Research Paper

Screening for Anti-Dengue Leads from *Euphorbia hirta* L. through *In Silico* Methods

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Dengue is a vector-borne viral disease caused by Flavivirus. Current treatment and medicine are inadequate to eradicate the vector mosquitoes or to prevent the disease. Traditional healers have remedies for almost all ailments using natural products and plants. The well-known medicinal plant *Euphorbia hirta* L. is one of the herbs conventionally used for anti-dengue treatment even if the active compound responsible for the exact activity of the plant against the disease is not known. The plant is a rich source of various secondary metabolites. *In silico* screening of 76 small molecules of drug-value belongs to alkaloids, polyphenols and terpenes derived from the plant against dengue virus non-structural protein-5 protease and human inosine 5'-monophosphate dehydrogenase-II determined 16 biologically active compounds with possible activity to fight against dengue virus. Based on the therapeutic importance and drug-likeness parameters, the two best leads, 2-beta, 16-alpha, 19-trihydroxy-ent-kaurane and kaempferol were selected as promising candidates for developing anti-dengue drugs.

Key words: Dengue, *Euphorbia hirta* L., flavivirus, *Aedes* mosquitoes, vector

Dengue fever is one of the severe health problems during the monsoon periods in India. It is a vector-borne disease transmitted by silent, female urban mosquitoes primarily of *Aedes aegypti* and *Aedes albopictus*. The disease spread to tropical and subtropical regions of the world, and 3.9 billion people inhabiting 128 countries are at risk. World Health Organization (WHO) classifies dengue as one of the 17th neglected tropical diseases. Symptoms of dengue start from the 5th d of the bite by an infected mosquito and the symptoms may last for a week or longer. It is self-limiting and characterized by high fever, headache, muscle and joint pain, skin rashes, pain behind the eyes, vomiting, and bleeding from the mouth and nose. The severe form of the disease as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) may lead to multisystem involvement and death. The etiological agent of dengue fever is a positive sense single stranded Ribonucleic Acid (RNA) virus that belongs to the family Flaviviridae and the genus Flavivirus. Every year 390 million people are infecting and not less than 96 million people develop the severe form of the disease and undergoing treatment[1]. India is one of the most vulnerable areas of dengue. An estimate of the National Vector Borne Disease Control Programme (NVBDCP) showed that 153 635 new cases with 226 deaths from India in 2017, of which 19 776 new cases and 37 deaths from God's own land Kerala[2].

The current treatment system of dengue is highly insufficient or practically nil and is limited to vector control measures. Vaccination is one of the unsurpassed dengue prevention methods of treatment. A tetravalent dengue vaccine called Chimeric Yellow Fever Virus-Dengue Virus (DENV) Tetravalent Dengue Vaccine (CYD-TDV or Dengvaxia®) developed by "Sanofi Pasteur" is recommended by some countries. But its low level of protection against serotype-2 and the low vaccine efficacy in young seronegative candidates are of concern. In traditional medicine, plants are used as antipyretic agents to treat various fevers caused by bacteria, viruses, protozoa and other microorganisms. They are the universal manufacturers of a variety of small molecules of therapeutic value. The chemical composition and active ingredients of each plant are different. Their formation depends on the environment and the plants under various stresses. The necessity for plant-based natural medicine is getting higher as they are usually measured safer, non-toxic and less detrimental than synthetic drugs. The healing effect

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of herbal preparations is an independent or collective action of various small molecules present in them. Anti-dengue active compounds present in plants have not often been studied scientifically by pharmacologists. Current investigation proposed structure based virtual screening and detection of a druggable molecule that formulated the potential medicinal plant *Euphorbia hirta* (*E. hirta*) using molecular docking and other analysis tools. The significant cost and time required to separate drug candidates from a collection of natural or synthetic compounds in the early stages of drug development has been greatly reduced by the use of advanced *in silico* screening methods.

MATERIALS AND METHODS

Source plant and ligand preparation:

E. hirta, a popular medicinal plant commonly known as 'asthma weed/snake weed' in English, Chithrapala/ Nelapala in Malayalam, Dugthika/Ksheerani in Sanskrit and Tawa-Tawa in Philipino, belongs to the family Euphorbiaceae (fig. 1). It is a common herbaceous plant seen on roadsides, garden paths and grasslands; widely distributed in pan-tropic and partly subtropic areas including Australia, Queensland, New South Wales, Central America, Africa, Indomalaysia, Philippines, China and India[3]. Ethnopharmacology and the traditional use of the plant are sorted out and well documented. Parts of this plant are widely used in medicine to treat respiratory disorders, gastrointestinal diseases, wound healing, urinogenital disorders, tumors, lactation in women, viral infections, etc. The infusion of the plant is using the indigenous communities of the Philippines for the treatment of dengue fever[4]. Studies have shown that this plant has various other pharmacological properties including, antimicrobial and antiviral activities[3,5,6]. The antiviral activity of the plant is probably due to the presence of high tannin content in the plant[7].

