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Anti-Dengue Viral Compounds from *Andrographis paniculata* by Insilico Approach

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Abstract: The present study deals with *the in silico docking* efficiency of dengue viral protein NS5, with the andrographolide and 14deoxy11oxoandrographolide, carried out using AutoDock software. In the first phase of the study andrographolide was purified from *Andrographis paniculata*, a well-known siddha plant and second phase deals with inhibitory effect with NS5 using *in silico* analysis. Andrographolide showed higher binding energy (-5.66 kcal/mol) than the standard drug, febuxostat (-6.26 kcal/mol). The andrographolide showed six interactions similar to febuxostat in number. Whereas 14deoxy11oxoandrographolide showed higher binding energy (-7.37kcal/mol) than the both drug candidates with only 5 interactions of amino acid. These results clearly indicate that andrographolide and 14deoxy11oxoandrographolide have binding interactions with NS5. Further investigations on the andrographolide, 14deoxy11oxoandrographolide compound and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of dengue.

Key words: Andrographolide · 14-Deoxy 11-Oxoandrographolide · Dengue · Ns5protein · Molecular Docking

INTRODUCTION

Drug lead screening using the *in silico methods* have been an active area of research for many years, due to the tedious and expensive nature of experimental screening procedures [1, 2]. These *in silico* methods evaluate the drugs based on their drug likeliness, ADME properties, structural complexity, functional group modified, the charges and interactions on the surfaces. The drug receptors are proteins located at the membrane or cytoplasm and involved in chemical signaling between and within cells to carry out the vital functions. The ligands are molecules especially, to bind the receptors to inhibit or antagonist their activities. A high throughput virtual screening by molecular docking can be used nowadays in screening millions of compounds rapidly, reliably and cost effectively [3]. There is a wide range of software packages available for conducting molecular docking simulations such as Auto Dock is the most recent version and has been widely used for virtual screening, due to its enhanced docking speed. Its default search function is based on Lamarckian Genetic Algorithm, a hybrid genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy. Each docking is comprised of multiple independent executions of LGA and a potential way to increase its performance in parallelizing the aspects for execution.

In recent days, dengue virus has re-emerged and as an endemic, more than 110 Countries worldwide and it has been the most prevalent arthropod-borne viral diseases in terms of morbidity and mortality [4].The dengue virus is found as four known strains Type I, II, III and IV (DEN-1, DEN-2, DEN-3 and DEN-4). Of these, the relatively milder strains include type-I, which is known as the classic dengue fever and type-III that causes high-grade fever without shock. Till now, there is no drug existing to completely curing the dengue fever. Nilavembu Kudineer Choornum (NKC) a siddha formulation for curing the dengue fever has been

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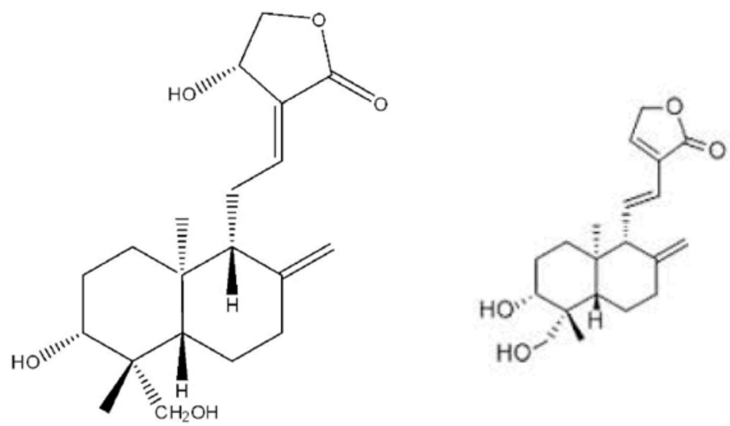


Fig. 1: Andrographolide and 14 deoxy 11 oxoandrographolide are main active compounds of the *A. paniculata,* play a major role in Nilavembu kudineer chooranam, an anti-dengue siddha formulae.

announced by Tamilnadu Govt. as anti- dengue remedy and accepted by Union Health Ministry. NKC is a combination of *Andrographispaniculata* (Seemainilavembu), *Vetiveriazizanoides* (Vettiver) *andropogonmuricatus* (Vilamicchaver), *Santalum album* (Sandhanam), *Trichosanthescucumerina (Peipudal)*, *Cyperusrotundus* (Koraikizahngu), *Zingiberofficialis* (Chukku), *Piper longum* (Milagu) and *Mollugocerviana* (Parapadkam).

