (2H, d, *J* = 6 Hz, H-10′) and 4.13 (2H, d, *J* = 6 Hz, H-10′).

Earlier literature review had revealed that genus *Mucuna* contains a number of steroids, terpenoids along with long alkyl chain [14,15]. Attempt was made to correlate compound **1** with those of reported in the literature and this is in line with the observation that compound **1** contains a number of alkyl chains in the molecule. ^{13}C NMR spectrum revealed the presence of 41 carbons in the molecule. ^{13}C NMR (DEPT) spectrum showed the presence of 18 methylene carbons and four methyl carbons in the molecule. Two carbonyl carbons appeared at δ 173.2 (C1′) and 173.1 (C1″), six olifinic carbons appeared at δ 131.8 (C5), 130.1 (C6), 129.9 (C8′), 127.8 (C9′), 129.9 (C8′) and 128.0 (C9″). Chemical shifts of all

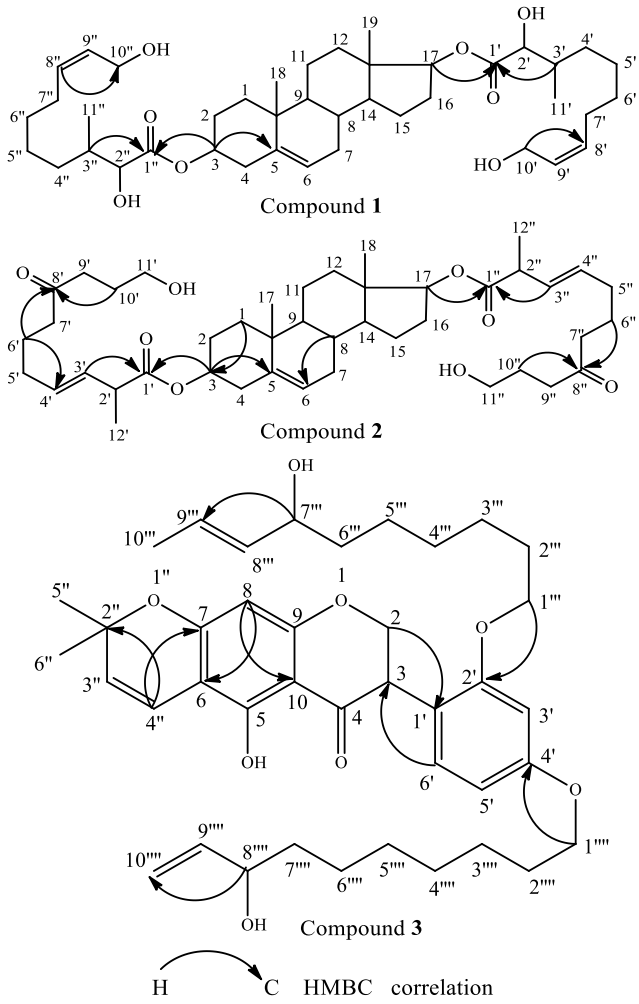

Fig. 1. Selected HMBC correlations of compounds **1**, **2** and **3**

TABLE-1
^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **1** ($CDCl_3$, δ, ppm, *J*, Hz)*

C atom	^{13}C, δ	^{1}H, δ	COSY	C atom	^{13}C, δ	^{1}H, δ	COSY
1	29.4	1.29; 1.24 (m)		3′	31.7	2.75 (m)	
2	29.6	1.59; 1.24 (m)		4′	29.0	1.29 (m)	
3	65.5	4.30 (m)		5′	29.2	1.29 (m)	
4	31.5	2.01; 1.59 (m)		6′	29.1	1.29 (m)	
5	131.8	–		7′	24.8	2.29 (m)	
6	130.1	5.26 (t, *J* = 4.8)	H-7	8′	129.9	5.27 (t, *J* = 4.8)	H-7′,9′
7	34.0	2.02; 1.59 (m)		9′	127.8	5.32 (t, *J* = 4.8)	H-8′,10′
8	33.9	1.59 (m)		10′	62.0	4.12 (d, *J* = 6.0)	H-9′
9	33.8	1.59 (m)		11′	14.2	0.90 (d, *J* = 4.0)	H-3′
10	31.8	–		1″	173.1	–	
11	31.9	1.29; 1.24 (m)		2″	68.5	4.28 (d, *J* = 4.4)	H-3″
12	29.6	1.29; 1.24 (m)		3″	31.8	2.75 (m)	
13	29.3	–		4″	29.0	1.29 (m)	
14	31.8	1.59 (m)		5″	29.1	1.29 (m)	
15	34.0	1.29; 1.24 (m)		6″	29.1	2.04 (m)	
16	34.1	2.04; 1.59 (m)		7″	24.8	2.29 (m)	
17	65.4	4.31 (m)		8″	129.9	5.36 (t, *J* = 4.8)	H-7″, 9″
18	14.0	0.88 (s)		9″	128.0	5.34 ((t, *J* = 4.8)	H-8″,10″
19	14.0	0.86 (s)		10″	64.9	4.13 (d, *J* = 6.0)	H- 9″
1′	173.2	–		11″	14.1	0.89 (d, *J* = 4.0)	H-3″
2′	68.9	4.31 (d, *J* = 4.4)	H-3′				

