

Apigenin And E-Viniferin from *Dioscorea Transversa* Leaves: Isolation and in Silico Evaluation Against HPV16 E6 And Inflammatory and Angiogenic Targets

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Abstract

Background and objectives: Long-term infection with HPV16 is the main cause of cervical cancer, and the viral E6 protein contributes by recruiting the host ligase E6AP, which marks p53 for destruction. Leaf extracts of *Dioscorea transversa* R. Br. have been reported to contain more polyphenols and to show stronger anti-inflammatory and pro-apoptotic effects than tuber extracts, but the compounds responsible were not known. This study aimed to identify them and to use molecular docking to examine how well they bind two sites on HPV16 E6 (the E6AP-binding site and the p53-binding site), as well as COX-2, TNF- α and VEGFR2.

Materials and methods: Aqueous-methanolic leaf and tuber extracts were compared by disc-diffusion assay and thin-layer chromatography. Bands present only in the leaf extract were purified by column chromatography and recrystallisation, and characterised by melting point, FTIR and HPLC co-injection with authentic standards. A library of 25 flavonoids and stilbenoids was docked using AutoDock Vina against the E6 oncoprotein of HPV16 at its E6AP- and p53-binding sites, the primary targets. Paclitaxel, hypericin and anabsinthin, previously reported as E6 ligands, were docked as reference compounds. The same 25 compounds were also docked against the secondary targets COX-2, TNF- α and vascular endothelial growth factor receptor 2 (VEGFR2), proteins involved in inflammatory and angiogenic signalling, with the respective co-crystallised ligands of each target as reference compounds. Physicochemical properties and the predicted clinical toxicity (ClinTox) probability of the two-site binders and the reference compounds were calculated in silico.

Results: The leaf extract gave wider inhibition zones than the tuber extract against *S. aureus* (13 vs 11 mm), *E. coli* (10 vs 9 mm) and *C. albicans* (14 vs 9 mm). The two purified compounds were identified as apigenin and ϵ -viniferin. Both scored better than paclitaxel at the two E6 sites (E6AP site/p53 site: apigenin -7.370/-6.098; ϵ -viniferin -7.900/-6.207 kcal/mol), although hypericin and anabsinthin scored better at the E6AP site. Apigenin also bound COX-2, TNF- α and VEGFR2 (-9.964, -9.061 and -9.825 kcal/mol), whereas ϵ -viniferin was largely limited to E6. Apigenin met all four Lipinski criteria and had a low predicted ClinTox probability (0.024).

Conclusion: Apigenin and ϵ -viniferin from *D. transversa* leaf emerge as computational leads against HPV16 E6, and apigenin may in addition act on inflammatory and angiogenic targets.

Keywords: *Dioscorea transversa*; apigenin; ϵ -viniferin; FTIR; HPV16 E6 oncoprotein; molecular docking; TNF- α ; ClinTox prediction

1. Introduction

Cervical cancer remains a major public-health problem for women, especially where health resources are limited. Global estimates indicate about 662,000 new cases and nearly 349,000 deaths each year, making it the fourth most common cancer, and the fourth leading cause of cancer death, among women [1,2]. The burden is uneven: countries with the lowest Human Development Index have roughly twice the incidence and almost five times the mortality of those with the highest, and among women aged 15–44 it ranks among the three most common cancers in most countries [1]. Without stronger action, annual cases are projected to pass 760,000 by the end of the decade [1].

Unlike most cancers, cervical cancer has a well-defined principal cause and can be prevented and detected early with proven tools. The World Health Organization's 2020 elimination strategy sets three targets for 2030, often called "90-70-90": vaccinating 90% of girls against HPV by age 15, screening 70% of women with a high-performance test by ages 35 and 45, and treating 90% of women with cervical disease [2]. Most low- and middle-

income countries remain far from these targets, so research on the biology of the disease and on new therapeutic options is still needed.

The association between HPV and cervical cancer is among the best established in oncology. Retesting of tumour samples with sensitive molecular assays detected HPV DNA in 99.7% of invasive cervical cancers worldwide, leading to HPV being classed as a necessary, though not sufficient, cause [3]. Only twelve to fourteen of the more than 200 known HPV types are high-risk, with types 16 and 18 responsible for most cervical cancers. The virus is small and non-enveloped, with a circular double-stranded DNA genome of about 8 kb carrying eight genes; E6 and E7 encode the main oncoproteins that drive cell transformation [4], and persistent HPV16 or HPV18 infection is the leading cause of cervical carcinogenesis [5,6].

HPV16 E6 and E7 inactivate the tumour suppressors p53 and retinoblastoma protein (Rb), permitting uncontrolled proliferation, a weakened DNA-damage response and escape from apoptosis [7]. E6 is an 18 kDa protein of about 150–160 residues arranged in two zinc-binding domains. It binds the ubiquitin ligase E6AP (E6-associated protein), and the E6–E6AP pair then captures p53, forming a three-protein complex that targets p53 for proteasomal degradation, as first shown by Scheffner and colleagues [4,8]. Since p53 normally halts the cell cycle, triggers repair or induces apoptosis after cellular stress, its continual removal lets cells with accumulating genetic damage survive and multiply [9,10]. E7 acts in parallel by binding hypophosphorylated Rb and releasing E2F transcription factors, pushing cells into S phase irrespective of growth signals [11]. Together, these actions drive progression from cervical intraepithelial neoplasia to invasive carcinoma, making the E6–p53 and E7–Rb interactions attractive, virus-specific drug targets that should spare HPV-negative cells [4,9,10]. Disrupting the E6–E6AP–p53 axis, either by blocking E6 directly or by stabilising p53, produces downstream changes that are the markers most often used to judge treatment effect in mechanistic studies, including our earlier cell-based work on *D. transversa* extracts [12].

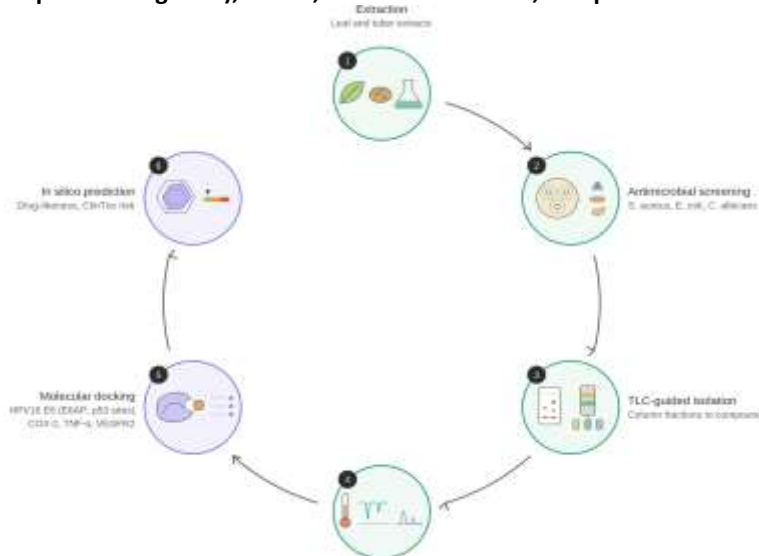
D. transversa R. Br., known as long yam or pencil yam, grows in open forests of northern and eastern Australia and along forest margins in southern India. Tribal communities in northern Kerala eat its tubers in times of food scarcity and use them, with other ingredients, in traditional postpartum preparations to help restore bone and muscle strength. Despite this long use, the chemistry behind these applications has been little studied.

Our earlier work showed that the leaf, rather than the traditionally eaten tuber, is the more active plant part, with higher total polyphenol content, stronger antioxidant and anti-inflammatory effects, and better modulation of cyclooxygenase-2 (COX-2) and tumour necrosis factor- α (TNF- α) in laboratory assays [13]. A follow-up study found that leaf and tuber extracts both induced ROS-dependent apoptosis in HPV-positive cervical cancer cells (HeLa and SiHa) but not in HPV-negative C33A cells or non-cancerous L929 cells, with restoration of p53 and dephosphorylation of Rb, suggesting interference with HPV oncoprotein activity [12]. Neither study identified the compounds responsible. Dioscorea tuber extracts also inhibit a range of bacterial and fungal pathogens [14].

