In-silico and *in vitro* evidence of anti-dengue viral action in selected Sri Lankan medicinal plants; a narrative review

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Abstract

More than half a century following the first isolation of the dengue virus, there is yet no effective antiviral agent for the treatment of dengue. For centuries, medicinal plants have been used by traditional medical practitioners for treating all types of infections. Plants are the direct or indirect sources of most approved drugs and synthetic drugs have been modelled on natural products. Screening of phytochemicals in vitro and/or by structure based computational studies are two approaches used in the search of a suitable antiviral agent for dengue. High throughput virtual screening by molecular docking allows for rapid and cost-effective screening of a larger number of compounds. This is faster and cheaper than using laborious in vitro assays for screening. Plant compounds identified by in silico assays, could subsequently be confirmed by in vitro assays. In this review, 52 medicinal plants used in Sri Lankan traditional medicine for fever patients, were identified. Literature search in PubMed and SCOPUS databases identified eight of 52 plants (*Acorus calamus, Aegle marmelos, Azadirachta indica, Carica papaya, Glycyrrhiza glabra, Psidium guajava, Syzygium aromaticum* and *Vetiveria zizanioides*) that had been studied by in silico methods. Phytochemicals of these eight plants with good docking activity for dengue virus, are described in this review. Out of these, phytochemicals epicatechin, kaempferol-3-o-β-rutinoside, rutin, catechin, quercetin, chalcones, hesperidin and naringin are the only compounds that have been studied by both in silico and cell culture methods. Except for rutin and hesperidin, in silico findings of all the other compounds were compatible with results of cell culture assays. Phytochemicals with good docking activity for dengue virus target proteins, but which require evaluation by cell culture assays, have been highlighted for consideration in future studies.

Keywords: Dengue, Sri Lanka, Plants, In silico, *In vitro*, Phytochemicals

Introduction

Dengue is a re-emerging infection mainly in tropical and subtropical regions of the world and a major public health problem.[1] Dengue virus (DV) belongs to the genus Flavivirus of the family Flaviviridae. It is an enveloped, single stranded positive sense RNA virus of approximately 11kb genome size. Dengue infection caused by any of the four serotypes (DV1, DV2, DV3 or DV4), may be followed by asymptomatic infection, dengue fever or severe dengue with haemorrhage and shock.[2] Majority of the evidence suggests that high virus loads lead to severe dengue infections.[2] Therefore, it follows that early treatment with an effective antiviral agent, would lead to lower viral loads and less of severe dengue cases.

The dengue virus genome codes for three structural proteins (capsid, membrane precursor, and envelope) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5).[2] Envelope (E) protein plays a vital role in the entry of the virus into host cells making it a major target for drug development.[2] NS3 has a protease and helicase domain. NS2B acts as a cofactor for NS3.[2] NS2B-NS3 serine protease performs the vital function of cleavage of viral polyprotein at the cleavage sites NS2A/NS2B, NS2B/NS3, NS3/NS4A, NS4B/NS5 and at the viral capsid.[2] NS5 protein has a

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methyltransferase domain at its N-terminal end and a RNA-dependent RNA polymerase (RdRP) at its C-terminal end.[2]

Research into dengue started in year 1943-44 when the virus was first isolated.[3] More than half a century later, there is yet no effective antiviral agent for the treatment of dengue. Several approaches have been used in the search for dengue antivirals: 1) repurposing of existing pharmaceuticals, 2) screening of compounds in vitro and 3) structure based computational studies.[4] A number of clinical trials with repurposed pharmaceutical agents, such as chloroquine,[5] prednisolone,[6] balapiravir,[7] celgosivir,[8] ribavirin,[9] and lovastatin[10] have had disappointing outcomes. Other investigators have selected medicinal plants used in traditional medicine, to search for antiviral agents[11].

There is a vast reservoir of lead compounds in nature that could be used either directly or serve as lead structures for the development of new anti-dengue viral agents.[4] Plants are the direct or indirect sources of approximately 50% of approved drugs and seven out of ten synthetic drugs are modelled on a natural product.[12] Medicinal plants have been used by traditional medical practitioners for treating infections for centuries. In Sri Lankan traditional medicine local plants have been used as remedies in fever patients.