*Assignments were based on COSY, HMBC, HSQC and DEPT (135º) experiments.

protons and carbons of compound **1** are given in Table-1. The mass spectrum (EI-MS**)** of compound **1** exhibited highest molecular ion peak at *m/z* 687.4824 $[M+1]^+$, consistent with the molecular formula $C_{41}H_{66}O_8$. Analysis of all spectral data structure **1** is suggested for compound **1** and characterised as [(8′*Z*,8″*Z*)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene-17-yl(2′,10′-dihydroxy-3′-methyldec-8′-enoate)-3-yl(2″,10″-dihydroxy-3″-methyldec-8″-enoate)] (**1**).

Compound **2** (Fig. 1b) also gave colour reactions for steroid and terpenoid [19,20]. IR spectrum of compound **2** showed a broad absorption band at ν_{max} 3420 cm^{-1} for O-H stretching. It also showed sharp absorption at ν_{max} 1736 cm^{-1} for >C=O (ester) and at 1704 cm^{-1} for >C=O (ketone). The EI-MS of compound **2** showed the highest molecular ion peak at *m/z* 711.4857 $[M+1]^+$ corresponding to the molecular formula $C_{43}H_{66}O_8$. The chemical shifts of protons showed the presence of five olifinic protons at δ 5.34 (1H, t, *J* = 4.8 Hz, H-6), 5.35 (1H, m, H-3′), 5.33 (1H, m, H-4′), 5.35 (1H, m, H-3″) and 5.33 (1H, m, H-4″). Two down fielded methine protons appeared at δ 4.27 (1H, m, H-3), 4.15 (1H, t, *J* = 4.8 Hz, H-17) and down fielded two sets of methylene protons appeared at δ 3.52 (2H, t, *J* = 4 Hz, H-11′) and 3.54 (2H, t, *J* = 4 Hz, H-11″). ^{13}C NMR spectrum of compound **2** showed the presence of four carbonyl carbons at δ 179.9 (C1′), 173.9 (C8′), 177.1 (C1″) and 173.8 (C8″). Six olifinic carbons appeared at δ 131.8 (C5), 130.1 (C6), 130.1 (C3′), 129.9 (C4′), 128.2 (C3″) and 127.2 (C4″). Carbon and proton content and their absorption patterns indicate compound **2** contains a number of side chains in the molecule. The connectivity of the side chains and location of functional groups are confirmed by HMBC, HSQC and COSY correlations. Absorptions of all protons and carbons of compound **2** are given in Table-2. Thus, on the basis of above spectral data structure **2** was confirmed to the compound **2**. Compound **2** is thus characterised as (3′*E*,3″*E*)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene-3-yl(11′-hydroxy-2′-methyl-8′-oxoundec-3′-enoate)-17-yl(11″-hydroxy-2″-methyl-8″-oxoundec-3″-enoate) (**2**).