HPV16 E6 promotes p53 loss through two surface regions: a hydrophobic pocket that accepts the LxxLL motif of E6AP, and a shallower, solvent-exposed groove that contacts p53 [15,16]. Following the site definitions of Elfiky et al. [17], we treated the E6AP-binding and p53-binding sites as two separate docking targets on one E6 structure. Molecules with affinity at both sites are of particular interest because they could, in principle, hinder E6AP recruitment and p53 capture simultaneously. Here, screened molecules scoring better than the reference drug paclitaxel at both sites are termed two-site binders. Because the p53-binding groove is shaped partly by E6AP binding [17], results at this site are considered exploratory.

D. transversa is used traditionally for ailments that could involve HPV-associated disease, yet the compounds behind this use are unidentified, and it is unknown whether any leaf constituent interacts with HPV16 E6. The anti-inflammatory activity of the leaf extract likewise lacks a compound-level explanation. To address these gaps, we performed bioactivity-guided fractionation of the leaf, isolating apigenin and ϵ -viniferin. Their binding at the two E6 sites was predicted by molecular docking and compared with that of structurally related flavonoids, stilbenoids and reference anticancer compounds. The same compounds were also docked to COX-2, TNF- α and vascular endothelial growth factor receptor 2 (VEGFR2), proteins involved in inflammatory and angiogenic signalling, to explore a possible secondary basis for the extract's anti-inflammatory activity. Drug-likeness and ClinTox-based toxicity predictions gave an early indication of translational potential. Taken together, these *in silico* findings point to candidate compounds and putative targets that may relate to the plant's traditional use, and offer a starting point for preclinical and, ultimately, clinical studies.

Figure 1. Overview of the study design: extraction of leaf and tuber, antimicrobial screening, TLC-guided isolation, structural confirmation (melting point, FTIR, HPLC), molecular docking against HPV16 E6 (E6AP-binding and p53-binding sites), COX-2, TNF- α and VEGFR2, and prediction of drug-likeness and ClinTox risk



2. Materials and Methods

2.1 Plant material and extraction

Fresh leaves and tubers of *D. transversa* R. Br. were collected in February–March at Kanayi, northern Kerala, India. The plant was identified using Australian Rainforest Plants (8th edition) and Edible Plants of the World, and the identification was confirmed by the Department of Botany, Periyar University, Salem, India (PU/BOT/2026/090). Leaf (AqML) and tuber (AqMT) extracts were prepared in aqueous methanol (1:1, v/v) by indirect ultrasound-assisted extraction, as described earlier [13].

2.2 Antimicrobial assay

The antimicrobial effect of AqML and AqMT was tested by agar disc diffusion [18] against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. Inocula were adjusted to 0.5 McFarland (about 1.5×10^8 CFU/mL) and spread on Mueller-Hinton agar (bacteria) or Sabouraud dextrose agar (*C. albicans*). Sterile 6 mm discs carrying extract (100 μ g/disc), a positive control (ampicillin 10 μ g/disc for bacteria; fluconazole 25 μ g/disc for *C. albicans*) or vehicle alone were placed on the inoculated surface. Plates were incubated at 37 °C for 24 h (bacteria) or 25 °C for 48 h (*C. albicans*). Inhibition zones, including the disc diameter, were measured in triplicate and are given as mean $\pm$ SD.

2.3 Thin-layer chromatographic profiling

AqML and AqMT were separated on silica gel 60 F254 plates using toluene:ethyl acetate:methanol:formic acid (6:4:1:0.5) as the mobile phase. Plates were viewed under UV light (254 and 366 nm) and after derivatisation with Fast Blue B salt followed by 10% NaOH, and were also scanned by densitometry to obtain an HPTLC fingerprint.

2.4 Isolation of apigenin and ϵ -viniferin

The two most intense, well-resolved bands found only in the leaf extract (R_f 0.48 and 0.53) were scraped off, adsorbed onto silica gel and purified by open-column chromatography (silica gel 60, 3 cm column). Elution used a stepwise hexane:ethyl acetate gradient (90:10 to 60:40), followed by ethyl acetate:methanol (95:5 to 80:20). Fractions were followed by TLC and pooled when their R_f values matched. The pooled material was recrystallised from hot methanol to give two purified compounds, whose melting points were measured in a capillary apparatus (348 °C and 151 °C) and compared with literature values.

2.5 FTIR spectroscopy

Each purified fraction (about 1–2 mg) was ground with roughly 200 mg of dry KBr and pressed into a pellet. Spectra were recorded on a Shimadzu FTIR spectrometer between 4500 and 650 cm^{-1} , with several scans averaged to improve the signal-to-noise ratio. Peaks were picked with the instrument software.

2.6 HPLC analysis

The purified fractions and authentic standards (apigenin, Merck/Supelco 41151; ϵ -viniferin, Phytolab 83350) were analysed on a Shimadzu LC-20AP Prominence system fitted with an SPD-M20A photodiode-array detector and reversed-phase C18 columns 250 $\times$ 4.6 mm, 5 μm (ϵ -viniferin) and 150 $\times$ 4.6 mm, 5 μm (apigenin). The mobile phases were methanol:water (65:35, v/v, ϵ -viniferin) and acetonitrile:0.1% formic acid (35:65, v/v, apigenin), at 1 mL/min. Identity was assigned by matching retention times with the standards and by co-injection (spiking), with the absence of peak splitting taken as evidence of co-elution.

2.7 Molecular docking

A library of 25 phytochemicals was assembled based on their structural and chemical class relatedness to ϵ -viniferin and apigenin, the stilbenoid and flavonoid isolated from *D. transversa*. The library comprised 13 stilbenoids (3-hydroxystilbene, pinostilbene, pterostilbene, resveratrol, oxyresveratrol, piceatannol, pinosylvin, isorhapontigenin, combretastatin A-4, ϵ -viniferin, ampelopsin A, miyabenols and hopeaphenol) and 12 flavonoids and chalcones (apigenin, chrysin, eupalitin, galangin, glabridin, kaempferol, luteolin, myricetin, naringenin, quercetin, EGCG and butein). Anabsinthin, hypericin and paclitaxel served as literature-based reference compounds for E6 [17]. Crystal structures of HPV16 E6 (4XR8) [17], COX-2 (PDB 3LN1) [19], the TNF- α trimer (6OP0) [20], and VEGFR2 (4AG8) [21] were taken from the RCSB Protein Data Bank. Receptors were prepared with Modeller v10.8 [22], which rebuilt missing residues, loops and side-chain heavy atoms; polar hydrogens were added at pH 7.4. Structures were then converted to .pdbqt format with Gasteiger charges using MGLTools v1.5.6 [23] for use with latest modified version (V.1.2.7) of AutoDock Vina v1.2.0 [24].

Ligand structures were taken from PubChem as SMILES strings. Three-dimensional conformers were generated with RDKit (ETKDG method- Landrum G's RDKit available as open source) [25] and optimised with the MMFF94 force field [26], and the files were prepared with Open Babel v3.2.0 [27] and MGLTools. For COX-2, TNF- α and VEGFR2, the grid boxes were centred on the co-crystallised ligands (celecoxib [19], A7A [20] and axitinib [21], respectively). HPV16 E6 acts through protein–protein contacts and has no co-crystallised inhibitor, so the boxes for its E6AP-binding and p53-binding sites were built from binding-site residues reported in the literature [17]. Grid centres and sizes are listed in Table 1 below.

