Novel anti-hepatitis B virus flavonoids sakuranetin and velutin from *Rhus retinorrhoea*

SARFARAZ AHMED[1], MOHAMMAD K. PARVEZ[1], MOHAMMED S. AL-DOSARI[1], MAZIN A.S. ABDELWAHID[2], TAWFEQ A. ALHOWIRINY[1] and ADNAN J. AL-REHAILY[1]

[1]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; [2]Department of Organic Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8678, Japan

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Abstract. Drug-resistance in hepatitis B virus (HBV), especially due to prolonged treatment with nucleoside analogs, such as lamivudine (LAM), remains a clinical challenge. Alternatively, several plant products and isolated phytochemicals have been used as promising anti-HBV therapeutics with no sign of resistance. Among all known *Rhus* species, *R. coriaria*, *R. succedanea* and *R. tripartite* have been widely studied for their anti-HBV efficacy, however, the effects of *R. retinorrhoea* have not been previously investigated. The current study reported the isolation of two flavonoids, namely sakuranetin (SEK) and velutin (VEL), from the dichloromethane fraction of *R. retinorrhoea* aerial parts using chromatography and spectral analyses. The two flavonoids (6.25-50 μg/ml) were pre-tested for non-hepatocytotoxicity using an MTT assay and their dose- and time-dependent inhibitory activities against HBV [hepatitis B surface antigen (HBsAg) and hepatitis B 'e' antigen (HBeAg)] in cultured HepG2.2.15 cells were assessed by ELISA. SEK and VEL at the selected doses (12.5 μg/ml) significantly inhibited HBsAg by ~58.8 and ~56.4%, respectively, and HBeAg by ~55.5 and ~52.4%, respectively, on day 5. The reference drugs LAM and quercetin (anti-HBV flavonoids), suppressed the production of HBsAg/HBeAg by ~86.4/~64 and ~84.5/~62%, respectively. Furthermore, molecular docking of the flavonoids with HBV polymerase and capsid proteins revealed the formation of stable complexes with good docking energies, thus supporting their structure-based antiviral mechanism. In conclusion, the present study was the first to demonstrate the anti-HBV therapeutic activities of SEK and VEL isolated from *R. retinorrhoea*.

Correspondence to: Professor Mohammad K. Parvez, Department of Pharmacognosy, College of Pharmacy, King Saud University, 23 King Khalid Road, Riyadh 11451, Saudi Arabia
E-mail: mohkhalid@ksu.edu.sa; khalid_parvez@yahoo.com

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Introduction

The genus *Rhus* (family Anacardiaceae) is composed of >250 species of flowering plants, which are widely distributed in temperate and tropical regions, including Saudi Arabia (1). Globally, several *Rhus* spp. are traditionally known for their medicinal value (2,3). While *Rhus tripartita* has been used to treat inflammatory, cardiovascular and gastrointestinal diseases (4-6), *R. glabra* is known for its anti-microbial activities (7) and *R. coriaria* for its wound healing capacity (8). In addition, previous studies on *R. tripartita, R. verniciflua* and *R. retinorrhoea* identified several pharmacologically significant flavonoids and anthocyanins (3,9-15). Notably, three *Rhus* spp., *R. abyssinica*, *R. retinorrhoea* and *R. tripartita* growing in Saudi Arabia have been documented (16). It has been reported that *R. retinorrhoea* exhibits anti-oxidative (17), as well as weak anti-malarial effects (11). Further phytochemical analyses identified several other compounds, such as persicogenin, velutin (VEL), trihydroxy-7-methoxyflavanone and homoeriodictyol (18), as well as di-*O*-methyltetrahydroamentoflavone, 7-*O*-methylnaringenin, 7,3'-*O*-dimethylquercetin, 7-*O*-methylapigenin and 7-*O*-methylluteolin (11). A previous study from our laboratory reported the identification of SEK in aerial parts of *R. retinorrhoea* by quantitative high-performance thin layer chromatograph (19).

Liver diseases constitute a major public health problem. Therefore, the use of herbal or plant products to treat hepatic disorders has gained increasing attention in phytomedicine (20). Globally, liver infection by hepatitis B virus (HBV), which may progress to cirrhosis and hepatocellular carcinoma, can cause fulminant and chronic conditions in >350 million individuals (21,22). Despite the efficacy of the currently available anti-HBV drugs, drug-resistance mediated by prolonged therapy with HBV polymerase (POL) inhibitors, such as lamivudine (LAM), acyclovir and adefovir, remains a clinical challenge (23). In view of this, several bioactive phytochemicals of different classes, such as alkaloids, flavonoids, polyphenols, lignans, terpenes and anthraquinones have been identified as promising and non-resistant anti-HBV drug candidates (24-30). Notably, a previous study showed that *R. coriaria* could inhibit the production of HBV proteins in cultured hepatocytes (31). In addition, robustaflavone derived from *R. succedanea* (32) and catechins derived from

R. tripartite (33) could serve as potential inhibitors of HBV activities in HBV-reporter HepG2.2.15 cells. Notably, to the best of the authors' knowledge, the anti-HBV efficacy of *R. retinorrhoea* or its phytoconstituents remain unknown. Therefore, the present study aimed to evaluate the inhibitory potential of the flavonoids SEK and VEL isolated from *R. retinorrhoea* against HBV in HepG2.2.15 cells, supported by structure-based molecular docking studies.