Compound **3** (Fig. 1c) gave positive colour test for flavonoid [20]. IR (KBr, ν_{max}, cm^{-1}): 3388 (O-H), 1695 (>C=O, α,β-unsaturated ketone). The EI-MS of compound **3** had a molecular ion peak at *m/z* 663.3864 $[M+1]^+$, indicative of the molecular formula $C_{40}H_{54}O_8$. ^{1}H and ^{13}C NMR data of compound **3** in $CDCl_3$ are given in Table-3. Compound **3** contains 12 aromatic carbons appeared at δ 151.8 (C5), 128.0 (C6), 142.8 (C7), 115.8 (C8), 137.4 (C9), 135.3 (C10), 129.1 (C1′), 142.9 (C2′), 124.0 (C3′), 136.0 (C4′), 123.4 (C5′), 126.3 (C6′). One carbonyl carbon and six olifinic carbons appeared at δ 179.1 (C4) and 130.9 (C3″), 130.9 (C4″), 130.9 (C8‴), 130.9 (C9‴), 130.9 (C9⁗), 130.9 (C10⁗), respectively. Four aromatic and six olefinic protons appeared at δ 8.15 (1H, s, H-3′), 7.09 (1H, d, *J* = 8.4 Hz, H-5′), 7.50 (1H, d, *J* = 8.4 Hz, H-6′), 8.15 (1H, s, H-8), 5.84 (1H, d, *J* = 8.4 Hz, H-C3″), 5.82 (1H, d, *J* = 8.4 Hz, H-C4″), 5.39 (1H, d, *J* = 6.4 Hz, H-C8‴), 5.37 (1H, d, *J* = 6.4 Hz, H-C9‴), 6.63 (1H, d, *J* = 6.4 Hz, H-C9⁗), 6.61 (1H, d, *J* = 6.4 Hz, H-C10⁗), respectively. One phenolic (OH) proton attached with C5 appeared at δ 8.15 (1H, s, HO-C5). Based on the above spectral data compound **3** was identified as (*E*)-5-hydroxy-3-(2′-((7‴-hydroxydec-8‴-en-1‴-yl)oxy)-4′-((8⁗-hydroxydec-9⁗-en-1⁗-yl)oxy)-phenyl)-2″,2″-dimethyl-2,3-dihydropyrano[3,2-*g*]chromen-4(8*H*)-one (**3**).

TABLE-2
^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **2** ($CDCl_3$, δ, ppm, *J*, Hz)*

C atom	^{13}C, δ	^{1}H, δ	COSY	C atom	^{13}C, δ	^{1}H, δ	COSY
1	29.4	1.30; 1.25 (m)		4′	129.9	5.33 (m)	
2	34.0	1.62; 1.25 (m)		5′	29.2	2.76 (m)	
3	68.9	4.27 (m)		6′	27.1	1.30 (m)	
4	34.0	1.62; 1.25 (m)		7′	29.0	2.07 (t, *J* = 4.8)	H- 6′
5	131.8	–		8′	173.9	–	
6	130.1	5.34 (t, *J* = 4.8)	H-7	9′	29.2	2.07 (t, *J* = 4.8)	H-10′
7	31.9	2.03; 1.62 (m)		10′	27.1	2.03 (m)	
8	29.5	2.31 (m)		11′	62.1	3.52 (t, *J* = 4.0)	H-10′
9	29.4	2.34 (m)		12′	20.7	0.96 (d, *J* = 7.2)	
10	29.3	–		1″	177.1	–	
11	29.0	1.30; 1.25 (m)		2″	31.5	2.76 (m)	
12	28.9	1.30; 1.25 (m)		3″	128.2	5.35 (m)	
13	34.0	–		4″	127.8	5.33 (m)	
14	29.2	2.05 (m)		5″	31.1	2.76 (m)	
15	29.5	1.30; 1.25 (m)		6″	27.1	1.30 (m)	
16	31.5	2.03; 1.62 (m)		7″	29.3	2.07 (t, *J* = 4.8)	H-6″
17	68.1	4.15 (t, *J* = 4.8)	H-16	8″	173.8	–	H-7″, 9″
18	14.0	0.88 (s)		9″	29.1	2.07 (t, *J* = 4.8)	H-10″
19	14.0	0.87 (s)		10″	24.6	2.03 (m)	
1′	179.9	–		11″	64.9	3.54 (t, *J* = 4.0)	H-10″
2′	31.8	2.76 (m)		12″	20.7	0.95 (d, *J* = 7.2)	
3′	130.1	5.35 (m)					

*Assignments were based on COSY, HMBC, HSQC and DEPT (135º) experiments.