Table 1. Docking grid-box parameters for primary and secondary target protein and binding site

PDB ID	Target	Reference ligand for box definition	Grid centre (Å)	Grid size (Å)
4XR8	HPV16 E6 – E6AP-binding site	Anabsinthin, hypericin and paclitaxel (Literature-defined)	–60.9, –36.0, –53.5	72 $\times$ 56 $\times$ 59
4XR8	HPV16 E6 – p53-binding site	Anabsinthin, hypericin and paclitaxel (Literature-defined)	–54.3, –40.7, –65.1	56 $\times$ 46 $\times$ 42
3LN1	COX-2	Celecoxib (co-crystallised)	30.989, –22.284, –16.507	22.4 $\times$ 17.5 $\times$ 20.9
6OP0	TNF- α (trimer)	A7A (co-crystallised)	–12.817, –1.618, 19.009	22.1 $\times$ 18.9 $\times$ 19.7
4AG8	VEGFR2	Axitinib (co-crystallised)	20.824, 25.535, 39.46	25.8 $\times$ 19.8 $\times$ 19.3

2.8 Interaction analysis

Contacts between each docked ligand and the receptor were identified with the Protein-Ligand Interaction Profiler (PLIP) [28]. For E6, the contacting residues were compared with the E6AP-binding residues reported previously (Tyr32, Leu50, Val53, Arg55, Val62, Asp64, Leu67, Tyr70, Arg102, Ile128 and Arg131) [17], and with the contacts reported for paclitaxel, hypericin and anabsinthin [17]. For the secondary targets, the interactions of the docked compounds were compared with those of the co-crystallised ligands: celecoxib for COX-2 and A7A for TNF- α . For VEGFR2, they were compared with the combined contacts of four reference inhibitors: axitinib [21], sunitinib [21], brivanib [29] and pazopanib [30].

2.9 Physicochemical properties and ClinTox prediction

Lipinski's Rule of Five [26] was applied to all molecules, and ADME properties were predicted with ADMET-AI v2 [31]. The ClinTox model in this platform is based on the approach of Gayvert et al. [32], and drug-likeness was expressed as the quantitative estimate of drug-likeness (QED) [33].

3. Results and Discussion

3.1 Antimicrobial activity of the leaf and tuber extracts

Both extracts inhibited all three test organisms, although less strongly than the positive controls (Table 2, Figure 2). Against *S. aureus*, ampicillin gave a 29 mm zone, compared with 13 mm for AqML and 11 mm for AqMT. The same order was seen for *E. coli* (ampicillin 14 mm, AqML 10 mm, AqMT 9 mm). Fluconazole produced an 18 mm zone against *C. albicans*, whereas AqML and AqMT gave 14 mm and 9 mm. The leaf extract therefore outperformed the tuber extract against every organism, and the difference was greatest for the yeast.

Figure 2. Zones of inhibition produced by *D. transversa* leaf (AqML) and tuber (AqMT) extracts against *S. aureus*, *E. coli* and *C. albicans*. Ampicillin was the positive control for bacteria and fluconazole for *C. albicans*

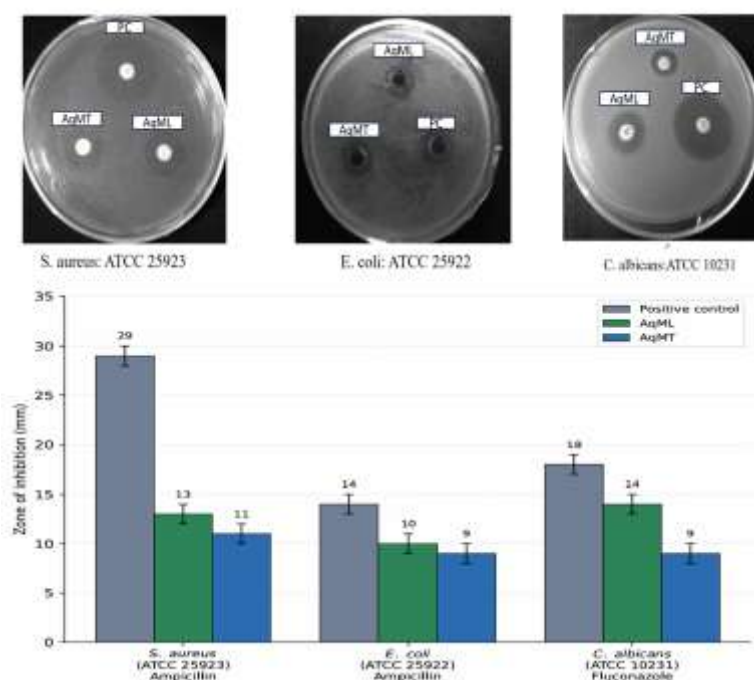


Table 2. Antimicrobial activity (zone of inhibition, mm) of *D. transversa* leaf (AqML) and tuber (AqMT) extracts.

Test organism	Positive control	Control zone (mm)	AqML (mm)	AqMT (mm)
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Test organism	Positive control	Control zone (mm)	AqML (mm)	AqMT (mm)
<i>S. aureus</i> (ATCC 25923)	Ampicillin	29 ±1	13 ±1	11 ±1
<i>E. coli</i> (ATCC 25922)	Ampicillin	14 ±1	10 ±1	9 ±1
<i>C. albicans</i> (ATCC 10231)	Fluconazole	18 ±1	14 ±1	9±1

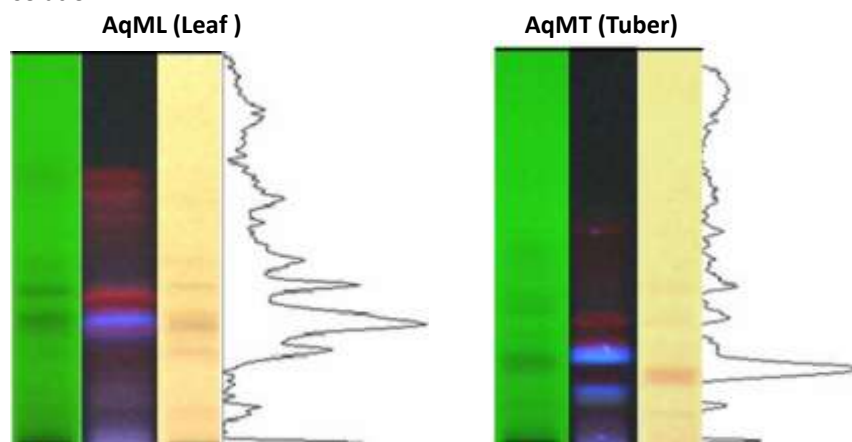
Values are means of three replicates. AqML, aqueous-methanolic leaf extract; AqMT, aqueous-methanolic tuber extract.

The larger zone of inhibition of the leaf agrees with its higher content of phenolics, tannins and flavonoids reported earlier [13], and with reports of phenolic-based antibacterial effects elsewhere in the genus, such as vanillic and isovanillic acids from *Dioscorea bulbifera* [34]. The large leaf–tuber difference against *C. albicans* is in line with earlier observations that semi-polar *Dioscorea* extracts act on this yeast better than purely aqueous ones [35, 36]. These results, together with the antioxidant and anti-inflammatory findings reported previously, supported the choice of the leaf for bioactivity-guided fractionation.

3.2 Chromatographic profile of the extracts

On normal-phase TLC, the leaf extract resolved into nine spots (R_f – 0.13, 0.24, 0.48, 0.53, 0.61, 0.69, 0.83, 0.85 and 0.97), whereas the tuber extract gave six (R_f – 0.13, 0.24, 0.48, 0.61, 0.69, 0.83) spots. Six R_f values were shared, and three (R_f 0.53, 0.85 and 0.97) were found only in the leaf (Figure 3). This chromatographic difference agrees with the earlier Folin–Ciocalteu and qualitative phytochemical results. The intense, closely spaced pair at R_f 0.48 and 0.53 was chosen for isolation because of its prominence and its peak height in the densitometric fingerprint.

Figure 3. HPTLC fingerprint of the leaf (AqML) and tuber (AqMT) extracts on normal-phase silica, viewed under UV light (254 and 366 nm) and in visible light after derivatisation. R_f 0.48 and 0.53 mark the bands selected for isolation.