Recent computational advances have opened up a new platform for drug development.[13] High throughput screening methods such as in silico experiments can identify substances specific for target sites on pathogens. Several steps in the dengue virus replication cycle have been targeted by in silico studies. The dengue envelope, non-structural proteins NS2B-NS3 and NS5 are the most common putative drug targets selected in studies. Recently a highly potent virus inhibitor, JNJ-AO7, which blocks the interaction between NS3 and NS4B viral proteins was reported.[14] Numerous pharmaceutical agents, phytochemicals and chemicals that have good docking activity with target sites on dengue virus have been reported.[13] These compounds could be used as natural leads or synthetic analogues and their derivatives to produce effective anti-dengue viral agents. However, only a few of these identified compounds have been further evaluated by cell culture or in vivo experiments. High throughput virtual screening by molecular docking allows for rapid and cost-effective screening of a large number of compounds, unlike the more expensive and laborious method of cell culture.

The objective of this review was to identify medicinal plants used in Sri Lankan traditional medicine for treating fever patients, and to describe those that have been studied by computational methods and found to have phytochemicals with good docking activity for dengue virus targets. Phytochemicals that require further confirmation by in vitro and/or in vivo studies have also been highlighted.

Fifty-two medicinal plants, used for treating fevers in Sri Lankan traditional medicine, were identified by consultation with traditional medical practitioners. A literature search for the 52 plants was done in PubMed and SCOPUS databases using the keywords “dengue AND (*name of plant*)”. Criteria for inclusion were: publications prior to the first June, 2020, English language original articles, plant derived bioactive compounds only and in silico studies of phytochemicals that bind to dengue virus targets. Review articles were excluded. A secondary search for additional articles was done manually by scrutinizing references of chosen articles.

The phytochemicals identified as having potential medicinal value were searched on PubMed for in vitro or in vivo studies using “(name of phytochemical) AND dengue” as the keywords. Phytochemicals that had undergone testing in cell culture were identified. Only studies where virus inhibition was measured by plaque reduction assays were included as that is considered the gold standard test for infectivity. An antiviral agent of clinical value should be capable of reducing the dengue virus titre by at least one log (90-100%).

The 52 medicinal plants used in Sri Lankan traditional medicine to treat fever patients were: *Acorus calamus, Aegle marmelos, Aerva lanata, Alastonia scholaris, Alysicarpus vaginalis,*

Andrographis paniculata, Azadirachta indica, Carica papaya, Carissa carandas, Cassia auriculata, Cassia fistula, Cedrus deodara, Cissampelos pareira, Coriandrum sativum, Coscinium fenestratum, Cuminum cyminum, Curcuma longa, Cyperus rotundus, Foeniculum vulgare, Glycyrrhiza glabra, Gmelina arborea, Gymnema sylvestre, Justicia adathoda, Mollugo cerviana, Munronia pinnata, Myristica fragrans, Oroxylum indicum, Phyllanthus emblica, Piper longum, Piper nigrum, Plumbago indica, Pongamia pinnata, Psidium guajava, Punica granatum, Saussurea lappa, Solanum melongena, Solanum xanthocarpum, Stereospermum suaveolens, Strychnos potatorum, Syzygium aromaticum, Tephrosia purpurea, Tephrosia tinctoria, Terminalia bellirica, Terminalia chebula, Tinospora cordifolia, Tragia involucrata, Tribulus terrestris, Trichosanthes cucumerina, Vetiveria zizanioides, Vitis vinifera, Withaniya somnifera and *Zingiber officinale.*

An initial search in PubMed and SCOPUS databases using "dengue AND (*plant name*)" as keywords turned up 139 and 254 articles respectively (Figure 1).