NS5 Protein: The DENV NS5 protein is a 900 residue peptide with a methyltransferase domain at its N-terminal end (Residues 1-296) and a RNA-dependent RNA polymerase (RdRp) at its C-terminal end (Residues 320–900). The methyltransferase domain consists of α/β/β sandwich flanked by N-and C-terminal sub-domains. The DENV RdRp is similar to other RdRps containing palm, finger and thumb subdomains and a GDD motif for incorporating nucleotides [4].

Andrographis paniculata is a plant that is native to South Asian countries such as India and Sri Lanka. The leaf and underground stems are used to make medicine. *A.paniculata* is a potent drug used in Ayurveda, Siddha and Homoeopathy in many formulations and is effective in the treatment of various diseases like malaria, diabetes, viral hepatitis, cirrhosis, liver cancer, etc. It stimulates the immune system and improves the blood cell counts in people with HIV also and helps in anti-allergy treatment. *A.paniculata* is also used as antibacterial, antiviral agent. It also acts as a painkiller and is used for the treatment of worms. *Andrographolide* is a labdane diterpenoid that is the main bioactive component of the medicinal plant *Andrographis paniculata*. Andrographolide is an extremely bitter substance extracted from the stem and leaves of *A. paniculata*. It is a major active constituent of *A.paniculata* [5]. The present study focuses to confirm the presence of andrographolide in anti-dengue active plant, *A. paniculata* using chromatography techniques and its inhibitory activity on NS5 protein on *in silico* method. Synthetic modification of andrographolides and anti-dengue effect of NKC will be carried out to get better leads.

MATERIALS AND METHODS

Extract and Isolation Andrographolide: 150 g of dried leaf powder of *A. paniculata*was taken with 800 ml of methanol and the extraction was carried out in Soxhletapparatus [7]. The obtained extract was concentrated using distillation apparatus to get crude extract (12.8 g).A charcoal treated methanolic extract (11 g) was subjected to column chromatography on silica gel (Merck, 60:120 mesh). Then the column was eluted with mixtures of hexane and ethyl acetate, in increasing polarity to obtain thirty fractions and andrographolide was eluted in the fractions 16-25 in the ratio of hexane: ethyl acetate (15:85).The collected fractions were crystallized and further re-crystallized in chloroform: methanol. The isolated compound was out-sourced and was identified as andrographolide using various spectroscopic techniques such as NMR and GC-MS and the results were compared with previous literatures.

Bio Informatics Approach

Preparation of Ligand Structures: The small-molecule topology generator PUBCHEM server [8] is used for ligand optimization (Figure 2, 3), a tool for high-throughput crystallography of protein-ligand complexes