3.3 Isolation of two compounds

Column chromatography on silica gel with the stepwise gradient described in Section 2.4, with TLC checks at each stage, gave fractions of matching R_f and purity. Recrystallisation produced two compounds with melting points of 348 °C (Isolated Fraction 1) and 151 °C (Isolated Fraction 2), close to the values reported for apigenin [37] and ϵ -viniferin (reported by multiple supplier's product page) respectively.

3.4 FTIR characterisation

FTIR was used to identify the functional groups of the two isolates before chromatographic confirmation (Table 3).

Fraction 1. The spectrum (Figure 4) showed a broad, strong band at 3312–3291 cm^{-1} from hydrogen-bonded phenolic O–H stretching, with weak aromatic C–H bands at 3106 and 3093 cm^{-1} . Weak absorptions at 2952–2625 cm^{-1} were attributed to overtone and combination bands of the hydroxyl system, as no strong aliphatic C–H band appeared. The most diagnostic feature was a strong, sharp band at 1654 cm^{-1} , assigned to a conjugated chromone C=O. Its position, 40–60 cm^{-1} below that of an isolated ketone, reflects conjugation with C2=C3 and the A ring plus hydrogen bonding to the 5-hydroxyl group, which is typical of 5-hydroxyflavones. Bands at 1608, 1590, 1557 and 1503 cm^{-1} arose from aromatic C=C stretching, the band at 1355 cm^{-1} from O–H bending, and those at 1269–1164 cm^{-1} from pyran C–O–C and phenolic C–O stretching. Bands at 831 and 806 cm^{-1} indicate a para-substituted B ring, consistent with a single hydroxyl at C-4'. These features fit a 5,7,4'-trihydroxyflavone, apigenin [38].

Fraction 2. The spectrum (Figure 5) was clearly different. A broad band at 3272 cm^{-1} indicated phenolic O–H, but the absence of significant absorption at 1650–1750 cm^{-1} rules out a flavone, flavonol or flavanone skeleton and points to a stilbenoid. Bands at 2921 and 2850 cm^{-1} indicate sp^3 C–H stretching. Because monomeric stilbenes such as resveratrol have no sp^3 carbons, these bands are consistent with the C-2 and C-3 methine carbons of a dihydrobenzofuran ring in resveratrol dimers, although aliphatic contaminants cannot be excluded by FTIR alone. Bands at 995 and 959 cm^{-1} show that the trans double bond is retained. Aromatic C=C stretching appeared at 1592, 1511, 1477 and 1440 cm^{-1} , O–H bending at 1363 cm^{-1} , and C–O bands at 1228, 1169, 1144, 1119 and 1050 cm^{-1} . The band at 828 cm^{-1} fits para-disubstituted rings, and those at 748, 720 and 691 cm^{-1} fit the meta-substituted resorcinol-type ring, both of which occur in ϵ -viniferin [39]. Weak signals at about 2680–1770 cm^{-1} lay on a flat baseline and were treated as noise.

In summary, Fraction 1 is a hydroxylated flavone with a conjugated chromone carbonyl and a para-substituted B ring, whereas Fraction 2 is a carbonyl-free dimeric stilbenoid with a trans olefinic bridge. These assignments agree with apigenin and ϵ -viniferin, the melting-point data (Section 3.3) and the HPLC results (Section 3.5).

Table 3. Principal FTIR bands (cm^{-1}) of the two isolated fractions and their assignments.

Fraction	Wavenumber (cm^{-1})	Assignment
1 (Apigenin)	3312–3291	O–H stretching, hydrogen-bonded phenolic OH
1	3106, 3093	Aromatic C–H stretching
1	1654	C=O stretching, conjugated chromone (5-OH hydrogen-bonded)
1	1608, 1590, 1557, 1503	Aromatic C=C stretching (A and B rings; C2=C3)
1	1355	Phenolic O–H bending
1	1269, 1245, 1224, 1183, 1164	C–O–C (pyran) and phenolic C–O stretching
1	831, 806	Out-of-plane C–H bending, para-substituted B ring
2 (ϵ - Viniferin)	3272	O–H stretching, phenolic OH
2	2921, 2850	sp^3 C–H stretching (dihydrobenzofuran C-2/C-3)

Fraction	Wavenumber (cm ⁻¹)	Assignment
2	1650–1750 region	No carbonyl absorption (excludes flavone/flavanone skeleton)
2	1592, 1511, 1477, 1440	Aromatic C=C stretching
2	1363	Phenolic O–H bending
2	1228, 1169, 1144, 1119, 1050	Aryl ether C–O–C and phenolic C–O stretching
2	995, 959	Out-of-plane C–H bending, trans olefin
2	828	para-Disubstituted aromatic ring
2	748, 720, 691	meta-Substitution (resorcinol-type ring)

The FTIR spectra of the two fractions pointed to different structural classes. Fraction 1 showed a strong conjugated carbonyl band at 1654 cm⁻¹, together with bands typical of a para-substituted B ring, as expected for a 5-hydroxyflavone such as apigenin [38]. Fraction 2 had no carbonyl absorption but showed trans C–H out-of-plane bending (995 and 959 cm⁻¹) and sp³ C–H stretching, as expected for a resveratrol dimer with a dihydrobenzofuran ring such as ε-viniferin [39].

Figure 4. FTIR spectrum of Isolated Fraction 1 (Apigenin).

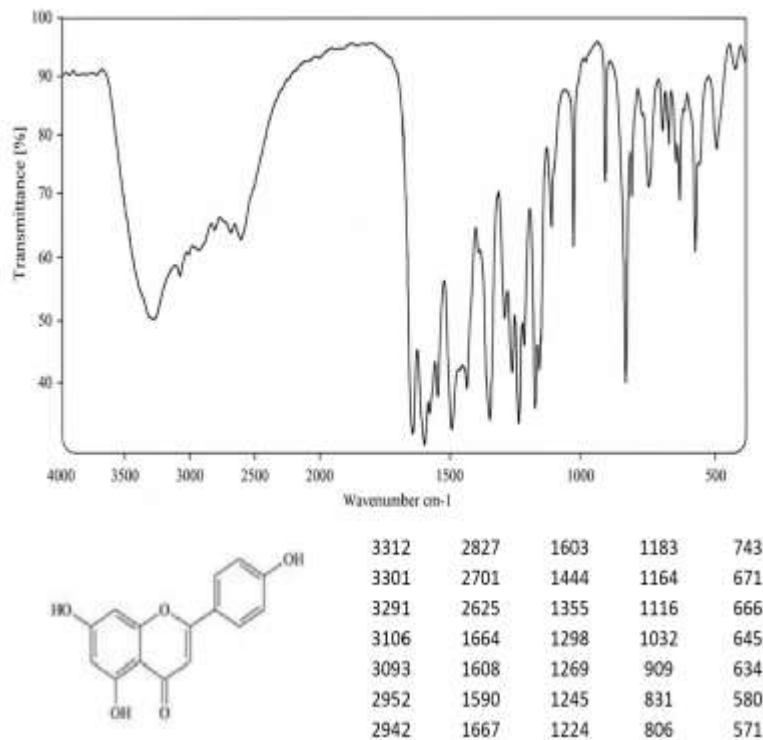
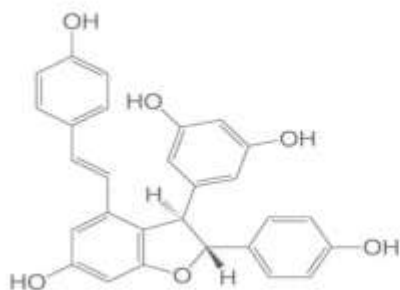
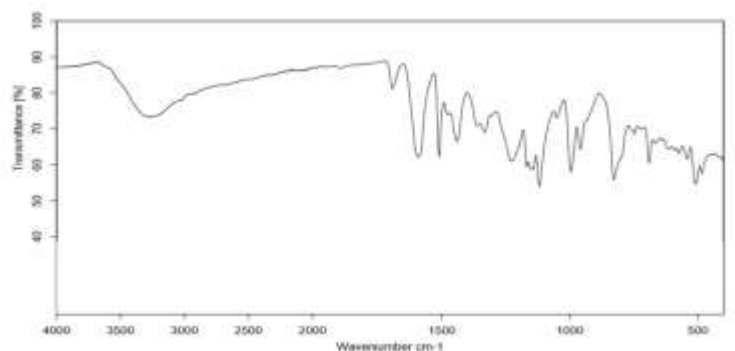


Figure 5. FTIR spectrum of Isolated Fraction 2 (ε-viniferin).