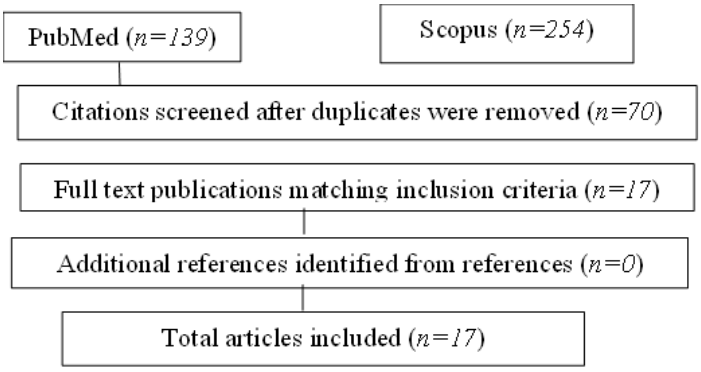

Fig:1: PubMed and SCOPUS databases using "dengue AND (plant name)

Only eight of the 52 plants had been screened by in silico methods for dengue virus (Table 1). Phytochemicals of these eight plants, identified as potential natural leads by docking studies, are listed in Table 2. These compounds were searched on PubMed to identify those that had also been studied *in vitro* and/or *in vivo* (highlighted in Table 2). Findings of these studies are described below.

Table 1. Medicinal plants used for treating fever patients in Sri Lankan traditional medicine and have been studied by in silico methods for dengue antiviral activity

Scientific name	Local name	Common English name	Used part of plant	References
Acorus calamus	Wada kaha	Sweet Flag, Calamus	Rhizomes	15
Aegle marmelos	Beli	Stone apple	Leaves	16
Azadirachta indica	Kohomba	Neem, Margosa	Leaves, seeds, roots, bark	16, 17, 18, 19, 20
Carica papaya	Papol	Papaya	Leaves	21, 22, 23, 24, 25, 26
Glycyrrhiza glabra	Welmee	Licorice	Root	27
Psidium guajava	Pera	Common guava	Leaves	28
Syzygium aromaticum	Karabu-neti	Cloves	Flower, buds	29
Vetiveria zizanioides	Seven-dara	Vetiver	Roots	30

Plant compounds identified with anti-dengue viral activity by in-silico methods and cell culture assays are shown in Table 2.

Table 2. Plant compounds identified with anti-dengue viral activity by in-silico methods and cell culture assays

No.	Scientific name	In silico target site	Compounds identified by in-silico studies	In silico binding		In vitro activity	
				+/-	Ref	Y/ND	Ref
1	*Acorus calamus*	NS5	Acoric acid 3	+	15	ND	
			B-asarone	+		ND	
			Calamusin D	+		ND	
2	*Aegle marmelos*	NS2B-NS3	Marmesinin	+	16	ND	
3	*Azadirachta indica*	NS2B-NS3	Desacetylnimbin	+	18	ND	
			Desacetylsalannin	+	18	ND	
			Epicatechin[#]	+	17	Y	17
			Hyperoside	+	17	ND	
			Kaempferol-3-O-β-rutinoside[#]	+	17	Y	17
			Nimbin	+	18	ND	
			Rutin[#]	+	17	Y	32, 33, 34
		NS3	Meldenin	+	16	ND	
		E	Nimbin	+	19	ND	
4	*Carica papaya*	RdRP	Cardenolide	+	26	ND	
			Caricaxanthin	+	26	ND	
			Carpaine	+	26	ND	
		NS2B	**Catechin**[#]	+	22	Y	28
		NS3					
		NS5					
		NS2B-NS3	Caffeic acid	-	21	ND	
		NS1		+	24		
		NS2B-NS3	Chlorogenic acid	-	21	ND	
		RdRP		-	26		
		NS1		+	24		
		E & NS1	Chymopain	+	23	ND	
		NS2B-NS3 & NS1	Crotonoyl bromide	+	31	ND	
		RdRP	Dehydrocarpaine I and II	+	26	ND	
		NS1	5,7 dimethoxycoumarin	+	24	ND	
		NS2B	Epigallocatechin	+	22	ND	
		NS3					
		NS5					