TABLE-3
^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **3** ($CDCl_3$, δ, ppm, *J*, Hz)*

C atom	^{13}C, δ	^{1}H, δ	COSY	C atom	^{13}C, δ	^{1}H, δ	COSY
2	65.1	3.95		1‴	65.0	3.71 (t, *J* = 6.0)	
3	33.8	2.80		2‴	34.0	1.43 (m)	
4	179.1	-		3‴	31.9	1.31 (m)	
5	151.8	-		4‴	29.3	1.31 (m)	
6	128.0	-		5‴	28.9	1.31 (m)	
7	142.8	-		6‴	31.5	2.37 (m)	
8	115.8	7.31 (s)		7‴	68.9	4.30 (d, *J* = 6.4)	
9	137.4	-		8‴	128.0	5.39 (d, *J* = 6.4)	
10	135.3	-		9‴	127.9	5.37 (d, *J* = 6.0)	
1′	129.1	-		10‴	19.4	1.13 (s)	
2′	142.9	-		1⁗	62.1	3.67	
3′	124.0	7.31 (s)		2⁗	33.8	1.43 (m)	
4′	136.0	-		3⁗	31.6	1.31 (m)	
5′	123.4	7.09 (d, *J* = 8.4)		4⁗	29.1	1.31 (m)	
6′	126.3	7.50 (d, *J* = 8.4)		5⁗	29.0	1.31 (m)	
2″	77.2	-		6⁗	28.8	1.31 (m)	
3″	130.9	5.84 (d, *J* = 8.4)		7⁗	29.6	2.36 (m)	
4″	130.2	5.82 (d, *J* = 8.4)		8⁗	70.2	4.17 (d, *J* = 6.0)	
5″	14.1	0.99 (s)		9⁗	139.2	6.63 (d, *J* = 8.4)	
6″	14.1	0.98 (s)		10⁗	114.0	6.61 (d, *J* = 8.4)	
5-OH	-	8.15 (s)					

*Assignments were based on COSY, HMBC, HSQC and DEPT (135º) experiments.

Absorption patterns, number of protons and carbons contain of compounds **1-3**, suggest the compounds contain side chains in the molecules [14,15]. The connectivity of the side chains were confirmed by analyzing 2D HMBC spectra (Fig. 1). All these compounds **1-3** are new natural products and were isolated for the first time from the seeds of the plant.

Antimicrobial activity: *In vitro* antimicrobial activities of the isolated compounds **1-3** were assayed against two Gram-positive and two Gram-negative bacteria along with two fungal strains by using agar disc diffusion method. The development of diameter of inhibition zones (mm ± S.D.) are shown in Table-4. Ceftriaxone and amphotericin B were used as standard. All the tested compounds **1-3** showed activities against bacterial and fungal strains. Compound **1** showed inhibition value (15.0 ± 0.6 mm) against *S. aureus* and inhibition value (18.0 ± 1.2 mm) against *B. subtilis*. Compound **2** displayed the highest inhibition value (16.0 ± 0.5 mm) against *S. aureus* and (19.3 ± 0.6 mm) against *B. subtilis*. Compound **3** showed inhibition value (15.0 ± 1.0 mm) against *S. aureus* and (16.0 ± 1.5 mm) against *B. subtilis.* Compound **1** and **3** also exhibited inhibition value (10.0 ± 0.6 mm) and (15.5 ± 0.6 mm) against *S. typhimurium* and inhibition value (17.5 ± 1.0 mm) and (13.0 ± 1.0 mm) against *E. coli*, respectively. Compound **2** also showed promising activities (17.0 ± 0.6 mm) and (19.5 ± 1.0 mm) against *S. typhimurium* and *E. coli*, respectively (Table-4). In antifungal study, compound **2** showed remarkable activities (16.0 ± 0.6 mm) and (6.0 ± 1.0 mm) against fungal strains *T. harzianum* and *A. niger*. Compound **1** and **3** showed moderate antifungal activities with inhibition value (14.0 ± 1.0 mm), (5.2 ± 1.5 mm) and (6.7 ± 1.3 mm), (5.3 ± 1.5 mm) against fungal strains *T. harzianum* and *A. niger*, respectively (Table-4). Thus, compound **2** exhibited excellent antimicrobial activity compared to the standards and may serve as a lead candidate for the development of more potent and safer drugs in the future.