Fig. 1: Habitat and inflorescence of the medicinal herb *E. hirta* L., (a) Habitat of the plant and (b) Enlarged view of cyathium inflorescence of the plant

This plant is a source of various chemicals such as flavonoids, terpenoids, phenols, essential oils, tannins, acids and other compounds such as alkaloids, saponins, amino acids and minerals[6]. Huang *et al.* in 2012[3] and Kausar *et al.* in 2016[8] reviewed the phytochemistry and pharmacology of the plant. According to Kausar *et al.* flavonoids in the plant include quercetin, quercitrin, quercitol and its derivatives; terpenoids include triterpenes: Alpha (α)-amyrin, beta (β)-amyrin, friedelin, taraxerol and its derivatives; tannins include the dimer rich hydrolysable dehydroellagitannins-Euphorbins A, B, C, E and terchebin; the monomeric hydrolysable tannins-Geraniin and acids include ellagic, gallic, tannic, maleic and tartaric acids[8]. Phytochemicals, their function and uses are also documented in the phytochemical database of Dr. Duke. Based on the literature, 76 small compounds with different levels of pharmacological activity, including antiviral properties, were selected for screening. Structural details of the selected compounds were obtained from the chemical database ChemSpider. The compounds, which have no structural deposits (6 out of 76) in databases and other popular sources, were drawn using an online tool, ChemSketch. Three-Dimensional (3D) structures of the compounds were generated in the CORINA 3D structure generator and subjected to docking against the target proteins.

Selection and preparation of target proteins:

A recent review on current and future flavivirus drug targets[9] provides possible therapeutic targets of DENV. Considering the crucial role of Non-Structural Protein-5 (NS5) in the genome replication and methylation and capping of RNA, they selected it as one of the targets in the current study. NS5 is the most conserved target expressed in the host during DENV infection with 900 amino acid residues (~102 kDa). It has a Methyl-Transferase (MTase) domain in the N-terminal and an RNA-dependent RNA polymerase (RdRp) domain in the C-terminal. The MTase is responsible for the capping of viral RNA and methylation of a N7 and 2'O ribose activity. On the other hand, RdRp replicates viral RNA. Moreover, it down-regulates the host immune interferon response and modulating RNA splicing within the host cell[10]. The present study chose the MTase domain of the NS5 as a receptor. Active-site of the receptor was determined based on the site where the known inhibitor molecules (S-Adenosyl-L-Homocysteine (SAH) and Guanosine-5'-Triphosphate (GTP)) were attached. The active site residues of the target include Lys14, Leu17, Asn18, Leu20, Phe25, Lys29, Ser56, Gly58, Gly81, Cys82, Gly83, Gly86, Trp87, Thr104, Lys105, His110, Glu111, Lys130, Asp131, Val132, Phe133, Asp146, Ser151, Pro152, Arg211, Ser213, Thr214, Arg499 and Lys656. The target structure available in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) with PDB ID: 4V0Q is used here for the docking study.

Human Inosine 5'-Monophosphate Dehydrogenase-II (IMPDH-II) is the enzyme in the biosynthesis of purine nucleotides is the second target. It is the enzyme that catalyzes the biosynthesis of guanine nucleotides from Inosine Monophosphate (IMP). Human IMPDH has two isoforms, type I and type II having 84 % sequence similarity. They exist in tetramers consisting of 55 805 Diacetylene (DA) Da monomers with 514 amino acid residues. In normal host cells, IMPDH-I is the prevailing type. IMPDH-II is predominant and up-regulated in the rapidly replicating cells (neoplastic) and pathogenic virus multiplication sites[11]. Selective catalyzing property articulate type two IMPDH which is a promising target for antiviral drug development. Inhibition of the enzyme could interfere with or even terminate its activity by altering or blocking the natural substrate's (IMP, Nicotinamide Adenine Dinucleotide (NAD^+)) binding site[12]. Nair *et al.* detailed the active site region of the enzyme. The IMP binding site is a cavity formed by 36 residues including Ser68, Pro69, Met70, Asp71, Thr72, Val73, Thr74, Ser276, Gln277, Asn303, Val304, Arg322, Val323, Ser327, Gly328, Ser329, Ile330, Cys331, Ile332, Thr333, Gln334, Glu335, Val336, Asp364, Gly365, Gly366, Ile367, Gln368, Met385, Met386, Gly387, Ser388, Leu389, Leu390, Tyr412 and Arg413. The structure of the IMPDH complexed with Ribavirin Monophosphate (RVP) in the RCSB PDB with PDB ID: 1NF7 is used here for the docking study.