		2B-NS3		-	21	Y	17, 35
		E & NS1	**Kaempferol**[#]	+	23		
		NS1			24		
		NS2B, NS3, NS5	Protocatechuic acid	+	22	ND	
		NS1			24		
		NS2B-NS3		-	21		
		NS2B-NS3		+	21	Y	28, 36
		NS1	**Quercetin**[#]		24		34
		E			25		
		RdRP	Violaxanthin	+	26	ND	
		RdRP	Zeaxanthin	+	26	ND	
5	*Glycyrrhiza glabra*	NS2B-NS3	3,3',5'- tetrahydroxy-5-prenylbibenzyl	+	27	ND	
			3,3',5'-trihydroxy-4-methoxy-5-prenylbibenzyl	+	27	ND	
			3-acetoxy-4',5-dihydroxy-3'-prenyldihydrostilbene	+	27	ND	
			Licobenzofuran	+	27	ND	
			Glycyrrhisoflavone	+	27	ND	
			4'-O-methylglycyrrhisoflavone	+	27	ND	
			Chalcones (kanazol Y)[#]	+	27	Y	37
		RdRP + E	**Chalcones (kanazol Y)**[#]	+	27	Y	37
		methyltransferase	Glabraisoflavanone*	+	27	ND	
6	*Psidium guajava*	E	**Catechin**[#]	-	28	Y	28
		NS5		+			
		E	**Hesperidin**[#]	+		Y	
		NS5		+			
		E	**Naringin**[#]	+		Y	
		NS5		+			
		E	**Quercetin**[#]	-		Y	
		NS5		+			
7	*Syzygium aromaticum*	NS2B-NS3	Eugeniin	+	*29*	ND	
			Isobiflorin	+			
			Biflorin	+			

8	*Vetiveria zizanioides*	NS2B-NS3	Ethyl 4-(4-methylphenyl)-4-pentenoate	+	30	ND

#Phytochemicals that have been studied in cell culture
+ good molecular docking
- poor molecular docking
ND – in vitro testing not done
Y – cell culture studies done
E – envelope protein
NS2B-NS3 serine protease
NS5 – non-structural protein 5
NS1 – non-structural protein 1
RdRP – RNA dependent RNA polymerase
Ref – references

Acorus calamus

Although in silico findings of this plant have not been confirmed by cell culture methods, good binding of dengue NS5 protein with calamusin D (-6.1 kcal/mol), acoric acid (-5.5 kcal/mol) and β-asarone (-4.7 kcal/mol) have been reported.[15]

Aegle marmelos

One computer modelling study was retrieved for marmesinin of *A. marmelos*. Marmesinin had good interactions (-42 kcal/mol) with dengue virus-2 (DV2) NS3.[16] This needs confirmation by cell culture methods.

Azadirachta indica

Five studies fitting the inclusion criteria were retrieved for this plant. Forty-nine bioflavonoids from *A. indica* were virtually screened in one study, resulting in the identification of kaempferol-3-O-β-rutinoside (-9.555 kcal/mol), rutin (-9.324 kcal/mol), hyperoside (-7.879 kcal/mol) and epicatechin (-7.622 kcal/mol) as potent dengue virus NS2B-NS3 inhibitors. These bioflavonoids had significant bioavailability and drug-likeness.[17] In-vitro antiviral activity of kaempferol-3-O-β-rutinoside and epicatechin against DV2 showed 77.7% and 66.2% inhibition in baby hamster kidney (BHK-21) cells, substantiating the findings of docking studies.[17] However, the degree of inhibition of dengue virus in vitro by these bioflavonoids was not adequate (<90%).

Three triterpenoids from neem i.e. nimbin (-5.56 kcal/mol), desacetylnimbin (-5.24 kcal/mol) and desacetylsalannin (-3.43 kcal/mol) had a good binding affinity with dengue virus NS2B-NS3 in another study.[18] Nimbin also showed high binding activity against the envelope protein of all four dengue serotypes and had increased absorption and oral bioavailability.[19] These triterpenoids have not been evaluated in cell culture.

Despite promising docking results with NS2B-NS3, the polyphenol rutin did not show significant inhibitory activity with macrophages infected with DV2 and dengue virus–3 (DV3).[17,33] Rutin did not inhibit DV2 replication in African green monkey kidney (Vero) and BHK-21 cells either,[32,34] Meldenin from *A. indica* had good interactions with DV2 NS3 protein but cell culture studies are needed for validation of these results.[16]

Carica papaya

Senthivel *et al* investigated seven compounds from *C. papaya* leaves and found that the flavonoid quercetin had the highest binding energy.[21] Farooq and others virtually screened, 900 bioactive phytochemicals of *C. papaya* resulting in the identification of nine compounds i.e. protocatechuric acid, genistein, epigallocatchin, baicalein, 1-hydroxy-2-propanone, catechin, fisetin, 2-methyl-propanoic acid and 2-methyl-butanoic acid, that had high affinity binding to NS2B, NS3 and NS5 proteins of DV2. Epigallocatchin (-13.2911 kcal/mol), catechin (-9.0122 kcal/mol) and protocatechuric acid (-7.5592 kcal/mol) were found to have the highest interaction with NS2B, NS3 and NS5 proteins.[22]