Molecular docking studies: Compound **2** showed significant inhibition of both fungal receptor proteins. Compound **2** was docked with the fungal protein receptors from *T. harzianum* (PDB ID: 5JBO) and *A. niger* (PDB ID: 1UKC) to assess its potential antifungal properties. A strong interaction

TABLE-4
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE COMPOUNDS (**1-3**)

Compounds	Antibacterial activity				Antifungal activity	
	Gram-positive bacteria		Gram-negative bacteria			
	S. aureus	*B. subtilis*	*S. typhimurium*	*E. coli*	*T. harzianum*	*A. niger*
1	15.0 ± 0.6	18.0 ± 1.2	10.0 ± 0.6	17.5 ± 1.0	14.0 ± 1.0	5.2 ± 1.5
2	16.0 ± 0.5	19.3 ± 0.6	17.0 ± 0.6	19.5 ± 1.0	16.0 ± 0.6	6.0 ± 1.0
3	15.0 ± 1.0	16.0 ± 1.5	15.5 ± 0.6	13.0 ± 1.0	6.7 ± 1.3	5.3 ± 1.5
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.007
Cef	40.3 ± 0.6	50.0 ± 1.0	42.7 ± 1.5	27.5 ± 1.2	–	–
Amp B	–	–	–	–	17.7 ± 0.6	8.3 ± 0.6

The data are mean ± SD (standard deviation)

was noted between the compound and the receptors, as suggested by the values of the inhibition constant and the free energy of binding. Based on a theoretical evaluation of its antimicrobial potential, compound **2** seems to possess a strong level of effectiveness against fungi (Table-5 and Fig. 2). This relationship implies that the molecular docking findings can reliably predict biological activity, reinforcing the hypothesis that strong interactions between ligands and receptors are critical factors for antifungal efficacy.

TABLE-5
INTRAMOLECULAR INTERACTIONS OF COMPOUND **2** AND TARGET RECEPTORS

Protein PDB ID	Binding affinity (kcal/mol)	Residues with H-bond interaction	Hydrophobic interaction
1UKC	-7.5	ASN134 LEU298	LEU444(Alkyl) TRP301(Pi-Alkyl) PHE342(Pi-Alkyl)
5JBO	-7.6	TRP357 GLU339 TYR179 GLN319	TYR179(Pi-Alkyl) TYR316(Pi-Alkyl) PHE333(Pi-Alkyl) TRP357(Pi-Alkyl)

***In silico* ADMET prediction:** *In silico* ADMET prediction is playing a crucial role in drug design and development process. Two well-known rules namely (i) Lipinski's rule of five and (ii) Veber's rule are generally applied to predict the pharmacokinetic properties of a drug candidate. There are various physicochemical descriptors include two rules such as molecular weight (MW ≤ 500), number of hydrogen bond acceptors (HBA ≤ 10), number of hydrogen bond donors (HBD ≤ 5) and lipophilicity (clogP ≤ 5), number of rotatable bonds (NROTB ≤ 10) and topological polar surface area (TPSA140 Å^2). The ADMET properties of a drug candidate are significantly influenced by lipophilicity and TPSA parameters. A drug candidate can easily pass through biological membrane having reasonable lipophilicity character. Molecules having higher lipophilicity (clogP > 5) showed good adsorption and solubility in fats and lipids. Tested compounds **1-3** showed lipophilicity values greater than 5 which indicates their preference for dissolving in fats and lipids rather than water. This characteristic leads to poor aqueous solubility and complications in drug absorption. TPSA value is correlated with H-bonding and is one of the important indicators for drug oral bioavailability. The TPSA value represents low oral bioavailability when it exceeds 140 Å^2. All compounds