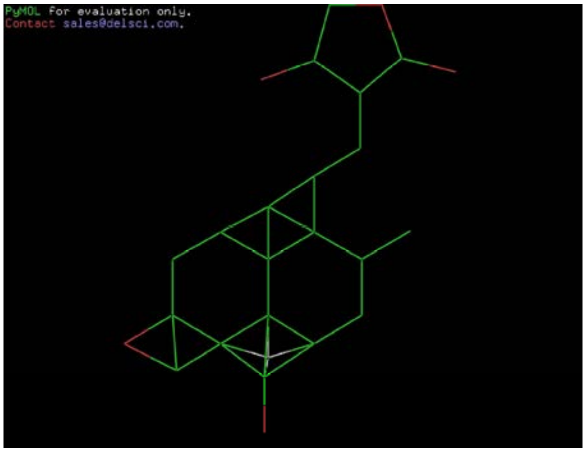


Fig. 2: Andrographolide

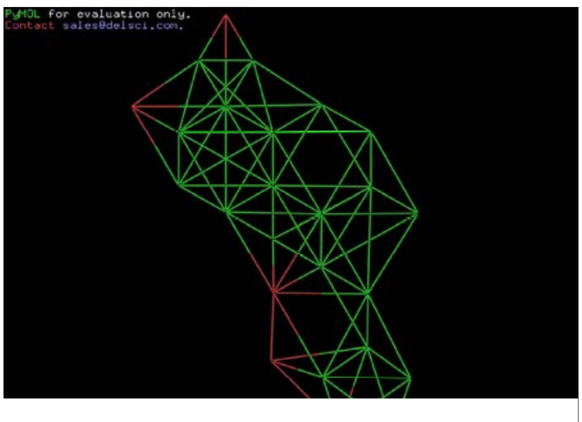


Fig. 3: 14-deoxy-11- oxoandrographolide

Fig. 5: NS5 Protein

which takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein-ligand complexes.

Preparation of Target Protein: Availability of several experimentally determined three dimensional structures of dengue virus NS5 protein (PDB ID: 3P97) was taken as the target protein for the docking studies. Febuxostat provide an excellent drug for using structure-based approaches for the discovery of andrographolide and 14-deoxy-11-oxoandrographolide as seen in previous literatures.

Protein- Ligand Interaction Using Auto Dock: The docking studies were approved by Auto dock tools version 4.2. The searching grid extended above the preferred target proteins; polar hydrogen was added to the ligand moieties. Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the non-polar hydrogen was merged with the carbons and the internal degrees of freedom and torsions were set. Andrographolide and 14-deoxy-11-oxoandrographolide compound were docked to target protein complex 3P97 with the molecule considered as a rigid body and the ligand being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375Å. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values (RMSD values), with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more [7].

Auto Dock-Docking and Visualization: Auto dock is an exhaustive search method for docking studies was used in this study. That able to rotation, torsions in breadth-first order are constructed and those poses that survive the torsion search are scored. The N-lowest energy poses were retained and the final set of poses undergoes coarse minimization, re-clustering and ranking. The Hydrogen bond and steric interactions were obtained using Molegro molecular viewer.

RESULTS AND DISCUSSION

Isolation of Andrographolide from *A. paniculata*: TLC analysis revealed that *A. paniculata* extract had fewer compounds when TLC plates were sprayed with the vanillin-sulphuric acid spray reagentThe presence of andrographolide from *A. paniculata* was confirmed by TLC technique in the mobile phase ratio is Chloroform ; Methanol (8.8 : 1.2) in parellel with andrographolide purchased from Sigma (Figure 6- 9).

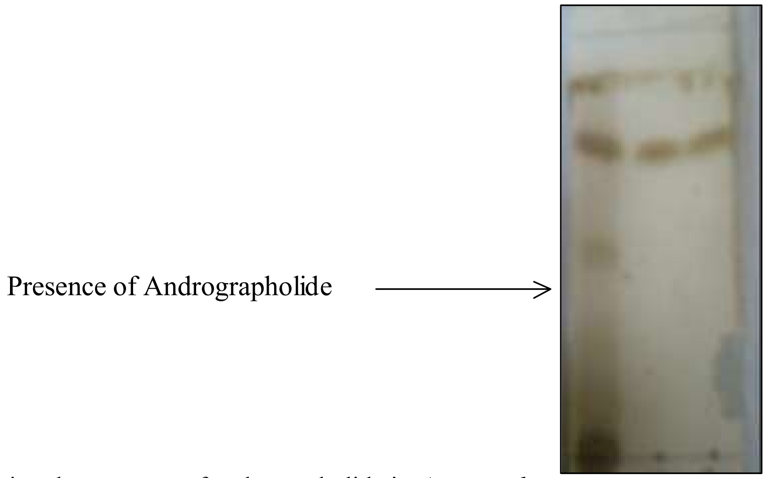


Fig. 6: TLC plate showing the presence of andrographolide in *A. paniculata* extract
E - *Andrographispaniculata,* A - crystallized Andrographolide
A1 - Recrystallized Andrographolide, Mobile Phase: Chloroform 8.8 : Methanol 1.2; Spray Reagent:20% Sulphuric acid. The solvent fronts and origins are marked with pencil lines at the top and bottom of the TLC plates respectively.