3272	1772	1050
3025	1693	996
2921	1592	959
2850	1511	828
2681	1477	748
2477	1440	720
2325	1363	691
2221	1333	666
2201	1308	640
1992	1294	592
1983	1228	574
1967	1169	509
1888	1144	485
1221 (Ctrl)	1119	451

3.5 HPLC confirmation

Each isolate gave one dominant peak. Isolated Fraction 1 eluted at 2.629 min matching the apigenin standard (Figure 6), and Isolated Fraction 2 eluted at 20.330 min, matching the ϵ -viniferin standard (Figure 7). Co-injection of each isolate with its standard gave a single, unsplit peak, showing co-elution and not a chance overlap of retention times. Retention-time matching, co-injection behaviour and melting-point agreement together support the identity of the isolates as apigenin (fraction 1), a widely distributed flavone, and ϵ -viniferin (fraction 2), a resveratrol-derived dimeric stilbenoid.

Figure 6. HPLC chromatograms confirming Isolated Fraction 1 as apigenin: (A) apigenin standard; (B) Isolated Fraction 1; (C) co-injection of Fraction 1 with the standard, showing a single unsplit peak. Retention times are given on each trace.

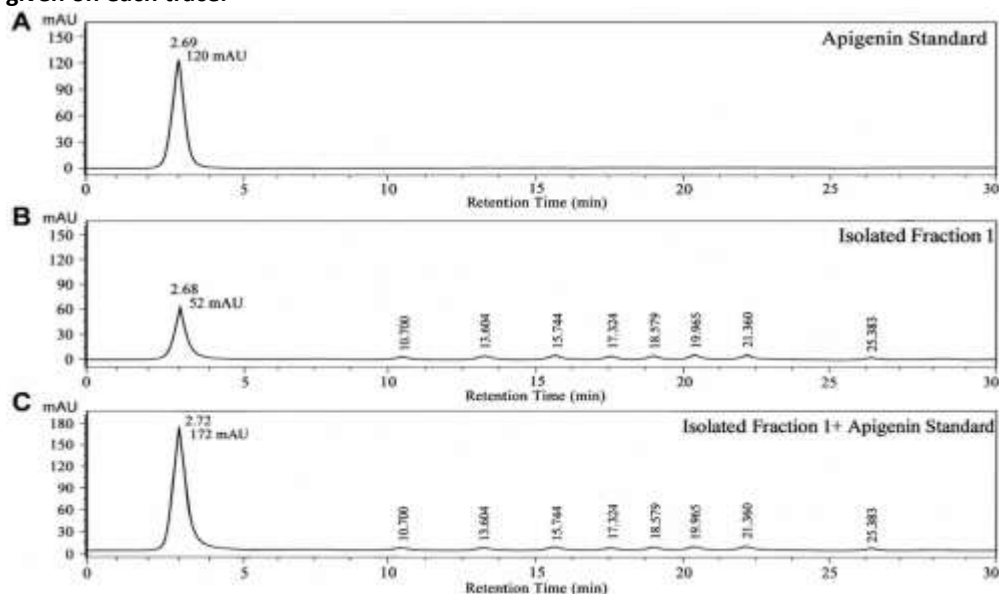
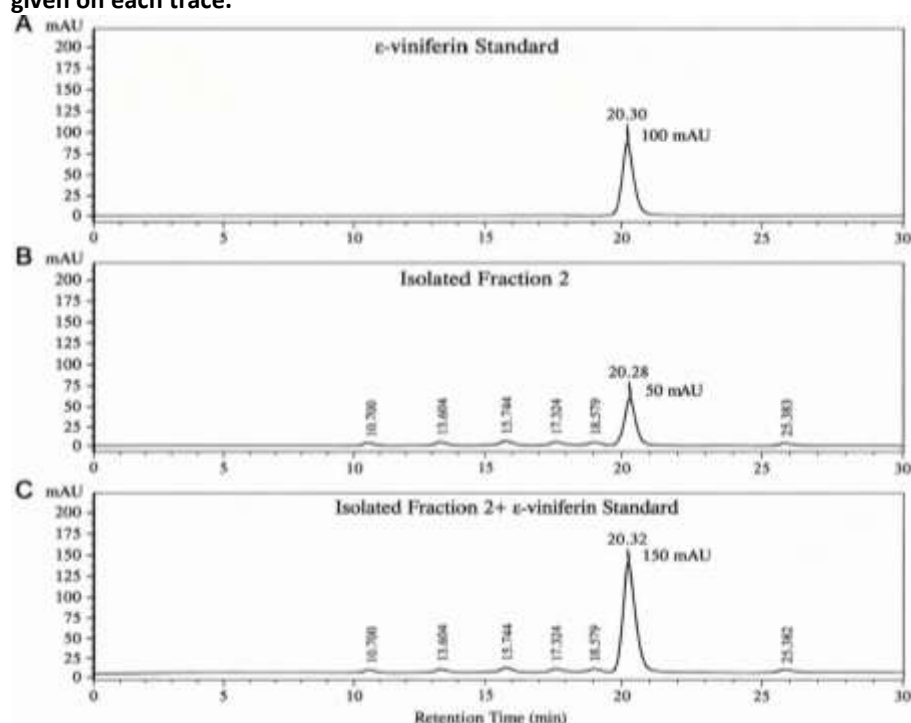


Figure 7. HPLC chromatograms confirming Isolated Fraction 2 as ϵ -viniferin: (A) ϵ -viniferin standard; (B) Isolated Fraction 2; (C) co-injection of Fraction 2 with the standard, showing a single unsplit peak. Retention times are given on each trace.



3.6 Docking at the two HPV16 E6 binding sites

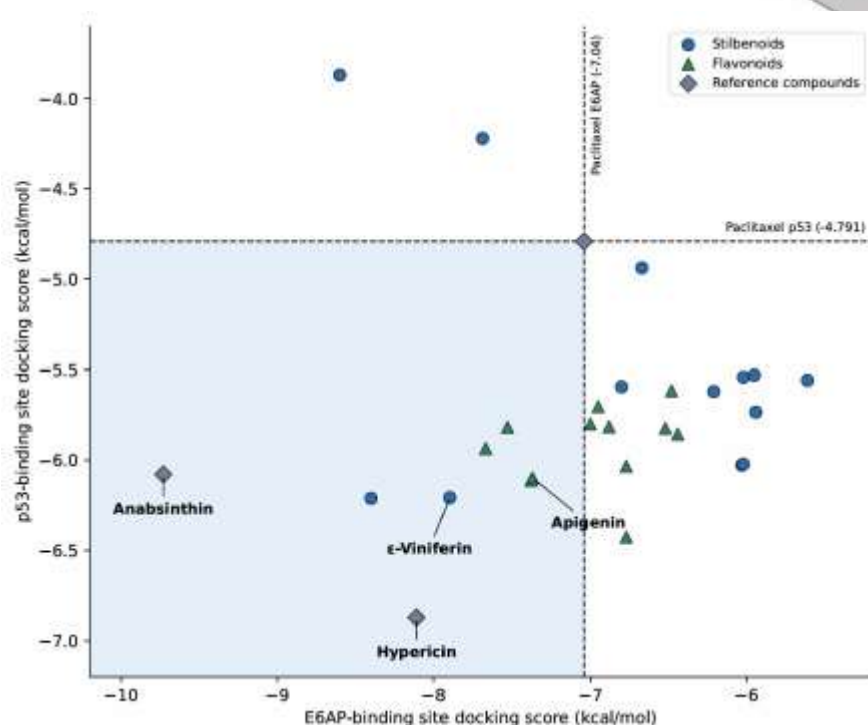
Scores for all the screened ranged from -5.610 to -8.600 kcal/mol at the E6AP site and from -3.870 to -6.426 kcal/mol at the p53 site. At these sites respectively, paclitaxel scored -7.040 and -4.791 , anabsinthin -9.730 and -6.078 , and hypericin -8.110 and -6.871 (Table 4).