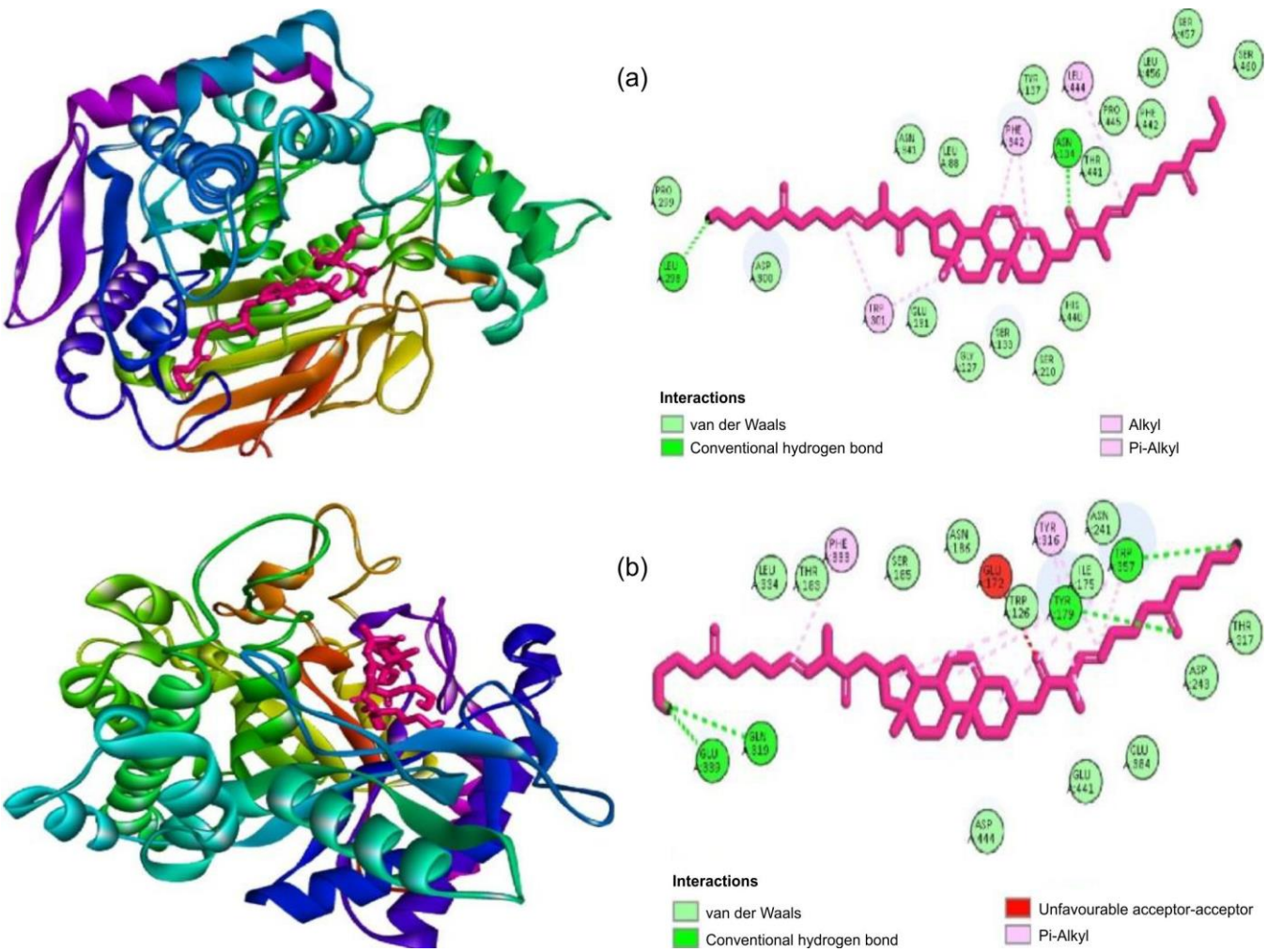

Fig. 2. 3D and 2D images of the binding mode between compound **2** with (a) *T. harzianum* (PDB ID: 5JBO) and (b) *A. niger* protein (PDB ID: 1UKC)

1-3 represent the TPSA values within the acceptable limit (114.6-133.5). The results obtained during ADME analysis are given in Table-6. Results show compounds **1-3** did not satisfy two parameters clogP ≤ 5 and NROTB ≤ 10 along with MW ≤ 500. Hence, compounds **1-3** showed moderate oral bioavailability by following partial Lipinski's rule of five and Veber's rule.

Toxicity risk factor is one of the crucial parameters in drug discovery and development process because most of the drugs could not pass in clinical trials due to their high toxicity risk factors. Generally, it is generated due to the presence of a particular group in the drug molecule. Toxicity of a drug depends on some parameters namely mutagenicity, tumorigenicity, irritancy and reproductive effects of the compounds. All the tested compounds **1-3** showed low mutagenic and tumorigenic values and moderate irritant and reproductive values. The tested compounds showed drug score ranging from 0.04-0.07. The predicted drug-likeness values of the compounds and standards are also presented in Table-7. Toxicity predictions of compounds **1-3** indicated low mutagenic and tumorigenic values, compounds **1** and **3** showing high irritancy and compound **2** displaying higher reproductive toxicity. These results indicate that the isolated compounds possess reasonable pharmacokinetic profiles but require structural optimization to improve drug-likeness and safety.