Validation of docking protocol:

The reliability of the docking protocols needs to be validated before the actual docking study begins. The Root Mean Square Deviation (RMSD) has been used to measure the quality of reproduction of a known binding pose by a computational method, such as docking. To perform docking validation, the ligand that is bound to the crystallographic structure of the target is separated from their active site and re-docks to the same binding site using a computational docking tool and superimposes them in PyMol to understand the RMSD of the docked molecules. RMSD less than 1.5 Å or even less than 1 Å is ideal and represents better reproduction of the correct pose. Here, the RMSD between the crystallographic and docked structure of SAH is 0.056 Å, and the RMSD between the crystallographic and docked structure of

RVP is 0.953 Å. It indicates the reliability of the docking method in reproducing the experimentally observed binding pose.

Molecular docking studies and drug-likeness assessment:

Docking calculations with both enzyme targets and the phytochemicals were carried out in AutoDock 4.2.6 with the AutoDock Tool (ADT) 1.5.6 using standard procedures. A 3D grid cavity is large enough to accommodate and center around the binding site of NS5 and that of IMPDH-II respectively, were positioned and set the grid point spacing of 0.375 Å. Before docking, remove water/solvent molecules and the co-crystallized compounds from the targets. Targets with rigid residues in the active sites were used and set default parameters in the AutoDock for performing docking analysis and scrutinized hit molecules with the least free energy of binding and inhibition constant was further evaluated. Top hit compounds were tested for their compliance with the Lipinski's rule of five and analyzed molecular properties such as logP, polar surface area, and the number of molecules capable of donating and accepting hydrogen bonds for their suitability for drug development. The bioactivity and drug-likeness is measured using Molinspiration, Molsoft and Swiss Absorption, Distribution, Metabolism and Excretion (SwissADME) online tools. The presence of toxic substructures in the hits and their toxicity were studied using the MCULE toxicity checker.

RESULTS AND DISCUSSION

The phytochemical profile of *E. hirta* revealed that the outstanding medicinal property of the plant is due to the presence of high potential secondary metabolites. 76 such compounds were subjected here for the *in silico* screening trial against both the MTase domain of NS5-protease and human IMPDH-II-oxidoreductase enzymes. Compounds with molecular weight greater than 500 g/mol (14 molecules) were kept away from the present phytochemical scrutiny.