Apigenin, ϵ -viniferin, kaempferol, naringenin and chrysin outsourced paclitaxel at both sites and are the two-site binders here (Table 4, Figure 8). Galangin ($-7.000/-5.799$) fell just short at the E6AP site and is treated as borderline. Hypericin and anabsinthin beat all five at the E6AP site, and hypericin did so at the p53 site too, so these hits are comparable to, not better than, the strongest literature references.

Four of the five are flavonoids (including a flavone, a flavonol and a flavanone). The fifth, ϵ -viniferin, is the only oligomeric stilbenoid at this level. None of the ten monomeric stilbenoids qualified, although pinosylvin and resveratrol matched or exceeded paclitaxel at the p53 site alone. In this series, dual-site affinity therefore appears to require a flavonoid skeleton or a cyclised dimeric stilbene rather than an open monomeric one.

Three resveratrol oligomers (ampelopsin, a trimer; miyabenol and hopeaphenol, tetramers) gave physically implausible positive COX-2 and TNF- α scores despite favourable E6 results. Their molecular weights (470–907) probably exceed what the small COX-2 and TNF- α grid boxes can hold, whereas the E6 boxes are much larger. This was not tested, and the three compounds were excluded from further analysis. Apigenin ($-7.370/-6.098$) and ϵ -viniferin ($-7.900/-6.207$), the two compounds isolated from *D. transversa* leaf, were both two-site binders.

Figure 8. Docking scores of the screened compounds at the E6AP-binding site and the p53-binding site of HPV16 E6. Dashed lines mark the paclitaxel scores; compounds in the lower-left quadrant (two-site binders) scored better than paclitaxel at both sites. Apigenin, ϵ -viniferin, and reference compounds are labelled.



3.7 Residue-level interactions at the two E6 sites

PLIP-derived contacts of the top compounds were compared with the eleven previously reported E6AP-site residues (Tyr32, Leu50, Val53, Arg55, Val62, Asp64, Leu67, Tyr70, Arg102, Ile128 and Arg131) [16,29] and with the contacts described for paclitaxel, hypericin and anabsinthin [17]. Redocked paclitaxel touched five of these (Leu50, Val53, Val62, Tyr32 and Arg102), whereas the original report described only Val53, through hydrophobic interaction. Hypericin (Tyr32, Tyr70) and anabsinthin (Val62, Leu67) were each reported to touch two residues, again hydrophobically.

Our compounds matched or exceeded this (Table 5, Figure 9). Apigenin and kaempferol contacted five reference residues, like redocked paclitaxel, while chrysin, naringenin and ϵ -viniferin contacted six. Several also formed direct hydrogen bonds, which are generally more directional than hydrophobic contact, with Tyr32 (kaempferol, naringenin, ϵ -viniferin), Tyr70 (chrysin), Arg102 (apigenin, ϵ -viniferin) and Arg131 (all five). Overall, the compounds engage the E6AP site at least as extensively as the positive control and the reported top hits.

At the p53 site, our poses involved Pro98 and Gly94, and ϵ -viniferin also hydrogen-bonded with Arg88 and Ser92. None of these matches the interface defined earlier [16,17]. However, the reference contacts reported there disagree with one another: paclitaxel (Arg55, Val62, Arg8), hypericin (Ile23, Ala46) and anabsinthin (Pro13, Cys16, Thr22, Ile23, Ala46) share only Ile23, and only between the last two. This absence of a reproducible footprint, even for the positive control, fits the structural evidence that the p53 groove is induced by E6AP binding rather than pre-formed on isolated E6 [16,17]. Within our panel, ϵ -viniferin made the most extensive contact at this site (two hydrogen bonds, versus a single Gly94 contact for the others). It is therefore the leading candidate here, pending docking against an E6–E6AP complex in which the p53 groove is properly formed.

Table 4. Docking scores (kcal/mol) of the two-site binders and reference compounds at the E6AP-binding and p53-binding sites of HPV16 E6.

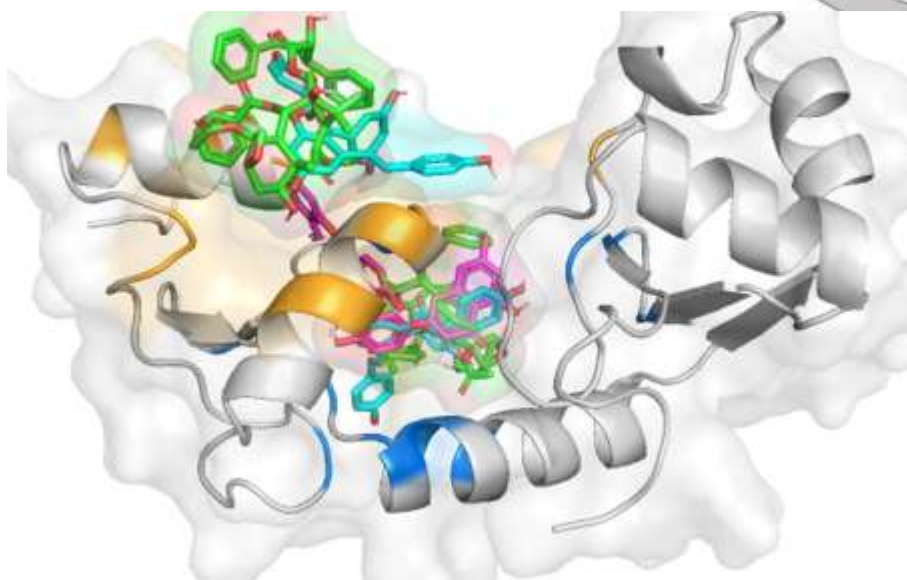
Compound	E6AP-binding site	p53-binding site	Remark
Apigenin	–7.370	–6.098	Isolated; two-site binder
ϵ -Viniferin	–7.900	–6.207	Isolated; two-site binder

Compound	E6AP-binding site	p53-binding site	Remark
Kaempferol	-7.530	-5.817	Two-site binder
Naringenin	-7.380	-6.111	Two-site binder
Chrysin	-7.670	-5.935	Two-site binder
Galangin	-7.000	-5.799	Borderline (E6AP site just above paclitaxel)
Paclitaxel	-7.040	-4.791	Reference compound; Two-site binder
Anabsinthin	-9.730	-6.078	Reference compound; Two-site binder
Hypericin	-8.110	-6.871	Reference compound; Two-site binder

Table 5. Contacts of the two-site binders and reference compounds with the eleven reported E6AP-site residues of HPV16 E6.

Compound	Reference residues contacted	Number (of 11)	Hydrogen bonds to reference residues
Paclitaxel (redocked)	Tyr32, Leu50, Val53, Val62, Arg102	5	—
Hypericin (reported [17])	Tyr32, Tyr70	2	None
Anabsinthin (reported [17])	Val62, Leu67	2	None
Apigenin	Leu50, Val62, Leu67, Arg102, Arg131	5	Arg102, Arg131
Kaempferol	Tyr32, Leu50, Val62, Arg102, Arg131	5	Tyr32, Arg131
Chrysin	Leu50, Val53, Val62, Leu67, Tyr70, Arg131	6	Tyr70, Arg131
Naringenin	Tyr32, Leu50, Val53, Val62, Leu67, Arg131	6	Tyr32, Arg131
ϵ -Viniferin	Tyr32, Leu50, Val53, Val62, Arg102, Arg131	6	Tyr32, Arg102, Arg131

Figure 9. Predicted binding of ϵ -viniferin (cyan), apigenin (magenta), and paclitaxel (green) at the E6AP-binding site and the p53-binding site of HPV16 E6 (PDB ID: 4XR8; grey cartoon). Active-site residues of the E6AP-binding site are shown in marine blue and those of the p53-binding site in orange.