Conclusion

In this study, three new compounds **1-3** were isolated from the seeds of *Mucuna pruriens.* All the compounds were characterised by using IR, 2D, 3D, NMR and mass spectral data. *In vitro* antimicrobial activities of the isolated compounds **1-3** were assayed against two Gram-positive and two Gram-negative bacteria along with two fungal strains by using agar disc diffusion method. Antimicrobial results showed that the compounds displayed moderate to good activity against various pathogens. Compound **2** showed the highest antibacterial activities with inhibition values (16.0 ± 0.5 mm) against *S. aureus* and (19.3 ± 0.6 mm) against *B. subtilis.* Compound **2** also showed promising antifungal activities with inhibition values (6.0 ± 1.0 mm) and (16.0 ± 0.6 mm) against *A. niger* and *T. harzianum*, respectively. To validate the antimicrobial results *in silico* ADMET prediction and molecular docking studies were performed. Compound **2** showed good binding affinity ranging between -7.5 to -7.6 and a variety of strong interactions with the effective binding sites of the target receptors. Pharmacokinetic profile of the isolated compounds **1-3** showed the moderate drug oral bioavailability and may contribute to the development of a more potent drug in the future.

ACKNOWLEDGEMENTS

The authors are highly grateful to Chittagong University of Engineering and Technology, Bangladesh for providing research fund to accomplish this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

TABLE-6
PREDICTED PHARMACOKINETIC PROPERTIES OF ISOLATED COMPOUNDS (**1-3**)

Compound	Lipinski's violations (≤ 1)	Lipinski's rule				Veber's rule		logS[g]	%ABS[h]
		MW[a] (≤ 500)	HBA[b] (≤ 10)	HBD[c] (≤ 5)	clogP[d] (≤ 5)	NROTB[e] (≤ 10)	TPSA[f] (140 Å^2)		
1	1	686.0	8	4	7.63	20	133.5	-7.2	62.82
2	2	710.0	8	2	8.47	22	127.2	-7.84	65.00
3	1	662.0	8	3	9.56	20	114.6	-8.5	69.35
Cef	2	554.0	14	4	-2.95	8	287.3	-2.95	9.62
Amp B	3	924.0	17	12	0.32	3	319.6	-5.08	-1.55
AA	0	176.12	6	4	-1.40	2	107.22	-0.55	72.00

[a]Molecular weight, [b]Number of hydrogen bond acceptors, [c]Number of hydrogen bond donor, [d]Lipophilicity, [e]Number of rotatable bonds, [f]Topological polar surface area, [g]Solubility parameter, [h]Percentage of absorption.

TABLE-7
In silico TOXICITY EFFECTS AND DRUG-LIKENESS VALUES OF ISOLATED COMPOUNDS (**1-3**)

Compounds	Toxicity effects				Drug-likeness	Drug-score
	Mutagenic	Tumorigenic	Irritant	Reproductive		
1	Low	Low	High	Medium	-8.36	0.04
2	Low	Low	Medium	High	-10.33	0.07
3	Low	Low	High	Low	-16.13	0.05
Ceftriaxone	Low	Low	Low	Low	16.69	0.63
Amphotericin B	Low	Low	Low	Low	-0.14	0.18
Ascorbic acid	High	High	Low	High	0.02	0.16