One study screened 103 lead compounds from 43 herbal sources by molecular docking. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one), a natural flavonol present in *C. papaya*, had good binding potential with envelope (-7.2 kcal/mol) and NS1 proteins (-7.5 kcal/mol). Chymopain (disodium;4,5-dihydroxybenzene-1,3-disulfonate) binding energy for envelope and NS1 proteins were -6.5 kcal/mol and -5.9 kcal/mol respectively.[23].

Mir and others identified quercetin (-8.48 Kcal/mol) as the flavonoid with best binding activity to envelope protein by screening baicalein, fisetin, hesperetin, naringenin/naringin and rutin. Baicalein and fisetin although binding well had poor bioavailability. Radakrishnan and others screened nine ligands from *C. papaya* leaves all of which docked well with RdRP. Violaxanthin had the highest interaction energy (-59.17 kcal/mol) and p-coumaric acid showed the least interaction energy. Carpaine, dehydrocarpaine I and zeaxanthin and cardenolide had the potential to dock with DV3 RdRP.[26]

Another study found good binding of six phytochemicals from *C. papaya* against DV2 NS1 protein i.e. kaempferol (-8.1kcal/mol), quercetin (-8.0 kcal/mol), chlorogenic acid (-7.6 kcal/mol), dimethoxycoumarin (-6.2 kcal/mol), caffeic acid (-6.0kcal/mol) and protocatechuic acid (−5.7 kcal/mol).[24] Crotonoyl bromide (-2.9 kcal/mol) of papaya had good affinity with target receptor NS2B-NS3 although it had the lowest inhibition constant value with NS1.[31]

Apart from kaempferol, catechin and quercetin, none of the other compounds of *C. papaya* have been investigated by in vitro studies. The flavonoid kaempferol did not have direct virucidal activity and did not inhibit the virus in the human origin cell line HEK293T/17.35. Surprisingly, when cultured in BHK-21 cells, there was a significant increase in both the level of infection and virus production as a consequence of treatment with kaempferol.[35] These findings were further confirmed by the demonstration of increased expression of envelope protein in DV infected cells treated with kaempferol.[35]

Three studies were retrieved which had investigated the inhibitory activity of quercetin on DV in cell culture assays.[28,34,36] Zandi and others studied the antiviral activity of four bioflavanoids against DV2 in Vero cells. The level of DV2 RNA production in the presence of quercetin was reduced by 67% compared to non-treated infected cells.[36] Quercetin inhibition post-treatment (quercetin added after inoculating virus) was more significant than in pre-treatment (quercetin added before virus inoculation) assays with a selectivity index (SI) value of 7.07.[36] Quercetin did not have significant direct virucidal activity.[36]

In silico findings for quercetin and catechin, have been confirmed in other studies as well. Vero cells infected with DV2 and treated with quercetin had the highest SI value (34.3) and catechin induced better viral inhibition when added before (100% inhibition) than after (91.8% inhibition) virus inoculation.[28] Quercetin from *Houttuynia cordata* displayed anti-DV2 activity with a SI of 0.88 in BHK-21 cells.[34]

Glycyrrhiza glabra

A virtual screening analysis by Powers and other, revealed that prenylated stilbenoids (3,3',5'-tetrahydroxy-5-prenylbibenzyl, 3,3',5'-trihydroxy-4-methoxy-5-prenylbibenzyl, 3-acetoxy-4',5-dihydroxy-3'- prenyldihydrostilbene, licobenzofuran), isoflavanoids (glycyrrhisoflavone, 4'-O-methylglycyrrhisoflavone) and chalcones (kanazol Y) demonstrated outstanding docking properties with DV target NS2B-NS3. In addition, kanazol Y docked well with RdRP and DV envelope protein while glabraisoflavanone bound well with methyltransferase[27].

