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ANTICANCER AND ANTIOXIDANT ACTIVITY OF PISCIDINOL-N, APOTIRUCALLANE TRITERPENE DERIVED FROM THE LEAVES OF *WALSURA TRIFOLIATA*

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ABSTRACT
Piscidinol - N, a new apotirucallane type terpenoid, isolated from the leaves of *Walsura Trifoliata* along with some known compounds. The new compound structure is determined by comprehensive spectroscopic (particularly 2D NMR and HRESI-Mass) investigation. On the basis of docking studies, new compound was screened for its antioxidant and anticancer activities. The docking study results of novel compound bioassay studies have shown higher binding affinity for the selected activities.

KEYWORDS: *Walsura trifoliata*, Piscidinol N, Anticancer activity, Antioxidant activity.

1.0 INTRODUCTION

On a worldwide platform, cancer is the leading cause of disease and mortality. Currently, there are over a hundred different forms of cancer, each with its own aetiology and natural history. Breast and ovarian cancers are the most common cancers in women, with breast and ovarian cancers being the most common. Because of the low selectivity of chemotherapeutic agents and the limited success of clinical therapies such as radiation, immunomodulation, and surgery, as evidenced by high morbidity and mortality rates, an urgent need for new and better therapeutic resources, including new chemotherapeutic agents, for cancer management is justified and timely.[1,2] Various anticancer research techniques, target mechanisms, and candidate drugs created against specific targets were summarised by Jayashree et al.[3]

Even normal metabolism can produce free radicals, or reactive oxygen species (ROS), which can cause substantial damage to cells and tissues. These reactive species are implicated in oxidative stress-related disorders such as heart disease, neurological disease, cancer, and the ageing process.[4] It has been suggested that active oxygen contributes to carcinogenesis through inducing gene alterations as a result of cell damage, as well as its effects on signal transduction and transcription factors.[3,5] All biomolecules, including DNA, phospholipids, proteins, and carbohydrates, are damaged by oxidative stress. Antioxidants are substances that operate as radical scavengers, preventing oxidation's radical chain reactions, and are known for their beneficial effects in boosting health and reducing the risk of cancer, hypertension, and heart disease.[6] Spices and herbs are known to be high in antioxidant components such phenols, flavonoids, tocopherols, ascorbic acid, and carotenoids, all of which have been shown to have antioxidant action.[7]

The genus *Walsura Roxb.* (Meliaceae), has proven to be a rich source of limonoids and triterpenoids with promising biological activities.[8] *Walsura trifoliata* (*synonym: Walsura piscidia Roxb*.) is an evergreen tree distributed widely in the tropical areas of Asia, such as Southern China, India, Malaysia, and Indonesia.[9] The plant bark is used as astringent to treat diarrhea and other diseases.[10] The triterpenoids are by far the most abundant metabolites of this plant and have been shown to possess antifeedant activity against important insect pests.[11] Previous chemical investigations on this plant have afforded a series of tirucallane and apo-tirucallane triterpenoids[12,13], we collected the twigs and leaves of *W. trifoliata* from Tirumala hills of chittor district and conducted detailed phytochemical study of its chemical constituents.

The apotirucallane triterpenoids discovered from the leaves of *W. trifoliata and* these possess anticancer activity. These structurally diverse and physiologically fascinating compounds have piqued our curiosity in continuing to investigate this plant's

phytochemistry.[14] Based on the research into the elements of this plant led the isolation of a novel apotirucallane triterpenoid named as Piscidinol N (1), (Fig. 1) along with some known compounds. The extraction, isolation, and structural characterization of novel apotirucallane triterpenoid, as well as its anticancer and antioxidant docking studies, are described in this work.

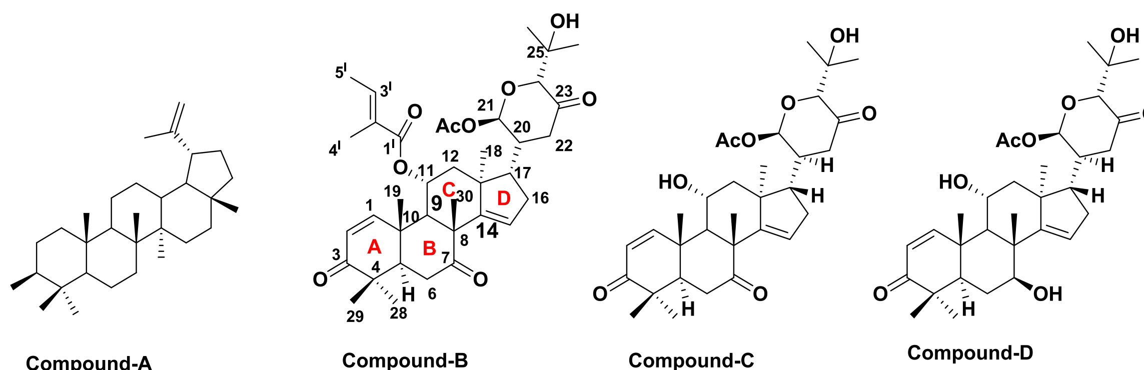


Fig 1: Isolated compounds from Walsura trifoliate.