3.8 Docking at Secondary targets - COX-2, TNF- α and VEGFR2

Apigenin was among the strongest COX-2 binders of the whole panel (–9.964 kcal/mol; redocked celecoxib –12.123 kcal/mol), kept strong affinity for TNF- α (–9.061 kcal/mol; A7A –12.897 kcal/mol) and bound VEGFR2 well (–9.825 kcal/mol). The VEGFR2 score was better than those of three of the four approved inhibitors tested (sunitinib –8.987, brivanib –9.382, pazopanib –9.379 kcal/mol) but not that of co-crystallised axitinib (–11.159 kcal/mol) (Table 6). Apigenin therefore shows affinity for all five binding sites examined. ϵ -Viniferin, by contrast, scored weakly at all three secondary targets (COX-2 –5.970; TNF- α –6.556; VEGFR2 –8.333 kcal/mol), which fits a profile restricted mainly to E6.

Table 6. Docking scores (kcal/mol) of apigenin, ϵ -viniferin and reference inhibitors at COX-2, TNF- α and VEGFR2.

Compound	COX-2	TNF- α	VEGFR2
Apigenin	–9.964	–9.061	–9.825
ϵ -Viniferin	–5.970	–6.556	–8.333
Celecoxib*	–12.123	—	—
A7A*	—	–12.897	—
Axitinib*	—	—	–11.159
Sunitinib*	—	—	–8.987
Brivanib*	—	—	–9.382
Pazopanib*	—	—	–9.379

*Reference or control compounds (ligands). More negative values indicate stronger predicted binding.

Interactions were compared with those of the reference ligands. At COX-2, celecoxib contacts Val318, Tyr324, Leu353, Trp356, Phe487, Val492 and Ala496 through hydrophobic contacts, forms hydrogen bonds with Gln161, Tyr324, Arg482, Ile486 and Phe487, and makes a π -cation interaction with Arg89 (eleven residues in all). ϵ -Viniferin reproduced the largest share of this pattern, contacting eight of the eleven residues (including Val318, Tyr324, Ile486, Phe487, Val492 and Ala496) and forming a hydrogen bond with Arg89, the residue that celecoxib engages by π -cation interaction. Apigenin, chrysin, kaempferol and naringenin each contacted six of the eleven residues, with a shared hydrophobic core (Val318, Trp356, Phe487) and a common hydrogen bond to Tyr324.

Figure 10. Docked poses of ϵ -viniferin (cyan) and apigenin (magenta) in the active site of human COX-2 (PDB ID: 3LN1; grey cartoon), overlaid on the co-crystallised ligand celecoxib (green). Active-site residues are shown in marine blue.

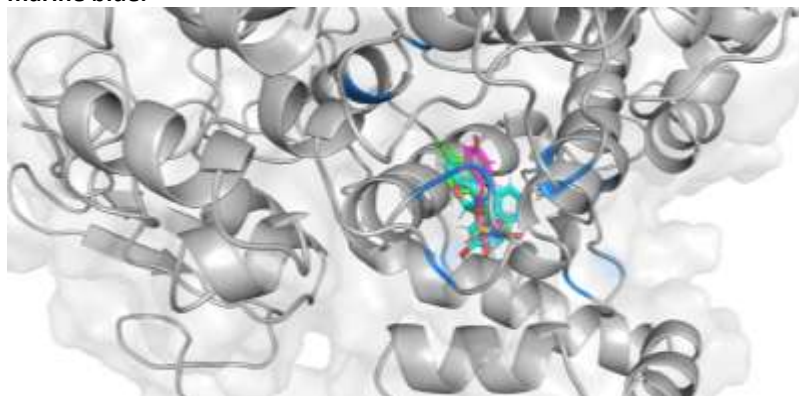
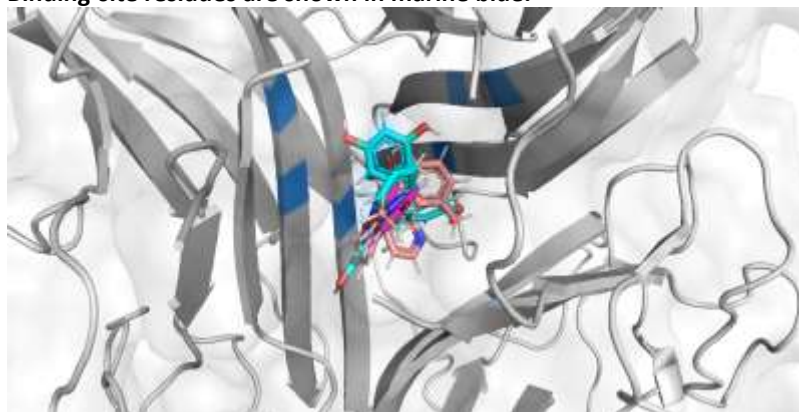


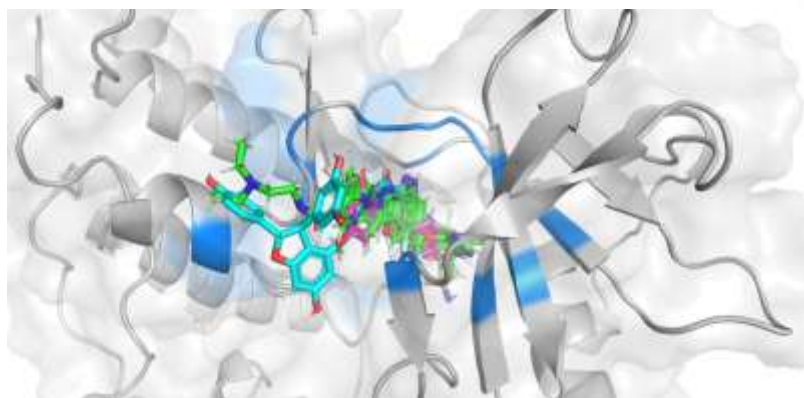
Figure 11. Docked poses of ϵ -viniferin (cyan) and apigenin (magenta) in the ligand-binding site of the TNF- α trimer (PDB ID: 6OP0; grey cartoon with transparent surface), overlaid on the co-crystallised ligand A7A (green). Binding-site residues are shown in marine blue.



At TNF- α , the co-crystallised inhibitor A7A occupies a compact six-residue footprint (Leu58, Tyr60, Tyr120, Val124, Leu158 and Tyr152) in a pocket at the interface of the TNF- α trimer, where it stabilises an asymmetric, signalling-incompetent form of the trimer [30]. ϵ -Viniferin contacted all six of these residues, including a hydrophobic contact with Val124 and π -stacking with Tyr60 that no other compound reproduced. Apigenin overlapped with five of six residues, chrysin with four, naringenin with three and kaempferol with two.

At VEGFR2, contacts were compared with the pooled contacts of axitinib, sunitinib, brivanib and pazopanib (seventeen residues). Kaempferol and naringenin overlapped most (eight residues each), followed by apigenin and chrysin (seven) and ϵ -viniferin (six). Every compound formed a hydrogen bond with Cys104, the hinge residue contacted by all four approved inhibitors, suggesting that all five adopt the ATP-competitive, hinge-binding orientation typical of type I/II VEGFR2 inhibitors. Lys53, which axitinib and brivanib engage by π -cation interaction, was contacted only by apigenin and kaempferol, and by hydrogen bonding, so the match at this residue is partial.

Figure 12. Docked poses of ϵ -viniferin (cyan) and apigenin (magenta) in the ATP-binding site of VEGFR2 (PDB ID: 4AG8; grey cartoon with transparent surface), overlaid on the control ligands (green). Active-site residues are shown in marine blue.