Except for chalcones none of the above-mentioned compounds of *G. glabra* have been evaluated in cell culture. Patil and others developed a group of structurally complex thienyl chalcones which were tested with DV2. Cyclopropylquinoline analog IV showed moderate inhibition of DV2 in cell culture.[37]

Psidium guajava

Trujillo-Correa and others studied five flavonoids (quercetin, catechin, naringin, gallic acid and hesperidin) from *P. guajava* by in silico methods. Out of the five ligands, only naringin (−8.0 kcal/mol) and hesperidin (−8.2 kcal/mol) had good docking scores with the envelope protein of DV. Except for gallic acid all others had good docking scores with DV NS5 protein.[28]

The same study demonstrated that quercetin had the highest SI value (34.3), when DV2 infected Vero cells were treated with quercetin.[28] Gallic acid, naringin and catechin were considered as highly selective (SI values ≥10) whereas hesperidin was considered non-selective (SI value <2) in this study. Gallic acid significantly inhibited viral activity when added both before and after virus inoculation but naringin inhibited DV only when added after virus inoculation. Catechin induced the best viral inhibition when added before (100% inhibition) or after (91.8% inhibition) virus inoculation.[28]

Syzygium aromaticum

Eugeniin (-10.2 kcal/mol), isobiflorin (-6.8 kcal/mol) and biflorin (-7.2 kcal/mol) from *S. aromaticum* underwent docking analysis with NS2B-NS3 of DV3 and eugeniin was identified as the most potent inhibitor while isobiflorin and biflorin showed moderate inhibition against dengue virus.[29] These findings have not been confirmed in vitro studies

Vetiveria zizanioides

Docking analysis of active compounds of *V. zizanioides* identified ethyl 4-(4-methylphenyl)-4-pentenoate as having the maximum binding affinity to NS2B-NS3 of all dengue serotypes.[30] In vitro studies were not retrieved for ethyl 4-(4-methylphenyl) -4-pentenoate.

Discussion

Computer modeling studies give detailed descriptions regarding the interactions of compounds with the target proteins. Computational approaches can be used to screen a large number of compounds at a time for antiviral action.[16] Narrowing down the likely compounds by in silico methods accelerates the screening process of compounds by cell culture. This review was intended to identify phytochemicals that had good docking activity with DV target proteins in order to facilitate further studies of these compounds.

Only eight plants out of 52 had been subjected to docking analysis in this review of Sri Lankan medicinal plants (*A. calamus, A. marmelos, A. indica, C. papaya, G. glabra, P. guajava, S. aromaticum* and *V. zizanioides).* DV target sites most commonly used in these studies were NS2B-NS3, NS1 and RdRP.

Virus life cycle

The virus life cycle consists of multiple steps which include viral entry, replication, viral assembly and release. Viral entry is initiated by the fusion of viral membrane with the host cell membrane, followed by endocytosis and the formation of endosomes. The low pH in the endosomes triggers fusion of viral and cell membranes which leads to the disassembly of the virion and the release of RNA into the cytoplasm. The envelope (E) protein is another target site of the dengue virus that has been utilized for the development of antivirals. The virus life cycle is initiated by binding to receptors on the envelope and internalization by endocytosis. The receptors involved in this process are not fully understood. The proposed host cellular receptors include dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), heparan sulphate receptors, mannose receptors and human C-type lectin like molecules.[41]

Following release of viral genome, the positive strand of RNA is translated into a single strand of polyprotein. This polyprotein is cleaved by viral and cellular proteases into three structural and seven non-structural proteins. Following several rounds of viral transcription viral assembly occurs at the endoplasmic reticulum and is released from the cells.[41]

NS5 is the largest and most conserved non-structural protein of flaviviruses which makes it an ideal target

for antiviral agents that could be used for several related viruses. More than 75% sequence homology is found among all four serotypes of dengue viruses. It is an important antiviral target because its enzymatic activity is crucial for virus replication. NS5 has methyltransferase and RNA-dependent RNA polymerase (RdRp) activities.[41]

NS3 protein is a multifunctional protein which has protease, helicase, nucleoside 5' triphosphatase activities. NS3 protease contains two domains: N-terminal protease which cleaves the viral polyprotein precursor into individual proteins and a C-terminal RNA helicase involved in dengue virus genome replication and viral RNA synthesis. Many compounds studied target NS3 protease domain. NS3 protease requires NS2 as a co-factor for its function.[41] Preventing the processing and release of viral proteins from the polyprotein precursor would inhibit viral genome replication.