Hit molecules obtained against DENV NS5-protease are shown as follow. Out of 62 phytochemicals docked with the DENV NS5 protease, 41 molecules shown strongly to moderate binding affinity with the target and those ligands with the best docking (11 molecules), based on the least binding free energy ($\Delta G_{bind} \leq -8$ kcal/mol), inhibition constant, number of H-bonds, bond strength and hydrophobic interactions, were screened as top hits (Table 1). All the hits showed ideal therapeutic properties. Betulin (ΔG_{bind}=-8.07) is one of hits and a pentacyclic triterpene that inhibit the activity of the target. It has antitumor activities when combined with cholesterol[13]. Cholesterol is another moderately binding molecule present in the plant. Derivatives of betulin are good anti-microbial agents[14]. Antiviral properties of betulinic acid derived from the precursor, betulin, emphasized its efficacy against Herpes simplex virus type 1 and 2, and Enteric Cytopathic Human Orphan (ECHO)-6 virus[15,16]. Ent-kaurane-diterpenoids are natural compounds displaying a broad spectrum of potential therapeutic effects on anticancer activity. 2-beta (β), 16-alpha (α), 19-trihydroxy-ent-kaurane (ΔG_{bind}=-8.38) and 2-β, 16-α-dihydroxy-ent-kaurane (ΔG_{bind}=-8.62) are two such recently isolated compounds[17]. They firmly bind the active site of the target by 4 and 5 H-bonds (bond length between 2.4 to 3.17 Å) and hydrophobic interaction of 7-8 residues. Triterpenoid cycloartenol (ΔG_{bind}=-8.61) is a phytosterol that showed a moderate binding affinity with the target. It forms one of the precursors for the biosynthesis of various sterols and has various pharmacological activities including, anti-inflammatory, anti-tumour, antioxidant, antibiotic and anti-Alzheimer's disease activities[18]. 24-methylenecycloartenol (ΔG_{bind}=-8.89) is the other triterpenoid that showed moderate activity against the target. Taraxerone (ΔG_{bind}=-9.03) and taraxerol (ΔG_{bind}=-9.41) are the two triterpenes showing considerable binding affinity with the target. Taraxerone can inhibit cancer cell colony formation and induce apoptosis[19]. It also exhibited weak antiviral activity against herpes simplex virus type 1 and 2[20]. Friedelin (ΔG_{bind}=-9.44) is another terpenoid having anti-microbial properties against *Mycobacterium tuberculosis, Candida* spp., etc. Moreover, it has anti-inflammatory and antipyretic properties besides gastro-protective, antioxidant and hepatoprotective activities[21]. α-amyrin (ΔG_{bind}=-9.61) and β-amyrin (ΔG_{bind}=-9.58) are two bioactive triterpenes that showed a strong binding affinity with the target. They have anti-inflammatory, anti-microbial, anti-fungal and antiviral activities[22]. It also protects from gastric ulcers and tumor formation. Stigmasterol (ΔG_{bind}=-10.56), an unsaturated sterol, is strongly bound with the least free energy of binding to the target. It has antiosteoarthritic, antihypercholesterolemic, antitumor, hypoglycaemic, anti-mutagenic, antioxidant, anti-inflammatory and Central Nervous System (CNS) activities[23].

Hit molecules obtained against Human IMPDH-II are shown as follow. The *in silico* screening of the phytochemicals with the target IMPDH-II protease

resulted in 44 active compounds. Out of them, eleven top hits (ΔG_{bind}≤-8 kcal/mol with at least one H-bond) have been identified (Table 2) following similar standards used for the first target NS5. Comparing the docking results of NS5 and IMPDH-II, betulin, 2-β, 16-α, 19-trihydroxy ent-kaurane, 2-β, 16-α-dihydroxy-ent-kaurane, β-amyrin α-amyrin and stigmasterol was found to be similar hits (fig. 2). The remaining five active hit compounds include three flavonoids, a diterpenoid and a phytosterol. Quercetin, kaempferol and cyanidin are the flavonoids that are water-soluble polyphenolic molecules and are generally antioxidants. Quercetin (ΔG_{bind}=-8.37) is the most abundant flavonoid that offers a variety of potential therapeutic uses. It prevents allergies, asthma, hay fever, rheumatoid arthritis and cancer cell growth. It exerts antibacterial activity and exhibit anti-infective and anti-replicative effect on virus[24]. Kaempferol (ΔG_{bind}=-8.35) has antioxidant, anti-inflammatory, anti-microbial, anticancer, antidiabetic, anti-allergic and analgesic activities[25]. Besides, it has potent anti-Human Immunodeficiency Virus (HIV)-1[26] and anti-Japanese encephalitis virus activities[27]. Cyanidin (ΔG_{bind}=-8.14), a water-soluble plant pigment anthocyanidin, is another antioxidant that protects against various types of cancer, heart disease and brain disorders. It prevents arthritis, fatty liver and eye diseases. It induces the immune system and supports the intestine, bone and joints[28,29]. All the three flavonoids are firmly bound to the target with seven to eight hydrogen bonds with a bond length between 2.5 to 3.29 Å. 16-α, 19-dihydroxy ent-kaurane (ΔG_{bind}=-8.97) is one of the three ent-kaurane compounds in the plant that interact firmly with the target. Campesterol (ΔG_{bind}=-10.53) is the simplest plant-derived steroid that helps to reduce cholesterol absorption in the intestine and prevent cancer[30].