Materials and methods

Plant material collection. The aerial parts of *R. retinorrhoea* Steud, ex Olive, locally known as 'Sumac/Heishar' were collected from the southern region of Saudi Arabia in March 2009. The plant material was authenticated (voucher specimen no. 15371) by Dr. Mohammad Yusuf, a plant taxonomist at College of Pharmacy, King Saud University Riyadh.

Extraction, fractionation and isolation of compounds from R. retinorrhoea. The ethanolic extract of the aerial parts of the plant was further fractionated in dichloromethane following the isolation of several known or new compounds belonging to different classes of phytochemicals. The majority of these were either obtained in very low quantity or were unsuitable candidate for testing against HBV. Based on available literature on their structural similarity and non-cytotoxic flavonoids reported against other viruses, two compounds were finally selected, namely C251 and C253, as previously described (11,18). For structure elucidation, ^{1}H and ^{13}C, and 2D nuclear magnetic resonance (NMR) spectroscopy of C251 and C253 were recorded at 700 and 175 MHz, respectively, on the Bruker Avance spectrometer (Bruker BioSpin GmbH) equipped with a 5-mm cryoprobe, in deuterated DMSO, using standard pulse programs. All organic solvents were purchased from Sigma-Aldrich (Merck KGaA).

Cell culture and drugs. HepG2.2.15 cells, which were established by stably transfecting human hepatoma HepG2 cells with the full genome of HBV, were generously provided by Dr S. Jameel (Virology Group, ICGEB, New Delhi, India). HepG2.2.15 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific Inc.) supplemented with 10% bovine serum albumin (Gibco; Thermo Fisher Scientific, Inc.) and 1X penicillin-streptomycin solution (HyClone; Cytiva) at 37˚Cin an incubator with 5% CO_2. Prior to treatment, cells at a density of $0.5x10^5$/100 μl/well were grown overnight in a 96-well plate (Corning, Inc.). LAM triphosphate (or 3TC) and quercetin (QRC; both from Sigma-Aldrich; Merck KGaA) served as positive controls, as previously described (26-30). For consistency and reproducibility, the assays were performed in duplicate.

Liver cell viability or toxicity assay. Although the *Rhus* spp., including *R. tripartite*, are known to be non-toxic (2,3), the optimal non-cytotoxic doses of the isolated compounds were first assessed in HepG2.2.15 cells. Briefly, compounds dissolved in DMSO (Sigma-Aldrich; Merck KGaA) were prepared in RPMI-1640 to produce four doses with 6.25, 12.5, 25 and 50 μg/ml of each compound. Following HepG2.2.15 cell incubation overnight, the culture medium was replaced with treatment media (in triplicate), including negative control (0.1% DMSO) medium, followed by incubation at 37˚C for 72 h. The cells were periodically monitored directly under an inverted microscope. Subsequently, cells were treated with MTT solution (TACS MTT Cell Proliferation Assay Kit; Sigma-Aldrich; Merck KGaA), according to the manufacturer's instructions. The optical density at a wavelength of 570 nm was measured using the Elx800 microplate reader (BioTek Instruments, Inc.). The results were analyzed in Excel 2010 (Microsoft Corp.) and presented in relation to the negative control.

HBV surface or envelop protein (HBsAg) inhibition assay. Initially, HBsAgs were dose-dependently inhibited (6.25, 12.5 and 25 μg/ml) by the isolated compounds to determine the maximally active concentration. HepG2.2.15 cells were cultured overnight and the culture medium was then replaced with treatment medium, including negative and positive control media, and incubated for an additional two days (a single time-point). Following the determination of the maximal dose, the time-dependent inhibition of HBsAg by the compounds was then assessed. HepG2.2.15 cells were treated with 25 μg/ml SEK or VEL and the corresponding controls, and incubated for several days. The culture was directly monitored every day under microscope and it was replenished with treatment media every alternate day. The culture supernatants collected and clarified (150 x g; 5 min; 22˚C) on day 1, 3 and 5 were quantitatively analyzed for HBsAgs using the diagnostic HBsAg ELISA kit (cat. no. 72348; Monolisa HBs Ag ULTRA assay; Bio-Rad Laboratories Inc.) in a microplate, according to the manufacturer's protocol. The optical density of the samples at a wavelength of 450 nm was measured and the results were then analyzed in relation to the negative control (Excel software 2010; Microsoft Corp.) and compared with the positive control.

HBV pre-core protein (HBeAg) inhibition assay. The treated culture supernatants collected and clarified (150 x g; 5 min; 22˚C) on day 1, 3 and day 5 were also quantitatively analyzed for HBeAg production using a HBeAg ELISA kit (cat. no. KAPG4BNE3; HBeAg/Anti-HBe Elisa Kit; DIAsource ImmunoAssays SA) according to the manufacturer's instructions. The recorded optical density (λ=450 nm) of the samples were analyzed in relation to the negative control (Excel software 2010; Microsoft Corp.), and compared with the positive control. All samples were tested in triplicate and the experiment was repeated for two times.