2.0 MATERIALS AND METHODS

2.1 Plant material

Walsura trifoliata plant material was gathered from the evergreen forest of the Seshachalam hills range near Tirupati, chittor district, Andhra Pradesh, India. Dr. K. Madhava Chetty verified it, and a voucher specimen (specimen # SVU-KM-261) was deposited in the Botany department's herbarium at Sri Venkateswara University in Tirupati, Andhra Pradesh, India.

2.2 Extraction and isolation

Walsura trifoliata leaves (2 kg) were air-dried and milled into powder before being extracted with 95 percent MeOH in cold soxhlet extraction. Filtered the MeOH layer, distilled the layer under plant vacuum. The crude extract was partitioned between water and chloroform, the chloroform fraction was evaporated under plant vacuum. The crude portion (15g) was then performed to column chromatography on a silica gel column (60-120 mesh, 150 X 15 cm) and eluted with a step-wise hexane/EtOAc (93:7, 90:10, 85:15, 80:20, 70:30 by volume) gradient to yield 95 fractions of 30 ml each.

TLC (silica gel 60 F_{254}, hexane/EtOAc, 85:15) was used to examine column fractions, and fractions with similar TLC patterns were aggregated to form five significant fractions (F1, F2, F3, F4 and F5). F_1 fraction was again subjected to the column chromatography with hexane/EtOAc (100:0 to 90:10) to get 35 sub fraction [F1a (1-18), F1b (19-25) & F1c (26-35)], no compounds were isolated from F1a & F1b. F1c fraction (26-35) was again performed the column chromatography with hexane/EtOAc (94:6 to 90: 10) to get 27 fractions, [B_1(1-5), B_2 (6-13) & B_3 (14-29)] obtained. Sub fractions B1 did not shown compounds in TLC analysis. B2 sub fractions have shown some compounds in TLC and were subjected to column chromatography (CC) on a silica gel (100-200 mesh) and eluted with a hexane/EtOAc (96:4) to get **compound-A** (10 mg). B3 sub fractions have also shown some compounds in TLC and were subjected to column chromatography (CC) on a silica gel (100-200 mesh) and eluted with a hexane/EtOAc (95:5) to get **compound-B** (5 mg)**.**

F_2 fraction was subject to column chromatography with hexane/EtOAc (96:4 to 90:10) to get 23 sub fractions [F2a (1-15) & F2b (16-23)]. F2a has not shown compounds in TLC analysis. F2b (16-23) have shown some compounds in TLC and sub fractions were subjected to column chromatography (CC) on a silica gel (100-200 mesh) and eluted with a hexane/EtOAc (88: 12) to get **compound-C** (7 mg).

F_3 fraction was subjected to column chromatography with hexane/EtOAc (90:10 to 85:15) to get 15 sub fraction [F3a (1-9) & F2b (10-15)]. F3a has not shown compounds in TLC analysis. F3b (10-15) sub fractions have shown some compounds in TLC and were subjected to column chromatography (CC) on a silica gel (100-200 mesh) and eluted with a hexane/EtOAc (85: 15) to get **compound-D** (8 mg).