TABLE 1: DOCKING RESULT OF HIT MOLECULES AGAINST NS5 PROTEASE

S. No.	Hit molecules	NS5 (Target-1)		
		ΔG_{bind} (kcal/mol)	Inhibition constant (Ki)	Residues with H-Bonds (bond length in Å)
1	Betulin	-8.07	1.22	Asp146 (3.23), Lys180 (3.23), Tyr218 (3.05)
2	2-ß,16-α,19-trihydroxy-ent-kaurane	-8.38	0.72	Glu149 (2.90), Val132 (3.17), Lys105 (2.65), Thr104 (2.85)
3	Cycloartenol	-8.61	0.49	Val132 (3.04), Asp131 (2.75)
4	2-ß-16-α-dihydroxy-ent-kaurane	-8.62	0.45	His110 (2.92), Glu111 (2.67), Arg84 (2.9), Gly85 (3.01), Cys82 (2.40)
5	24-methylenecycloartenol	-8.89	0.3	Asp131 (2.50), Val132 (2.95)
6	Taraxerone	-9.03	0.24	Trp87 (2.68)
7	Taraxerol	-9.41	0.13	Cys82 (2.70), Arg84 (3.07), Gly85 (2.87), Gly86 (3.19)
8	Friedelin	-9.44	0.12	Trp87 (2.76)
9	ß-amyrin	-9.58	0.09	Cys82 (3.10), Trp87 (3.24)
10	α-amyrin	-9.61	0.09	Nil
11	Stigmasterol	-10.56	0.02	Cys82 (2.85)

Note: Top hits obtained against the target NS5 with their free energy of binding (ΔG_{bind}), Inhibition constant (Ki) and the target residues established H-bonds with bond length

TABLE 2: DOCKING RESULT OF HIT MOLECULES AGAINST HUMAN IMPDH-II

S. No.	Hit molecules	IMPDH-II (Target-2)		
		ΔG_{bind} (kcal/mol)	Inhibition constant (Ki)	Residues with H-Bonds (bond length in Å)
1	Quercetin	-8.37	0.73	Gly324 (2.95), Gly326 (2.62), Ser68 (2.92), Ser68 (2.99), Ile367 (3.00), Ile367 (2.79), Ser388 (2.65), Leu389 (3.08)
2	Kaempferol	-8.35	0.76	Met70 (3.14), Ser68 (2.99), Ser68 (3.09), Gly324 (2.89), Gly326 (2.68), Ser388 (3.00), Leu389 (3.20), Ile367 (2.74)
3	Cyanidin	-8.14	1.08	Ser68 (3.29), Met70 (3.16), Gly326 (2.83), Ile367 (2.98), Ile367 (2.51), Leu389 (3.27), Ser388 (2.70)
4	16-α,19-dihydroxy-ent-kaurane	-8.97	0.27	Ser68 (2.79), Arg322 (3.19), Gly324 (2.89), Ser276 (2.94)
5	Campesterol	-10.53	0.019	Met385 (3.14), Gly365 (2.78), Asp364 (2.56)
6	Betulin	-10.23	0.032	Asp364 (2.78), Met420 (3.01)
7	2-ß,16-α,19-trihydroxy-ent-kaurane	-9.33	0.145	Asp364 (3.31), Ser68 (2.45), Ser388 (2.69), Ile367 (3.16)
8	2-ß-16-α-dihydroxy-ent-kaurane	-8.95	0.275	Asp274 (3.07), Asn303 (2.87), Arg322 (2.80), Asp364 (2.47), Gly365 (3.06)
9	ß-amyrin	-11.01	0.008	Ala419 (2.66)
10	α-amyrin	-10.95	0.009	Asp364 (2.85)
11	Stigmasterol	-10.25	0.031	Tyr411 (2.61), Met70 (2.89)

Note: Top hits obtained against the target IMPDH-II with their free energy of binding (ΔG_{bind}), Inhibition constant (Ki) and the target residues established H-bonds with bond length