E A A1

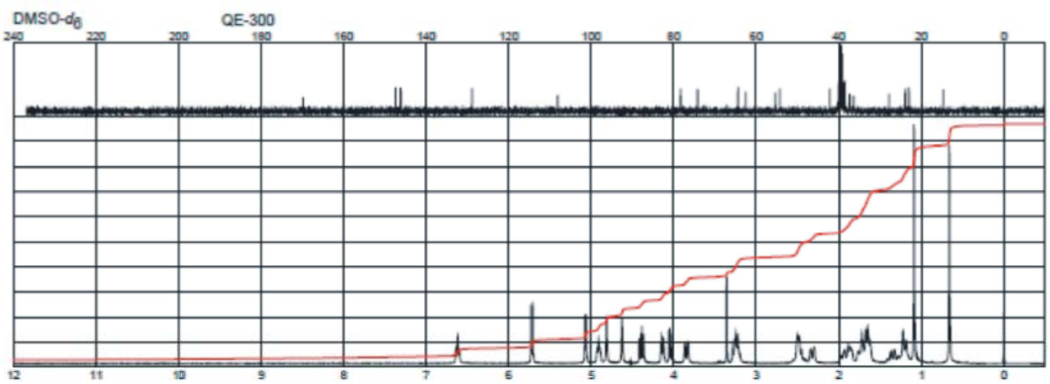


Fig. 7: Standard NMR Spectra of andrographolide (Purchased from Sigma Aldrich).

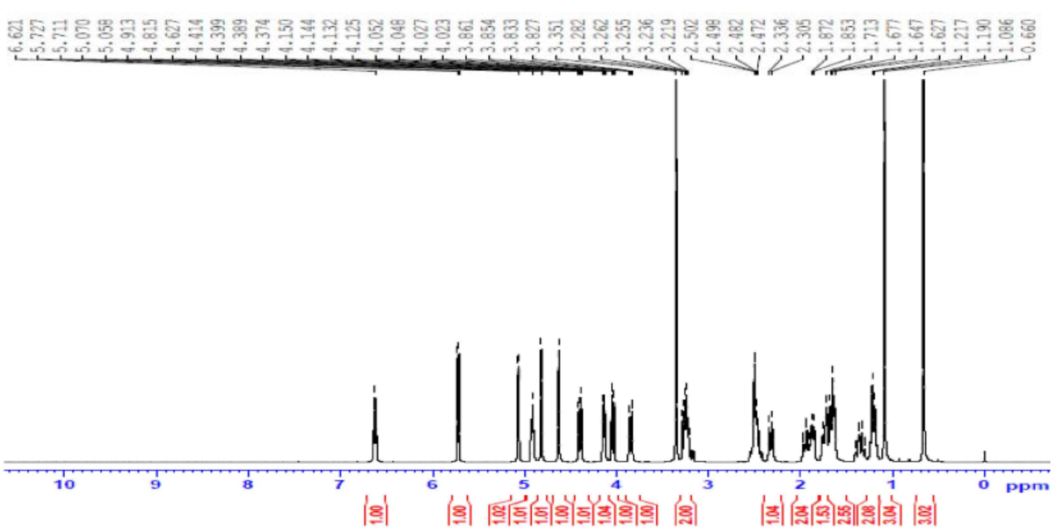
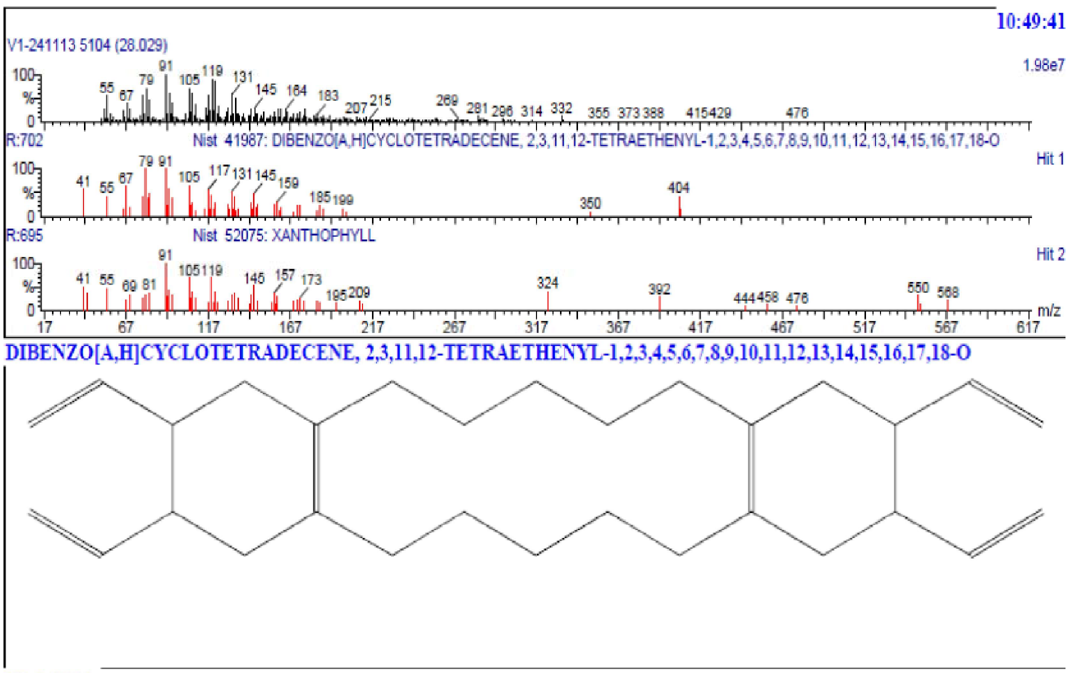