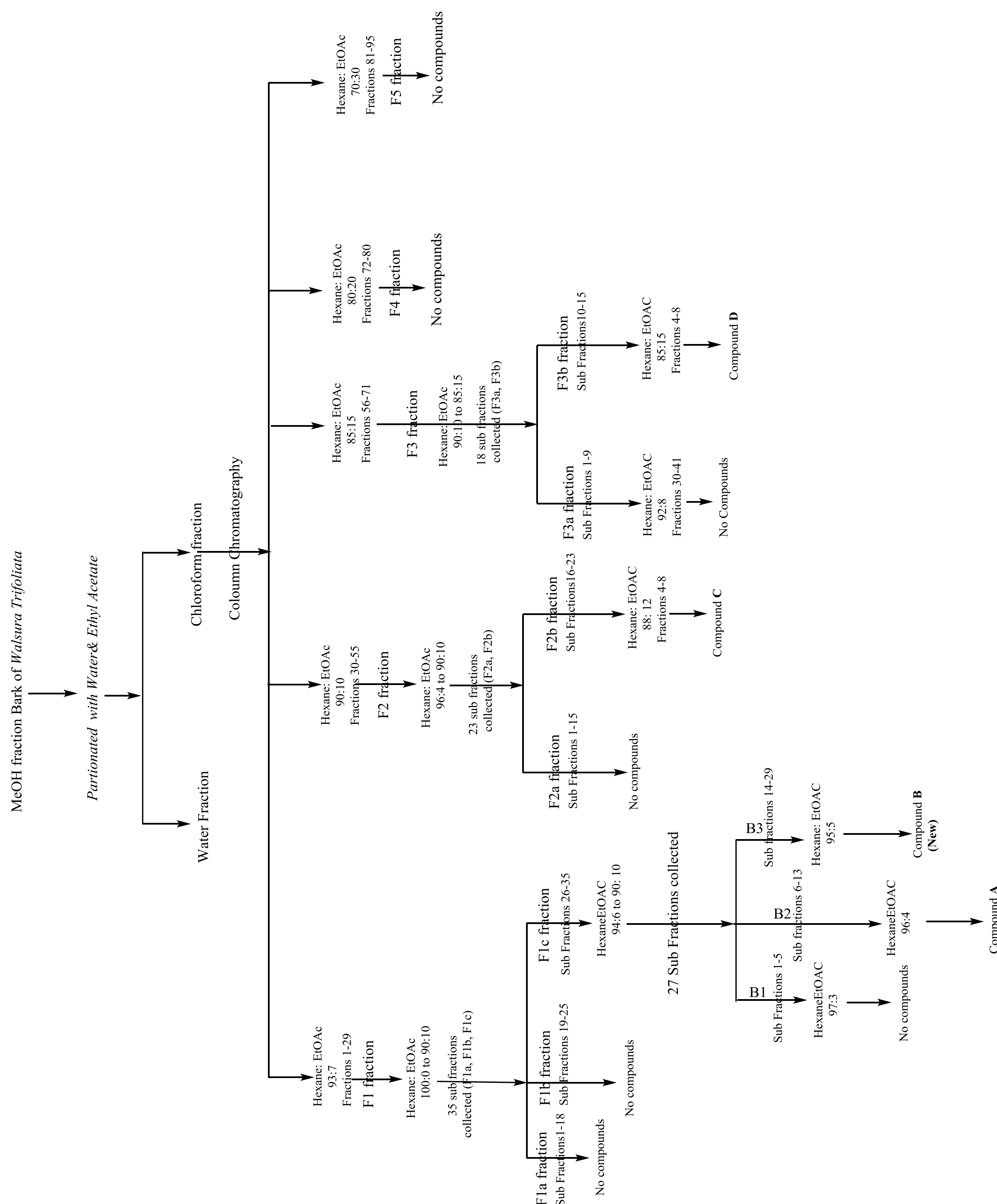


Fig (2): Schematic diagram for isolation of compounds from *Walsura trifoliata* leaves.

2.3 Characterization studies

Optical rotations were measured using a JASCO DIP 300 digital Polarimeter and at 1 ml cell at 25^{0}C. IR spectra were recorded on a Nicolet-740 spectrometer with KBr pellets. The NMR spectra were recorded on a Bruker FT-300 MHz spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively, using TMS as internal standard. The coupling constants (J) are supplied in hertz (Hz) and the chemical shifts are expressed as d values in parts per million (ppm).

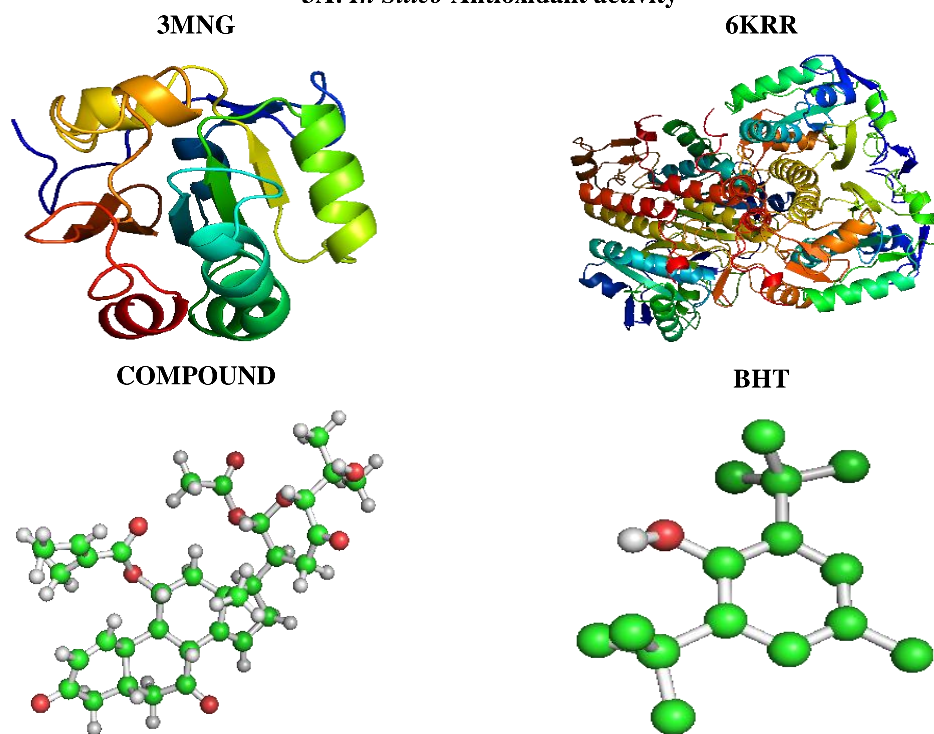
An LC-MS/MS (Agilent Technologies 6510) Q-TOF Mass spectrometer was used to collect mass spectra. Standard Bruker microprograms were used for the 2D experiments (1H-1H COSY, HSQC, HMBC, and NOESY). A silica gel column was used for chromatography (100-200 mesh, ACME make, 230-400 mesh Sigma-Aldrich). A Phenomenex Luna C18 (250 X 10 mm 10 μ) column was used in semi-preparative HPLC on an Agilent 1100 series LC/MSD Trap SL. On a Dionex P680 equipped with a PDA detector and a Shimadzu PRC- ODS (K) column, preparative

HPLC was performed: SB Zorbax (C18, 9.4 X 50 mm, 5 µ), Analytical TLC was carried out on pre-coated Merck plates (60 F254, 0.2 mm) using the solvent system EtOAc-hexane (50:50), and compounds were viewed under a UV lamp and sprayed using 10% H_2SO_4 followed by heating.