REFERENCES

1. M. Rahmatullah, N.K. Azam, M. Rahman, S. Seraj, M.J. Mahal and S.M. Mou, *Am.-Euras. J. Sust. Agric.*, **5**, 350 (2011).
2. P.K. Sadh, P. Chawla and J.S. Duhan, *Food Biosci.*, **22**, 113 (2018); https://doi.org/10.1016/j.fbio.2018.01.011
3. S.O. Majekodunmi, A.A. Oyagbemi, S. Umukoro and O.A. Odeku, *Asian Pac. J. Trop. Med.*, **4**, 632 (2011); https://doi.org/10.1016/S1995-7645(11)60161-2
4. Y. Rajeshwar, M. Gupta and U.K. Mazumder, *Iranian J. Pharmacol. Therap.*, **4**, 46 (2005).
5. S.L. Johnson, H.Y. Park, N.A. DaSilva, D.A. Vattem, H. Ma and N.P. Seeram, *Nutrients*, **10**, 1139 (2018); https://doi.org/10.3390/nu10091139
6. R.E. Laviada-Castillo, M.R. Segura-Campos, I. Chan-Zapata, J.C. Torres-Romero, J.L. Guillermo-Cordero and V.E. Arana-Argáez, *J. Food Biochem.*, **43**, e12834 (2019); https://doi.org/10.1111/jfbc.12834
7. M.A. Jimoh, O.A. Idris and M.O. Jimoh, *Plants*, **9**, 1249 (2020); https://doi.org/10.3390/plants9091249
8. T. Dhanani, R. Singh, S. Shah, P. Kumari and S. Kumar, *Green Chem. Lett. Rev.*, **8**, 43 (2015); https://doi.org/10.1080/17518253.2015.1075070
9. G. Shanmugavel and G. Krishnamoorthy, *Int. J. Herb. Med.*, **2**, 7 (2015).
10. A.K. Rayavarapu and D.S. Kaladhar, *Asian J. Biochem. Pharm. Res.*, **12**, 593 (2011).
11. N.H. Tan, S.Y. Fung, S.M. Sim, E. Marinello, R. Guerranti and J.C. Aguiyi, *J. Ethnopharmacol.*, **123**, 356 (2009); https://doi.org/10.1016/j.jep.2009.03.025
12. V.B. Mutwedu, R.B.B. Ayagirwe, S.B. Bacigale, L.M. Mwema, S. Butseme, T. Kashosi, B. Mitima, G.J. Manyawu and A.W. Nyongesa, *Trop. Anim. Health Prod.*, **51**, 1195 (2019); https://doi.org/10.1007/s11250-019-01808-2
13. J.S. Ashidi, F.O. Owagboriaye, F.B. Yaya, D.E. Payne, O.I. Lawal and S.O. Owa, *Heliyon*, **5**, 2716 (2019); https://doi.org/10.1016/j.heliyon.2019.e02716
14. Y. Lin, W.-J. Tsai, I.-S. Chen and Y.-H. Kuo, *J. Chin. Chem. Soc.*, **45**, 213 (1998); https://doi.org/10.1002/jccs.199800035
15. A.J. Demuner, L.C.A. Barbosa, J.C. Nascimento, J.J. Vieira and M.A. Santos, *Quim. Nova*, **26**, 335 (2003); https://doi.org/10.1590/S0100-40422003000300009
16. L.T. Renata, S.S. Alexandre, R.N.C. Ana, R.P.S. Alexandre and S.A. Jailane, *Afr. J. Biotechnol.*, **14**, 676 (2015); https://doi.org/10.5897/AJB2014.14354
17. D. Das, S. Das, M. Pandey and D. Bhattacharyay, *Eur. J. Med. Plants*, **31**, 19 (2020); https://doi.org/10.9734/ejmp/2020/v31i430226
18. L. Misra and H. Wagner, *Phytochemistry*, **65**, 2565 (2004); https://doi.org/10.1016/j.phytochem.2004.08.045
19. J.B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Dordrecht, Netherlands: Springer, edn 3 (1998).
20. M. Singh, I.P. Kumhar and M. Salim, *J. Med. Plants Stud.*, **10**, 103 (2022).
21. A.W. Bauer, W.M. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45(4_ts)**, 493 (1966); https://doi.org/10.1093/ajcp/45.4_ts.493
22. D.S. Goodsell, M.F. Sanner, A.J. Olson and S. Forli, *Protein Sci.*, **30**, 31 (2021); https://doi.org/10.1002/pro.3934
23. H.M. Berman, *Nucleic Acids Res.*, **28**, 235 (2000); https://doi.org/10.1093/nar/28.1.235
24. O. Trott and A.J. Olson, *J. Comput. Chem.*, **31**, 455 (2010); https://doi.org/10.1002/jcc.21334
25. M.M. Alnoman, S. Parveen, R.B. Alnoman, A. Khan, M.M. Khaleil, M. Jaremko, I. Al-Younis and A.-H. Emwas, *J. Mol. Struct.*, **1307**, 138021 (2024); https://doi.org/10.1016/j.molstruc.2024.138021