Fig. 8: NMR Spectra of our isolated andrographolide from *A. paniculata*extract.



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Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	702	557	DIBENZO[A,H]CYCLOTETRADECENE, 2,3,11,12-TETRAETHENYL-1,2,3,4,5,6,7,8,9,10,	404	C30H44	56613-34-4
2	695	450	XANTHOPHYLL	568	C40H56O2	127-40-2
3	689	481	2-NAPHTHALENECARBOXYLIC ACID, 8-ETHENYL-3,4,4A,5,6,7,8,8A-OCTAHYDRO-5-	218	C14H18O2	1451-36-1
4	682	560	ANDROGRAPHOLIDE	350	C20H30O5	5508-58-7
5	662	476	CORTICOSTERONE, BIS(PENTAFLUOROPROPIONATE)	638	C27H28O6F10	900352-22-6
6	658	482	RETINOIC ACID, METHYL ESTER	314	C21H30O2	339-16-2
7	656	497	1-PHENANTHRENECARBOXYLIC ACID, 7-ETHENYL-1,2,3,4,4A,4B,5,6,7,9,10,10A-DO	316	C21H32O2	1686-54-0
8	655	463	1-PHENANTHRENECARBOXYLIC ACID, 7-ETHENYL-1,2,3,4,4A,4B,5,6,7,8,10,10A-DO	316	C21H32O2	1686-62-0
9	651	501	I-PROPYL 5,8,11,14,17-EICOSAPENTAENOATE	344	C23H36O2	900336-72-2
10	649	506	BUTYL 4,7,10,13,16,19-DOCOSAHEXAENOATE	384	C26H40O2	900336-80-3

Fig. 9: GC-MS spectra of the isolated andrographolide from *A. paniculata*extract.

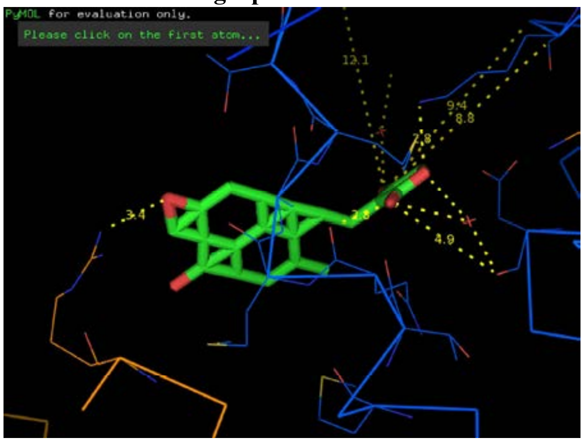
NMR–Results: The isolated sample is outsourced and NMR analysis was done and the obtained result was confirmed with the standard pure isolated compound andrographolide from Sigma Aldrich.