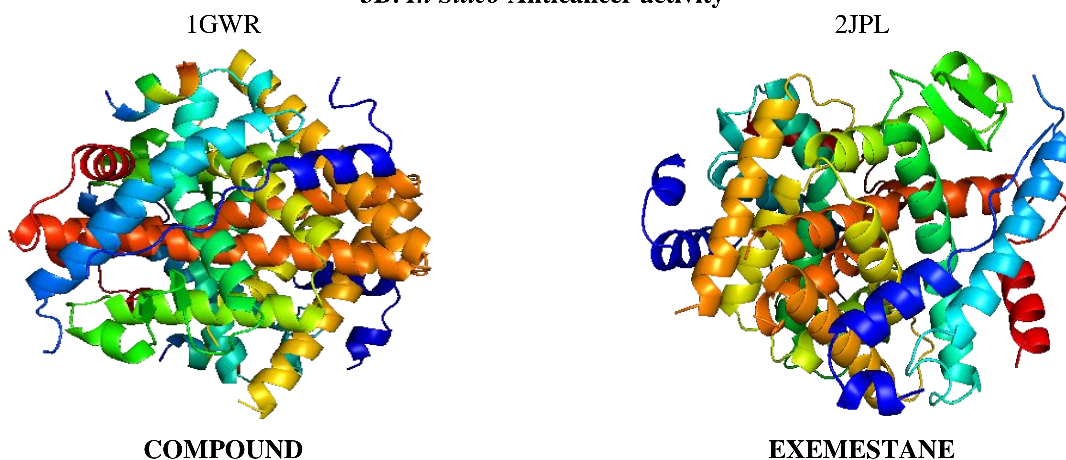
2.4 Anticancer and antioxidant activity by Molecular docking

The three-dimensional structures of Peroxiredoxins (PDB ID: 3MNG and 6KRR) and Human estrogen receptor such Alpha (HERA) (PDB ID: 1GWR and 2JPL) was downloaded from the RCSB protein Data Bank**.** The atomic coordinates of the protein was estranged and geometry optimization was done using Argus Lab 4.0.1 (Morris et al., 2009).[15] The ligands such as Butylated hydroxytoluene (BHT), Exemestane and Compound were converted into Pdbqt file format and atomic coordinates were generated using Pyrx 2010.12. The active binding sites of target protein were analyzed using the Drug Discovery Studio version 3.0 and 3D Ligand Site virtual tools (Ter Haar et al., 2001).[16] The structures of Target proteins and Reference compound were shown in figure 3(A & B).

3A: *In Silico* Antioxidant activity



3B: *In Silico* Anticancer activity



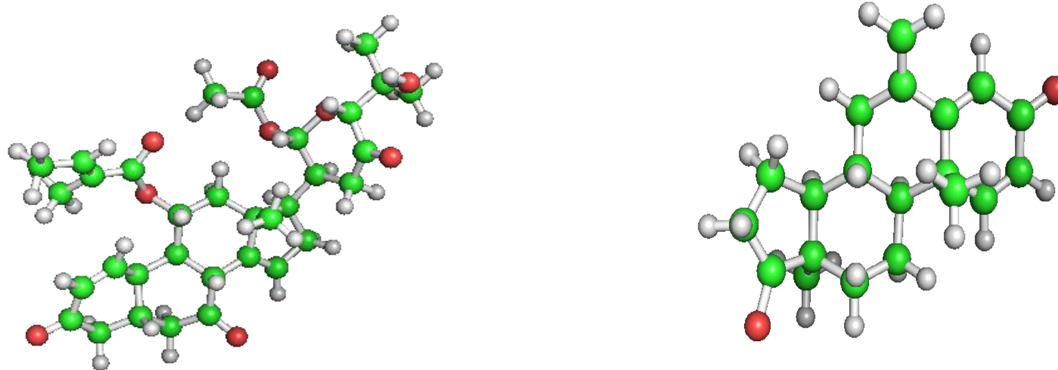


Fig. 3: 3D structures of Target proteins and Ligands.

Molecular docking studies were carried against all the target proteins (3MNG, 6KRR, 1GWR and 2JPL) with ligands such as Compound, BHT and Exemestane using the docking module implemented in Pyrx 2010.12. Flexible docking was employed, the inhibitor binding site residues were softened and highlighted through the "Site Finder" module implemented in the Pymol software. The grid dimensions were predicted as X: 28.27, Y: 27.13, Z: 28.51 for all the target proteins respectively. The docking was carried out with the default parameters *i.e.,* placement: triangle matcher, recording 1: London dG, refinement: force field and a maximum of 10 conformations of each compound were allowed to be saved in a separate database file in a .mdb format. After the docking process, the binding energy and binding affinity of the protein-ligand complexes interactions were calculated using Pymol viewer tool *(www.pymol.org).*

3.0 RESULTS AND DISCUSSION

3.1 Isolation and structure elucidation of new compound

The concentrated chloroform extract of *Walsura trifoliata* plant was chromatographed on silica gel, and the fractions were subjected to repeated column chromatography, resulting in the isolation of new compound (Pisicidinol - N / Compound - B), this structure was conformed based on IR, HRESI-MS, 1D and 2D NMR (HSQC, HMBC, COSY, and NOESY) spectroscopic methods. The other 3 known compounds[17] were identified as Lupeol (compound-A), Piscidinol-H (Compound-C) & Piscidinol-C (Compound-D) from mass, ^{1}H NMR & ^{13}C NMR data.