Although ϵ -viniferin had the weakest scores at all three secondary targets, its pose reproduced the reference contacts most closely at COX-2 and TNF- α , including the whole A7A footprint, so it occupies the same hot spots as established inhibitors even though its computed binding energy is less favourable. Kaempferol and naringenin, in contrast, matched the pooled VEGFR2 footprint best. The best-performing compound therefore depends on the target: ϵ -viniferin by pose similarity at COX-2 and TNF- α , kaempferol and naringenin at VEGFR2, and apigenin and chrysin as consistent second-line performers across all three.

3.9 Physicochemical properties and predicted toxicity

Comparison was restricted to the five two-site binders and the reference compounds. The four flavonoids (apigenin, kaempferol, naringenin, chrysin) met all four Lipinski criteria, whereas ϵ -viniferin met three of four, its logP (5.7) being slightly above 5 (Table 7). Several references broke more rules: hypericin met only one criterion and paclitaxel two, reflecting their larger and more complex structures (MW 504.5 and 853.9).

QED scores were higher for the flavonoids (apigenin 0.632, naringenin 0.742, kaempferol 0.546, chrysin 0.700) than for ϵ -viniferin (0.255), paclitaxel (0.130) or hypericin (0.123). By this composite measure, the flavonoid hits are predicted to be more drug-like than the benchmark compounds against which they were screened.

Predicted ClinTox probabilities were low for all four flavonoids (apigenin 0.024, kaempferol 0.029, chrysin 0.014, naringenin 0.080) and lower than those of every clinically used comparator: paclitaxel (0.345), celecoxib (0.334), axitinib (0.373), brivanib (0.167), pazopanib (0.426) and sunitinib (0.744), as well as the TNF- α reference A7A (0.539). Hypericin (0.019) was the only reference with a similarly low value. ϵ -Viniferin's score (0.075) was also favourable despite its larger size. These values are model-based predictions and not experimental toxicity data.

Table 7. Physicochemical properties and predicted ClinTox probability of the two-site binders and reference compounds.

Compound	MW	logP	HBA	HBD	TPSA ($\text{\AA}^2$)	Lipinski criteria met (of 4)	ClinTox
ϵ -Viniferin	454.5	5.7	6	5	110.4	3	0.075
Apigenin	270.2	2.6	5	3	90.9	4	0.024
Naringenin	272.3	2.5	5	3	87.0	4	0.080
Chrysin	254.2	2.9	4	2	70.7	4	0.014
Kaempferol	286.2	2.3	6	4	111.1	4	0.029
Hypericin*	504.5	5.1	8	6	155.5	1	0.019
Paclitaxel*	853.9	3.7	14	4	221.3	2	0.345
Axitinib*	386.5	4.6	4	2	70.7	4	0.373
Brivanib*	370.4	3.5	6	2	84.7	4	0.167

Compound	MW	logP	HBA	HBD	TPSA (Å ²)	Lipinski criteria met (of 4)	ClinTox
Sunitinib*	398.5	3.3	3	3	77.2	4	0.744
Pazopanib*	437.5	3.1	8	2	119.0	4	0.426
A7A*	407.5	5.1	5	0	48.5	3	0.539
Celecoxib*	381.4	3.5	4	1	78.0	4	0.334

*Reference or control compounds for the respective targets. MW, molecular weight; HBA, hydrogen-bond acceptors; HBD, hydrogen-bond donors; TPSA, topological polar surface area.

4. Discussion

This work extends our earlier characterisation of *D. transversa*, in which the leaf extract exceeded the traditionally eaten tuber in polyphenol content and in antioxidant, anti-inflammatory and anticancer activity. The leaf extract resolved into nine TLC bands against six for the tuber, three of them present only in the leaf, which indicates a broader chemical profile rather than simply a higher concentration. Selecting the most intense leaf-specific band pair led to apigenin and ϵ -viniferin, whose identity was supported by consistent melting-point, FTIR and HPLC co-injection data.

The larger inhibition zones of the leaf extract against *S. aureus*, *E. coli* and *C. albicans* are consistent with reported antimicrobial properties of flavonoids such as apigenin [40,41], stilbenoid oligomers such as ϵ -viniferin [42,43], and phenolic-based activity in other *Dioscorea* species [44]. Because crude extracts were tested at a single dose, this activity cannot be attributed to the isolated compounds.

The docking results provide a possible compound-level context for the extract's earlier anti-inflammatory and anti-cervical-cancer findings. E6 has been reported to raise VEGF promoter activity independently of p53 through Sp1 sites [45], and VEGFR2 has been linked to cervical cancer progression through DLL4/Notch signalling and to prognosis in early-stage disease [46]. Chronic inflammation involving COX-2 and TNF- α is also regarded as a co-factor in HPV persistence and progression [47,48]. Against this background, the predicted affinity of apigenin at both E6 sites and at COX-2, TNF- α and VEGFR2 raises the hypothesis that it may interact with several of these pathways, in line with the multi-activity profile of the extract. Any such effect would need experimental confirmation. ϵ -Viniferin showed a narrower, E6-focused profile. Although it scored lowest at the three secondary targets, it reproduced the reference-ligand contacts most closely at COX-2 and TNF- α . Docking scores and pose fidelity therefore ranked the compounds differently, and neither should be over-interpreted.

Several limitations apply. The screen used one scoring function, rigid receptors and a single pose per ligand. The large search boxes at the E6 sites (Table 1) may require higher exhaustiveness, and the three oligomers excluded for non-physical scores suggest that box size or exhaustiveness may need adjustment for large, flexible ligands. The p53-binding site was docked on E6 alone, although the groove forms fully only with E6AP bound, so findings at this site are tentative. Hypericin and anabsinthin outscored the isolated compounds at the E6AP site. No compound was tested experimentally against E6, and identities rest on comparison with authentic standards without NMR or mass spectrometry confirmation. The candidates (Tables 4–7) are therefore best regarded as hypotheses for prioritisation.

Future work could include molecular dynamics with MM-PBSA free-energy estimation for the two-site binders (apigenin, kaempferol, naringenin, chrysin and ϵ -viniferin), docking against an E6–E6AP complex, and laboratory studies such as a p53 rescue assay in HPV-positive cells. Finally, the leaf, rather than the tuber used in postnatal preparations in northern Kerala, appears to be the source of these two phytochemicals, which suggests it merits further attention as an under-used part of this ethnomedicinal species.

5. Conclusion

Zones of inhibition against *S. aureus*, *E. coli* and *C. albicans* were larger for the leaf extract of *D. transversa* than for the tuber extract, in agreement with earlier reports of stronger antioxidant, anti-inflammatory and anticancer

properties in the leaf. Fractionation guided by TLC of the two most intense leaf-specific bands gave apigenin and ϵ -viniferin, which were identified by melting point, FTIR, and HPLC retention time with co-injection. In docking against HPV16 E6 (E6AP-binding and p53-binding sites), COX-2, TNF- α and VEGFR2, both compounds scored better than paclitaxel at the two E6 sites. Apigenin showed binding to the three host targets, while ϵ -viniferin was largely confined to E6. Apigenin additionally fulfilled Lipinski's criteria and had low predicted ClinTox risk. Based on the docking results, apigenin and ϵ -viniferin are regarded as computationally prioritized leads, not experimentally validated anti-HPV agents. The findings suggest a possible compound-level basis for the traditional use of *D. transversa*. Further evaluation by molecular dynamics simulations, laboratory experiments, and preclinical and clinical studies would help to assess these predictions

6. Author Contributions

V.M. and K.G.G. conceived and designed the study. K.G.G. and N.C. carried out the extraction, antimicrobial activities; M.R. performed the chromatographic and spectroscopic experiments; A.M and K C. performed the molecular docking and the in silico physicochemical and ClinTox analyses; V.M. supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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8. Conflict of Interest:

The authors declare no conflict of interest.

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