GC-MS Results: Docked pose of dengue virus involved protein NS5 enzyme with andrographolide and 14-deoxy-11-oxoandrographolide ligands as shown in clearly demonstrated the peculiar inhibitory activity. The probable binding sites of preferred target 3P97 receptors were searched using protein data bank to predict the ligand-binding site. It works by binding hydrophobic probes to the protein and finding clusters of probes with the most favorable binding energy. These consist of active sites on protein surfaces and voids covered in the interior of proteins. The individual probe sites relate most closely to the favored high-affinity binding sites on the protein surface. These favorable binding sites relate to locations where a putative ligand could bind and optimize its van derwaals interaction energy.

The crystal structure of the NS5 protein was derived from PDB and used as a target for docking simulation. Ligands were created and prepared for the docking procedure using Auto dock. The goal of ligand-protein docking is to analyze the binding mode based on its energy values. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. Several potential inhibitors have been identified through the docking simulation. Thus the present study is one of its kind.

Analysis of the receptor/ligand complex models generated after successful docking of the andrographolide and 14-deoxy-11-oxoandrographolide were based on the parameters such as, hydrogen bond distance, amino acid interactions, binding energy and orientation of the docked compound within the active site. As a general rule, in most of the potent both hydrogen bond and hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity.

NS5 Protein Andrographolide Interaction



Protein NS5 Ligand Interaction on Andrographolide

The interaction between andrograhalide and NS5 protein showed, the binding energy (-5.66 kcal/mol)higher than the standard, febuxostat (-6.26 kcal/mol). Andrographolide showed six interactions (*viz.*Arg737, Gly339, Lys946. Asp953, Leu947 and Arg352) with hydrogen bond distance 3.4Å, 2.8Å, 2.8Å, 4.9Å, 8.4Å, 12.7Å respectively.

NS5 Protein 14-deoxy-11-oxoandrographolide interaction

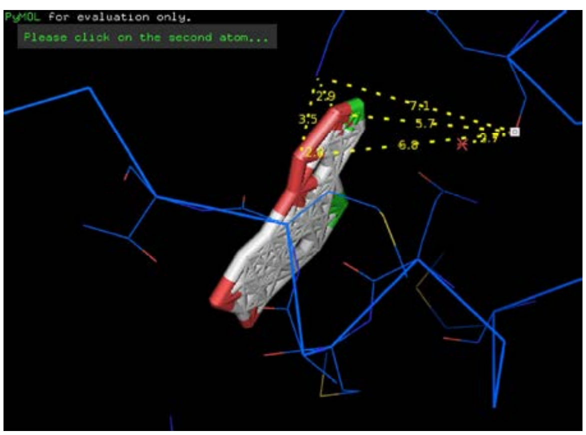


Fig. 10: Protein NS5 ligand interaction on 14 deoxy11oxoandrographolide

In 14-deoxy-11-oxoandrographolide there were 2amino acid interactions namely Lys946, Asp953 with hydrogen bonds distance 3.5Å, 2.9 Å, 7.1 Å, 5.7 Å, 6.8 Å and 1.216 Å respectively.

CONCLUSION

Molecular docking is a key technique to enhance the structural biological predictions to reduce the cost and time in drug discovery programs. The present study aimed to isolate the andrographolide and 14-deoxy-11-oxoandrographolide from *A. paniculata* methanolic extract and to confirm their anti-dengue activity using *insilico* analysis.The NS5 methyltransferase a key protein responsible for flavivirus pathogenesis was used and docked with the respective ligands using Auto dock software. The binding analysis showed those ligands had significant inhibitory activity against the target and could be a valuable drug candidates. As a conclusion, the present study confirmed the further molecular level analysis to reveal the nature of these ligands to eradicate the dengue fever.

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