Compound **B** is a pale yellow solid that was isolated. The absorptions of hydroxyl (3444cm^{-1}), α,β-unsaturated carbonyl (1668cm^{-1}), and ester (1719cm^{-1}) functionalities were evident in the IR spectra of compound **B**. HRESIMS determined the molecular formula as $C_{37}H_{51}O_9$, with a molecular ion peak at m/z 639.3559 $[M+H]^+$ (calcd *m/z* at 639.3533). The ^{1}H NMR spectra of 1 in $CDCl_3$ that seven methyl groups resonating at δ_H 1.11, 1.21, 1.24, 1.25, 1.26, 1.31, and 1.32, (3H each, s) in (Table 1), which correlated with the carbons at δ_C 21.6, 28.5, 25.7, 19.8, 30.0, 29.1, 20.1, 29.1, and in the HSQC spectrum, were ascribed to methyl groups. The occurrence of two well separated doublets at δ 7.02 (1H, d, *J* = 9.9 Hz), 5.61 (1H, d, *J* = 9.9 Hz) indicates a endo-cyclic double bond connection to the ketone at A ring, while another proton resonating at *δ* 5.56 (s, H-15) was ascribed the proton on double bond at D-rind. Moreover, additional proton signals appeared at [δ 1.93 (3H, s), 1.90 (3H, d, *J* = 7.1 Hz), 6.94 (1H, dd, *J* = 6.9 &13.1 Hz)] was tigloyl group in compound-B. In addition, analysis of the ^{1}H NMR spectrum (CDCl3) (Table 1) indicated presence of acetyl group δ 2.23 (3H, s)], one oxymethine proton 3.93 (1H, brs), one tri-substituted olefin [5.51 (1H, brs)], and acetal ring [H-20 (δ_H 2.18-2.20 (1H, m), H-21 (δ_H 6.22, (1H, brs), and H-24 (δ_H 3.93, 1H, brs)] were identified in the ^{1}H NMR spectrum (CDCl3) (Table 1). The ^{13}C NMR spectrum displayed 37 carbon resonances, and DEPT and HSQC tests categorized the carbon resonances in the ^{13}C NMR spectrum as 10 methyls, 4 methylenes, 11 methines, and 7 quaternary carbons including 5 carbonyl carbons.

All of the following spectroscopic evidence, as well as comparisons to earlier literature, pointed to **compound-B** being an apotirucallane triterpene with a tetracyclic core and acetal ring.

When the ^{1}H and ^{13}C NMR spectroscopic data of **Compound-B** (Table 1) were compared to those of Piscidinol-H[18], it was clear that their structures were quite similar, with the exception of the presence of tigloyl group [δ 1.93 (3H, s), 1.90 (3H, d, *J* = 7.1 Hz), 6.94 (1H, dd, *J* = 6.9 &13.1 Hz)] at C-11 (C-ring) rather than an hydroxyl group. This was confirmed by the presence of a signal at high deshilding position δ 5.71 (1H, d, *J* = 7.9 Hz) compare to the Piscidinol-H at δ 4.51 (1H, t, *J* = 6.8 Hz) (ref), tigloyl position at 11th position at C-ring was confirmed based HMBS study. Using COSY (Fig. 4), HMBC (Fig. 5) NOESY (Fig. 6), studies, all protons and carbon resonances of compound-B are completely assigned.

HMBC (Fig. 3) correlations of H-11 [δ 5.71 (1H, d, J=7.9 Hz)/C-11 (δ 166.6), C-9 (δ_C 40.5), C-10 (δ_C 40.3) indicate the of tigloyl group position at 11th carbon. And Further, HMBC correlations from H-2 (δ 5.61, d, *J* = 9.9 Hz)/C-3 (δ_C 203.2) is indicate the endocyclic double bond connection to the keto group at A ring. H-6 (δ 1.83-1.87, m,)/C-7 (δ_C 208.6), C-4 (δ_C 43.4) is indicate the carbonyl position at C-7 which is neighbor to the 6th carbon of B-ring. Tri-substituted olefin position was confirmed by the correlations from H-15 (δ 5.56, 1H, brs)/C-16 (δC 31.6), C-14 (δ_C 170.2).

The existence of six-membered acetal ring was also proved by the presence of HMBC correlations H-21 (δ 6.22, 1H, brs)/C-24 (δ_C 71.8), C-20 (δ_C 41.8); H-20 (2.18-2.20, 1H, m)/C-22 (δ_C 36.2), C-23 (δ_C 208.9); H-24 (δ 3.93, 1H, brs)/C-25 (72.0), C-23 (δ_C 208.9); H-24 (δ 3.93, 1H, brs)/C-25 (δ_C 72.0),C-23 (δ_C 208.9). In addition, the HMBC correlations of H-17 (δ 1.69-1.66, 1H, m) with C-20 (δ_C 41.8), C-21 (δ_C 91.3), and C-15 (δ_C 118.2) indicated a link between the tetracyclic moiety and the acetal ring.