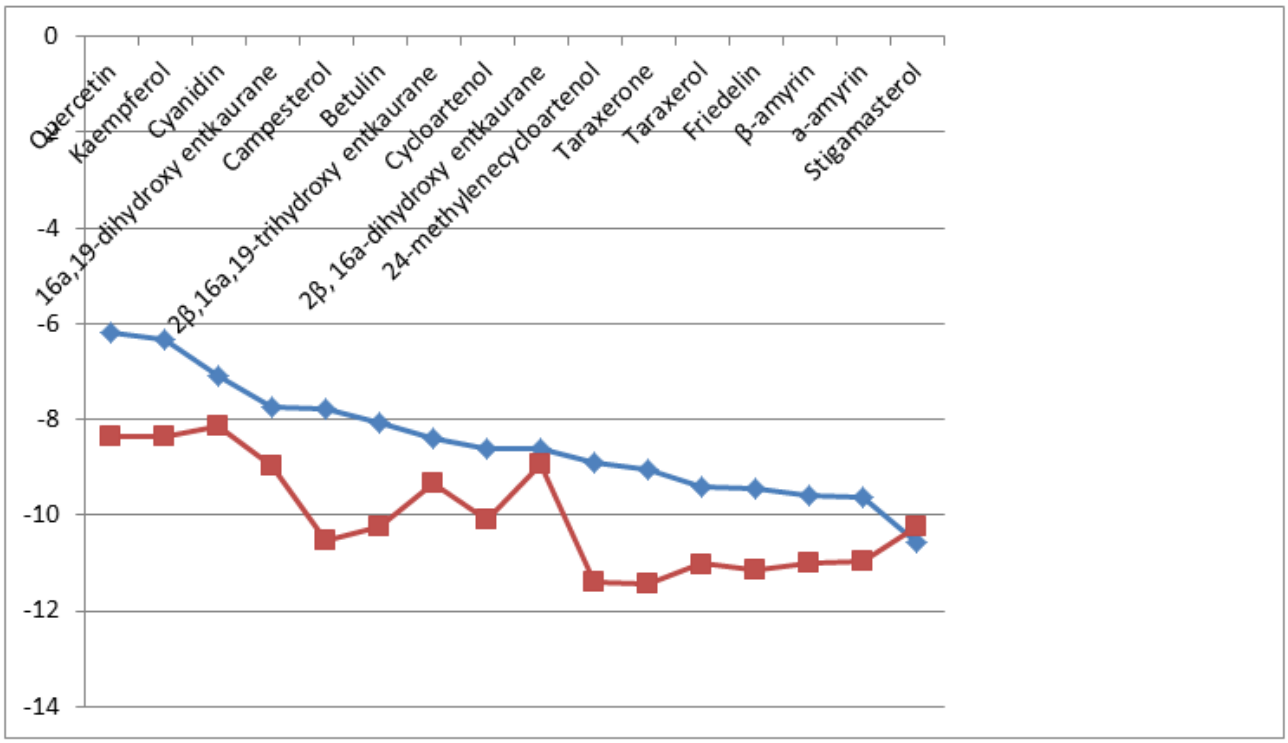

Fig. 2: Comparative competence of hits against the targets based on free energy of binding, Y-axis: Free energy of binding score (ΔG_{bind}) and X-axis: Hit molecules, (◆) DG_{bind} (NS5) and (■) DG_{bind} (IMPDH-2)

Structure of hit molecules obtained against NS5 protease and IMPDH-II are shown in fig. 3. The following molecules are represented as (1) Betulin; (2) 2-β-16-α-19-trihydroxy-ent-kaurane; (3) Cycloartenol; (4) 2-β-16-α dihydroxy-ent-kaurane; (5) 24-methylenecycloartenol; (6) Taraxerone; (7) Taraxerol; (8) Friedelin; (9) β-amyrin; (10) α-amyrin; (11) Stigmasterol; (12) Campesterol; (13) Cyanidin; (14) Kaempferol; (15) Quercetin and (16) 16-α-19-dihydroxy-ent-kaurane.

Top hit compounds (fig. 3) that showed firmly to moderate proximity on both targets were subjected to pharmacokinetic and toxicity analysis. A comparative description of them in Table 3 shows 2-β, 16-α, 19-trihydroxy-ent-kaurane and kaempferol isolated from the whole plant were agreed with drug-likeness and ADME scores. It further evidenced the absence of a toxic substructure. Gibbs free energy level of 2-β, 16-α, 19-trihydroxy-ent-kaurane with both NS5 (-8.38 kcal/mol) and IMPDH-II (-9.33 kcal/mol) underlined the potential to formulate it as a drug. Kaempferol is also equally prospective though its free energy of binding level with NS5 (-6.33 kcal/mol) is comparatively lower than with IMPDH-II (-8.35 kcal/mol). The bioactivity of the biological compounds are either significantly active (score>0.00), moderately active (score between -0.50 and 0.00), or inactive (score<-0.50)[31]. The bioactivity of the hits towards Glycoprotein-Coupled Receptor Ligand (GPCRL), Ion Channel Modulator (ICM), Nuclear Receptor Ligand (NRL), Protease Inhibitor (PI), Kinase Inhibitor (KI) and Enzyme Inhibitor (EI) were observed. Based on the observations 2-β, 16-α, 19-trihydroxy-ent-kaurane is significantly active (>0.00) towards GPCRL, ICM, NRL, PI and EI while moderately active (<0.00) to KI. Kaempferol is moderately active to all except KI, NRL and EI. The structure of the leads, molecular interaction between the active site of targets and leads, and the Molsoft graph showing the drug-likeness of leads are depicted in fig. 4.