NS1 is non-structural protein found in different cellular locations. It is present as the endoplasmic reticulum resident form, membrane anchored form and the secreted form. Intracellular NS1 is involved in early viral replication although its specific function is not well understood.[42] NS1 is also postulated to be involved in the development of severe dengue. Any compound capable of suppressing the activity of NS1 should therefore be capable of reducing the number of severe dengue cases.

Antiviral activity of phytochemicals

Phytochemicals listed in Table 2 were searched in PubMed for studies with dengue virus infected cell lines. Epicatechin, kaempferol-3-O-β-rutinoside, rutin, catechin, caffeic acid, quercetin, chalcones, gallic acid, hesperidin and naringin were the only phytochemicals that had been studied for anti-dengue viral activity in cell culture assays. Except for rutin and hesperidin, in silico-positive findings for all other compounds were confirmed by the results of cell culture methods. Phytochemicals that have not been evaluated by in vitro assays have been highlighted in Table 2 for consideration in future studies.

Plants have been the sources of approximately 50% of approved drugs and synthetic drugs are usually modelled on a natural product.[13] Phytochemicals with anti-dengue viral activity are important for the identification of natural leads or for drug development from its analogues. As evidenced by some studies computational methods are not always successful in identifying compounds with inhibitory action.[32,33,34] At the same time some compounds identified as promising have not produced adequate inhibition of viruses in cell lines.[17,35] Despite these drawbacks, in silico screening is useful for accelerating the process of screening numerous compounds in a relatively short time.

Molecular docking identified calamusin D, acoric acid and β-asarone of *A. calamus* as having good free energy of binding with NS5.[15] All three phytochemicals identified are present in *A. calamus* rhizomes which is used in Sri Lankan medicine.[38] Further evaluation of these phytochemicals by in vitro experiments are necessary to confirm their usefulness.

Neem leaves, seeds, roots and bark are used in Sri Lankan traditional medicine. About 135 phytochemicals have been isolated from different parts of neem but only a few have been studied.[18,39] Dwivedi and others reported good inhibitory potential of rutin with NS2B-NS3, but this was not evident in any of the three in vitro studies which used human and mammalian cell lines for growing the DV.[32,33,34]

Fruit, flower, seed, leaf, bark and root of papaya tree are known to possess many biologically active compounds. Aqueous papaya leaf extracts have been used as treatment for dengue fever.[40] Several compounds from *C. papaya* have been shown to have potential inhibitory activity by computational methods. However, only catechin and quercetin have been confirmed as potential inhibitors in cell culture.[28,34,36] Kaempferol although detected by docking methods as a good inhibitor of the envelope and NS1 proteins, did not demonstrate inhibitory activity on HEK293T/17 or BHK-21 cells,[17,35]

Several phytochemicals of *G. glabra* listed in Table 2 were found to have good binding energy with

several target proteins of DV.[27] Derivatives of chalcones have been studied in vitro but *G. glabra* phytochemicals need further evaluation in cell culture.[37]

Despite anecdotal evidence for its medicinal properties, *P. guajava* has not been screened for its antiviral phytochemicals until recently.[28] Trujillo-Correa and others confirmed that catechin (91.8% inhibition), naringin and quercetin (100% inhibition) were good inhibitors of NS5 by docking methods and found similar results with Vero cells and DV2. Hesperedin was the only phytochemical that did not perform well in vitro studies.[28]

Only a few of these computer modelling studies have used target proteins from all four dengue serotypes for analysis.[19] Most investigators have used only the DV2 protein structures. In vitro studies with plant extracts have shown differential activity with the four serotypes. As such, in silico studies based on all four dengue serotypes would lead to more accurate predictions and may explain the occasional contradictory findings of in silico and in vitro studies.

As evident by some of these studies, individual phytochemicals had less inhibitory action than the crude extract of the plant. This demonstrates the importance of synergism between compounds in crude extracts.[34] Soil and climate in different geographical locations affect the chemical composition of plants. Therefore, decoctions prepared from herbal aqueous extracts could vary. This could be overcome by combining compounds that act on different viral target proteins to formulate pharmaceutical formulations that can be regulated.

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