Table 1: ^{1}H and ^{13}C NMR data of compound (in $CDCl_3$, 300 MHz and 75 MHz)

S. No	1H NMR data	13C NMR data	HMBC correlations
1	7.02 (1H, d, *J* = 9.9 Hz)	157.9	C-2
2	5.61 (1H, d, *J* = 9.9 Hz)	124.1	C-1 &C-10
3	----	203.2	----
4	----	43.4	----
5	2.62 (1H, dd, *J* = 2.4, 9.3Hz)	45.1	C-7 &C-29
6	1.83-1.87 (2H, m)	23.9	C-7 &C-29
7	----	208.6	----
8	----	45.3	----
9	2.31-2.28 (1H, m)	40.5	C-11
10	----	40.3	----
11	5.71 (1H, d, *J* = 7.9 Hz)	70.1	C12 & C-13
12	1.80-1.85 (2H, m)	42.8	C-11
13	----	39.9	----
14	----	170.2	----
15	5.56 (1H, brs)	118.2	C-17
16	2.03-1.99 (1H, m)	31.6	C-15 & C-17
17	1.69-1.66 (1H, m)	56.1	C-21
18	1.25 (3H, s)	19.8	C-13 & C-17
19	1.31 (3H, s)	20.1	C-10
20	2.18-2.20 (1H, m)	41.8	C-23
21	6.22 (1H, s)	91.3	C-20 & Acetyl carbonyl
22	2.45-2.49 (2H, m)	36.2	C-23
23	----	208.9	----
24	3.93 (1H, brs)	71.8	C-23
25	----	72.0	----
26	1.32 (3H, s)	29.1	C-24
27	1.26 (3H, s)	30.0	C-24
28	1.11 (3H, s)	21.6	C-3
29	1.24 (3H, s)	25.7	C-3
30	1.21 (3H, s)	28.5	C-7 & C-14
CO	----	168.4	
CH3	2.23 (3H, s)	21.1	Acetyl carbonyl
11	----	166.6	----
21	----	127.9	----
31	6.94 (1H, dd, *J* = 6.9, 13.1 Hz)	139.1	C-4' & C-1'
41	1.93 (3H, s)	11.9	C-5'
51	1.90 (1H, d, *J* = 6.9 Hz)	15.2	C-3'

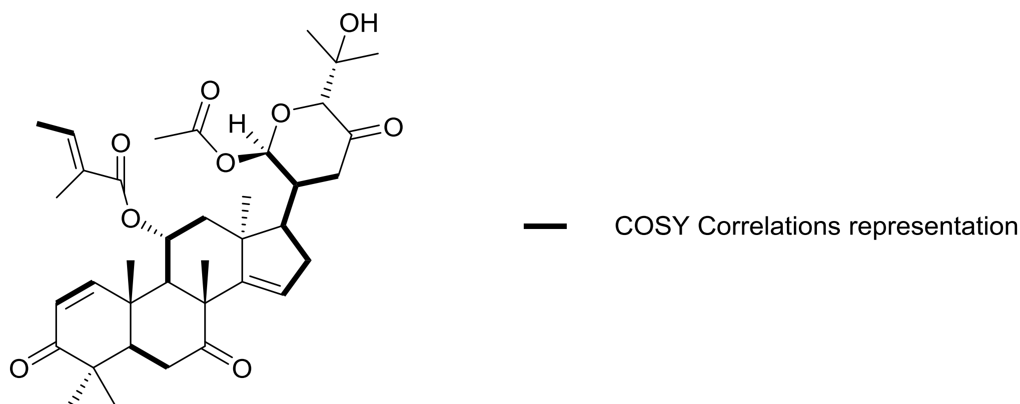


Fig 4: Key COSY correlations of Compound-B.

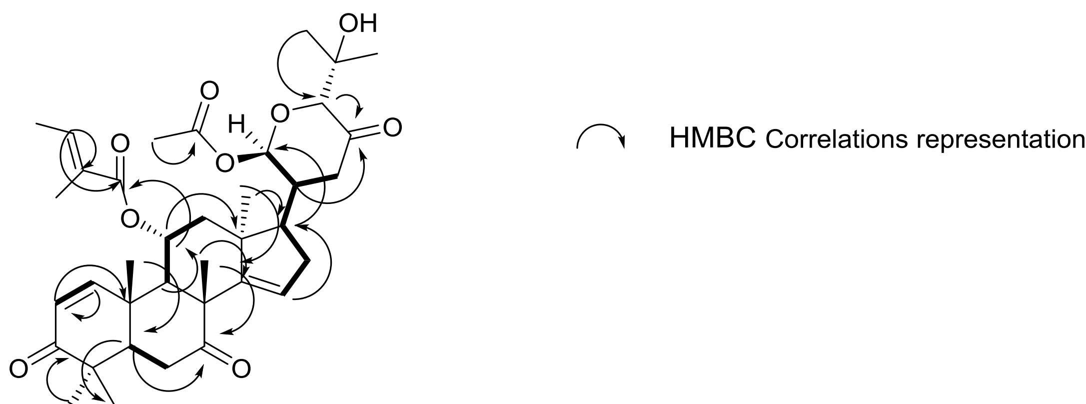


Fig 5: Key HMBC correlations of Compound-B.