Overall the plant *E. hirta* is a valuable herb conventionally using for anti-dengue treatment. The chemistry of the plant revealed its biochemical richness and the presence of various flavonoids that make the plant an antioxidant herb and be a nutritional supplement to fight against free radicals. *In silico* screening of 76 small molecules derived from the plant with DENV-NS5 protease and human IMPDH-II predicted 16 biologically active compounds with possible activity to fight against the DENV. Based on the therapeutic importance and pharmacokinetic properties of selected leads, 2-β, 16-α, 19-trihydroxy-ent-kaurane and kaempferol are the two best candidates for anti-dengue drugs. Formulation of a nutraceutical with pharmaceutical-grade and standardization of nutrients from the herb as well as the development of a safe and effective medication from the leads to fighting with DENV need further *in vitro* and *in vivo* analysis based on the *in silico* result.

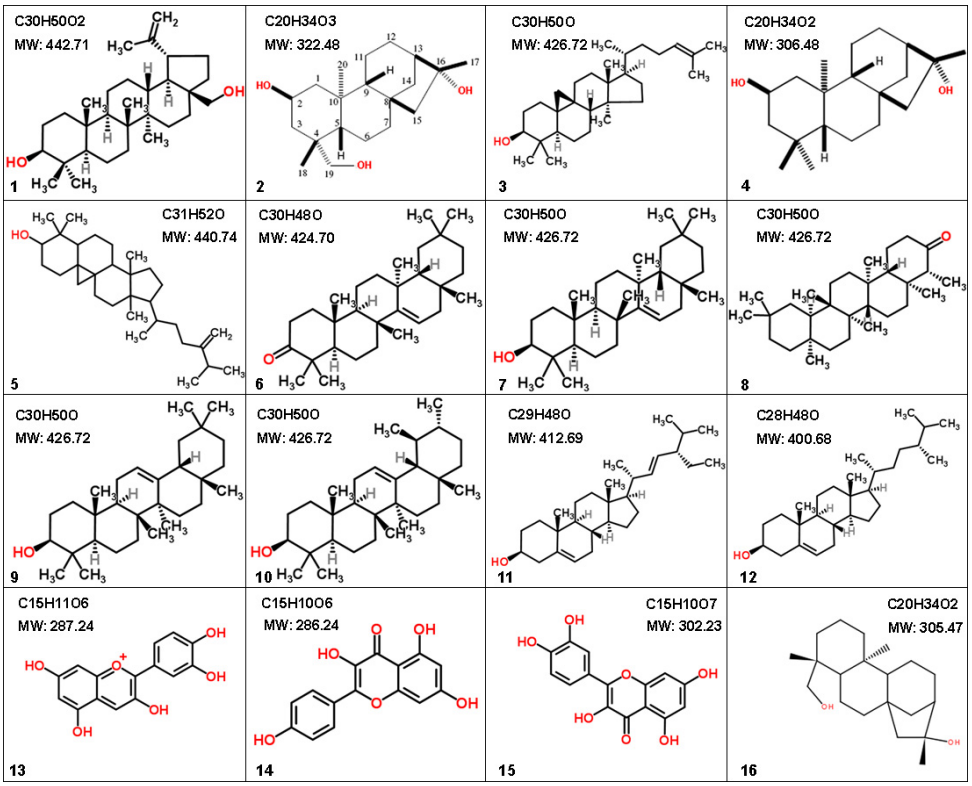

Fig. 3: Structure of hit molecules obtained against NS5 protease and IMPDH-II

TABLE 3: DRUG LIKENESS ASSESSMENT USING DIFFERENT TOOLS

S. No.	Hit molecules	Molinspiration bioactivity score						Molsoft	MCULE	ADME
		GPCRL	ICM	KI	NRL	PI	EI	DLS	TS	LL
1	Betulin	0.21	-0.04	-0.41	0.85	0.09	0.51	-0.09	F	No
2	2-β,16-α,19-trihydroxy-ent-kaurane	0.34	0.13	-0.10	0.87	0.25	0.60	-0.94	NF	Yes
3	Cycloartenol	0.21	0.10	-0.40	0.86	0.14	0.66	-0.31	F	No
4	2-β-16-α-dihydroxy-ent-kaurane	0.29	0.22	-0.15	0.77	0.13	0.52	-1.22	NF	No
5	24-methylenecycloartenol	0.14	0.11	-0.37	0.90	0.06	0.60	-0.51	F	No
6	Taraxerone	0.07	-0.10	-0.40	0.43	-0.14	0.37	-0.89	NF	No