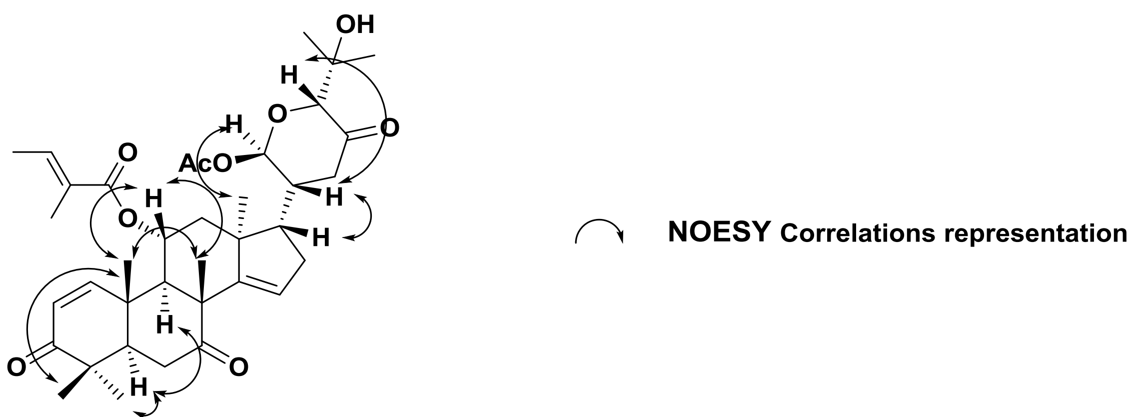


Fig 6: NOE correlations of Compound-B.

The biogenetic consideration and interpretation of NOE data were used to define for **compound-B** relative configuration (Fig. 6). The basic apotirucallane part (A, B, C and D rings) of compound-B was believed to have the same configuration as previously isolated apotirucallane triterpenes from *W. piscidia* with the same skeleton (ref). The NOE correlations between H_3-19/H-11 and H_3-30/H_3-19 are shown in Fig. 7 (Diagnostic NOE correlations from 3D diagram), demonstrating that these protons were co-facial and assigned to the β-orientation, as previously described for apotirucallanes. The α-orientation of H-5, H-21, H_3-28, and H-20 was determined by NOE correlations between H-5/H_3-28 and H-20/H-21. The structure of **Compound-B** was confirmed as an apotirucallane triterpenoid based on these findings, and it was given the name **Piscidinol - N**.

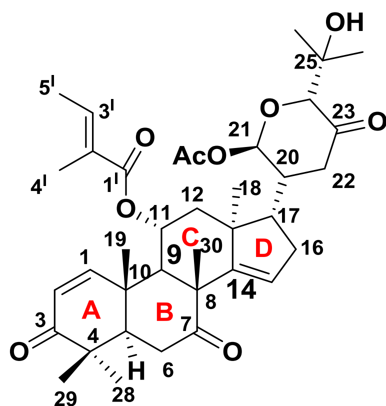


Fig 7: Piscidinol-N.

3.2 *In silico* antioxidant and anticancer activity

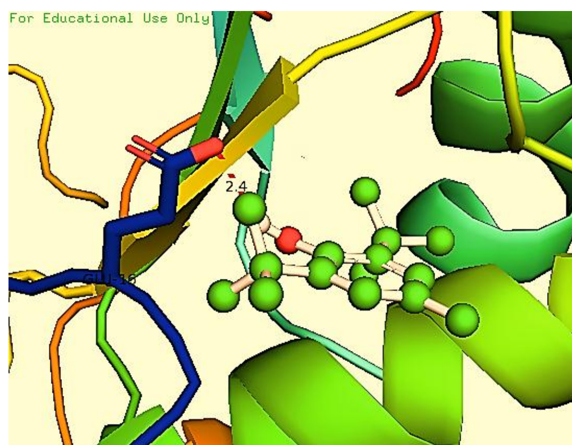
***In silico* studies**

The docking study results of the ligands have shown significant binding modes against all the target proteins. The results revealed that the compound has exhibited higher dock scores than both the reference compounds against *in silico* antioxidant and anticancer target proteins. The H-bonds, binding affinities and energy profiles of compound and reference compounds towards the active site amino acids of the enzyme are summarized in Table 2 and their 3D modelled interactions of the lead title compounds with HERA protein were shown in Figure 8. Hence, the present investigation demonstrate that the synthesised compound will be the promising next generation chemotherapeutic drug, which can be effectively used in the treatment of cancer and other related disorders. The compound and exemestane have formed hydrophobic interactions with 2JPL protein.