7	Taraxerol	0.21	0.02	-0.20	0.54	0.00	0.49	-0.91	NF	No
8	Friedelin	0.02	-0.06	-0.39	0.39	0.02	0.21	-0.48	NF	No
9	β-amyrin	0.22	-0.05	-0.31	0.67	0.11	0.56	-0.23	NF	No
10	α-amyrin	0.22	-0.02	-0.41	0.79	0.19	0.60	0.09	NF	No
11	Stigmasterol	0.12	-0.08	-0.48	0.74	-0.02	0.53	0.73	FF	No
12	Campesterol	0.11	0.01	-0.48	0.71	0.01	0.50	0.71	NF	No
13	Cyanidin	-0.13	-0.09	0.02	0.09	-0.30	0.01	-0.1	F	Yes
14	Kaempferol	-0.10	-0.21	0.21	0.32	-0.27	0.26	0.77	NF	Yes
15	Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28	0.93	F	Yes
16	16-α,19-dihydroxy-ent-kaurane	0.30	0.18	-0.13	0.75	0.14	0.49	-0.94	NF	No

Note: GPCRL: G-Protein Coupled Receptor Ligand; ICM: Ion Channel Modulator; KI: Kinase Inhibitor, NRL: Nuclear Receptor Ligand; PI: Protease Inhibitor; EI: Enzyme Inhibitor; DLS: Drug Likeness Score; TS: Toxic Substructure; F: Found; NF: Not Found and LL: Lead Likeness

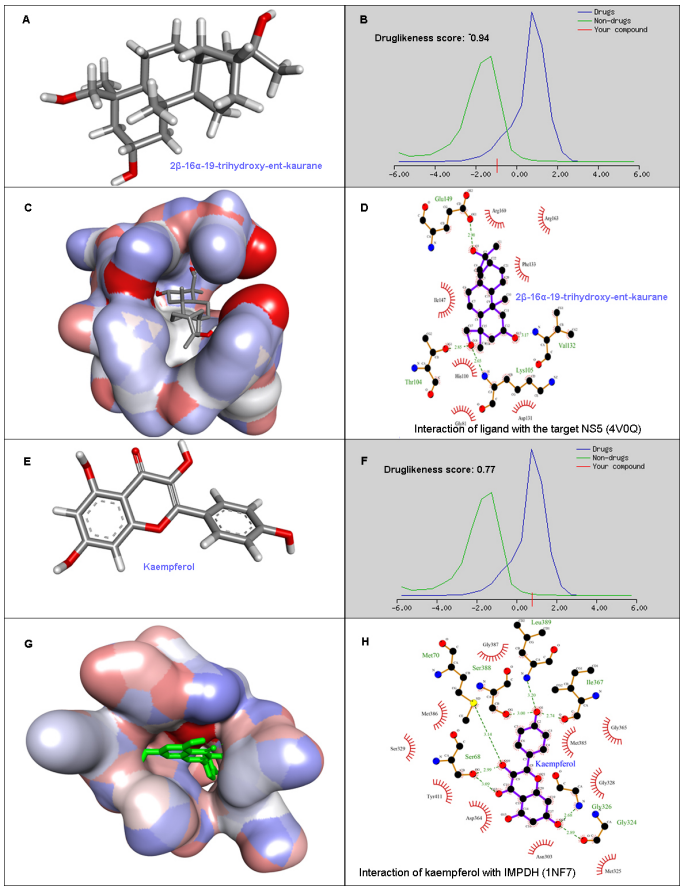

Fig. 4: Lead molecules, their interaction and drug-likeness properties

Note: (A) 2-β,16-α,19-trihydroxy-ent-kaurane (lead-1); (B) Molsoft graph showing drug likeness of the lead-1, (—) Drugs; (—) Non-drugs and (—) Compound; (C) 3D view of MTase active site of NS5 with the lead-1 in discovery studio; (D) Interaction of lead-1 in LigPlot; (E) Kaempferol (lead-2); (F) Molsoft graph showing drug likeness of the lead-2, (—) Drugs; (—) Non-drugs and (—) Compound; (G) 3D view of IMPDH-II active site binding with Kaempferol in discovery studio and (H) Interaction of lead-2 in LigPlot

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Conflict of interests:

The authors declared no conflict of interest.

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