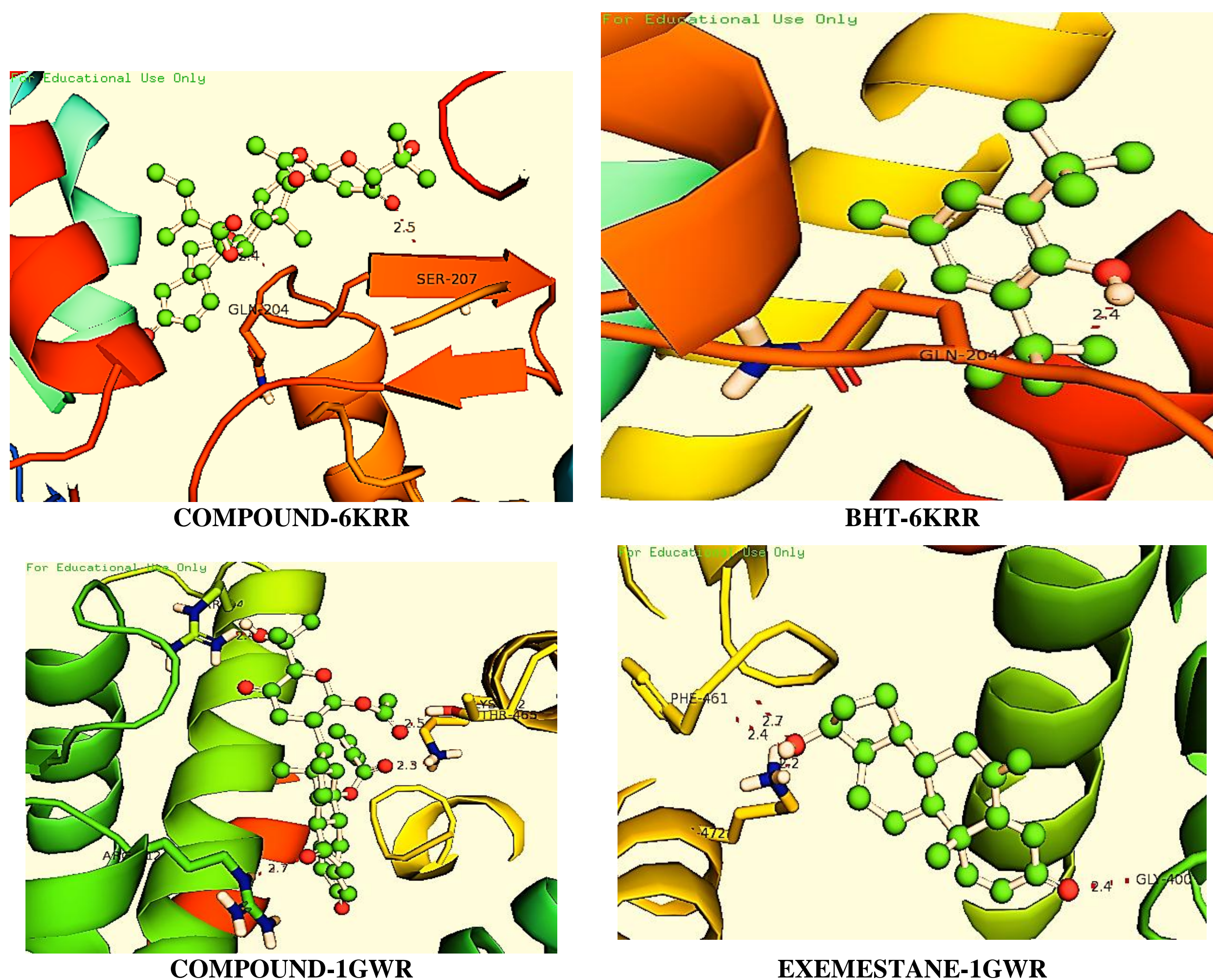
Table 2: Bonding characterization of ligands against target proteins.

Name	Tareget	BE	Binding interaction	Bond Length (Å)	Bond Angle (°)	Bond Type
***In silico* antioxidant activity**						
Com	3MNG	-7.0	Lys 126 CZ ….OC Val 142 CZ ….OC	2.0 2.3	89.1 107.9	H-don H-don
BHT	3MNG	-5.6	Glu 16 CA ….HO	2.4	91.9	H-don
Com	6KRR	-8.2	Gln 204 CB ….CO Ser 207 CA ….HO	2.4 2.5	118.6 103.7	H-acc H-don
BHT	6KRR	-5.7	Gln 204 CZ ….HO	2.4	106.0	H-don
***In silico* anticancer activity**						
Com	1GWR	-8.4	Thr 165 CB ….OC Lys 472 CA ….OC Arg 412 CA ….OC Arg 436 CZ ….OH	2.5 2.3 2.7 2.4	136.3 127.8 110.0 106.0	H-don H-don H-don H-acc
EXE	1GWR	-7.2	Gly 400 CB ….OC Lys 472 CA ….OH Phe 461 CB ….OH Phe 461 CB ….OH	2.4 2.2 2.7 2.4	106.1 86.0 90.8 104.5	H-don H-acc H-acc H-acc
Com	2JPL	-8.1	Gln 486 CA ….OC	2.4	96.1	H-don
EXE	2JPL	-7.3	Arg 485 CZ ….OC	2.2	131.7	H-don

COMPOUND-3MNG



BHT-3MNG



COMPOUND-6KRR **BHT-6KRR**

COMPOUND-1GWR **EXEMESTANE-1GWR**

Fig 8: 3D Modelled interactions of the lead title compounds with HERA protein.

4.0 CONCLUSION

In conclusion, we explored *W. trifoliata* leaves in our pharmacological-phytochemical integrated investigations on Meliaceae family & Walsura genus, which resulted in the identification of new apotirucallane triterpenoid compound called Piscidinol-N. The presence of the compound in *W. trifoliata* demonstrates the utility of these metabolites as Meliaceae family chemotaxonomic indicators. Molecular docking studies of the Piscidinol-N against all the target proteins revealed that the compound showed higher binding affinity than the reference ligands. Hence, the Piscidinol-N can be considered as promising therapeutic candidates for further optimization and development of potential anticancer and antioxidant drugs in near future.

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