Molecular docking analysis. Based on their promising anti-HBV activities in cultured cells, VEL and SAK were further subjected to virtual structure-activity analysis to uncover the potential mechanisms underlying their inhibitory effects. The viral POL and CORE proteins served as target drugs, while their respective inhibitor molecules LAM and heteroaryldihydropyrimidine (HAP) acted as standard ligands (28,34). Notably, in the absence of crystallographic data or 3D model for HBV POL, an in-house constructed POL structure was used, as previously described (28). The available 3D structures of HBV CORE (PDB code, 5E0I; https://www.rcsb.org/) and the ligands LAM, VEL and

Table I. The ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy data for the isolated compounds C251 and C253 in deuterated DMSO.

	C251 (Sakuranetin)		C253 (Velutin)	
Carbon no.	^{1}H(J in Hz)	^{13}C	^{1}H(J in Hz)	^{13}C
2	5.51 dd (2.8,2.8)	79.1	-	164.3
3	2.7dd (2.8, 2.8) 2.53 (merge in solvent peak)	42.4	6.98 s	103.8
4	-	197.5	-	182.4
5	-	163.4	-	161.6
6	6.09 d (2.8)	95.1	6.38 d (2.1)	98.4
7	-	167.9	-	165.6
8	6.11 d (2.8)	94.3	6.38 d (2.8)	93.1
9	-	163.7	-	157.7
10	-	103.1	-	105.1
1'	-	129.2	-	121.8
2'	7.34 d (8.4)	128.9	7.60 brs	110.7
3'	6.81 d (8.4)	115.6	-	148.5
4'	-	158.3	-	151.3
5,	6.81 d (8.4)	115.6	6.94d (8.4)	116.2
6'	7.34 d (8.4)	128.9	7.61d (2.1)	120.9
OCH3	3.79 s	56.5	3.88 s	56.5
OCH3	-	-	3.91 s	56.4
5-OH	12.12	-	12.98	-
4'-OH	9.61	-	10.01	-

SAK (https://pubchem.ncbi.nlm.nih.gov/) were retrieved. The target proteins were prepared by removing any solvent molecules or co-crystallized ligands and via adding hydrogen atoms and Kollman charges (28). For docking, the published catalytic or active residues of LAM (28) and CORE (34) were confirmed using the SEINA program (35). The two target proteins were prepared and energy-minimized in Maestro software (36). The ligand-target interactions were visualized using the 2D (Maestro) and 3D (UCSF ChimeraX) modes (37). The ligands were docked onto their corresponding target binding pocket or active site using AutoDock Vina 1.2.3 software (38,39).

Statistical analysis. All data were analyzed using SPSS 17.0 (SPSS Inc.). Data are expressed as the mean ± SEM of three independent experiments. The results were compared with the negative control group using one-way ANOVA followed by Dunnett's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Structure determination of the compounds isolated from R. retinorrhoea. The two isolated compounds, 251 and 253, which were subjected to ^{1}H and ^{13}C, and 2D NMR analyses (Table I; Figs. S1-S12), were identified as the structurally-related flavonoids SEK (4',5-dihydroxy-7-methoxyflavanone) and VEL (5,4'-dihydroxy-7,3'-dimethoxyflavone), respectively (Fig. 1; upper panel).

Non-cytotoxic effects of SEK and VEL. MTT assay demonstrated that the flavonoids SAK and VEL did not show any hepatotoxicity in cells cultured for 72 h even at the maximal dose tested (Fig. S13). This was consistent with the microscopic observation of cells, as treated cells exhibited intact morphology as with negative cells. Therefore, the 50% cytotoxicity concentration (CC_{50}) values could not be determined.

SEK and VEL inhibit HBsAg synthesis. Optimal dose assessment revealed that SAK and VEL at a dose of 25 μg/ml showed the maximal inhibition of HBsAg on day 2 (Fig. 1; lower panel). However, at a dose of 50 μg/ml, no significant increase in the inhibitory activities of VEL and SAK was observed (data not shown). Therefore, a dose of 25 μg/ml was selected as the optimally active dose for the time-course study. Among the three selected time-points (day 1, 3 and 5), the maximal inhibition rate of SAK and VEL on HBsAg synthesis was ~58.8 and ~56.4%, respectively, on day 5 (Fig. 2). In comparison, LAM and QRC inhibited HBsAg by ~86.4 and ~84.5%, respectively. Notably, since cell treatment with flavonoids at the maximal dose also enhanced cell proliferation and overgrowth-mediated apoptosis (data not shown), the assay was carried out at day 5.

SEK and VEL suppress HBV replication. Synthesis of HBeAg is a serological gold marker of HBV DNA replication in patients with HBV (21). Therefore, the inhibitory effect of SEK and VEL (25 μg/ml, each) on HBeAg expression in treated HepG2.2.15 cells was further analyzed. Of the analyzed

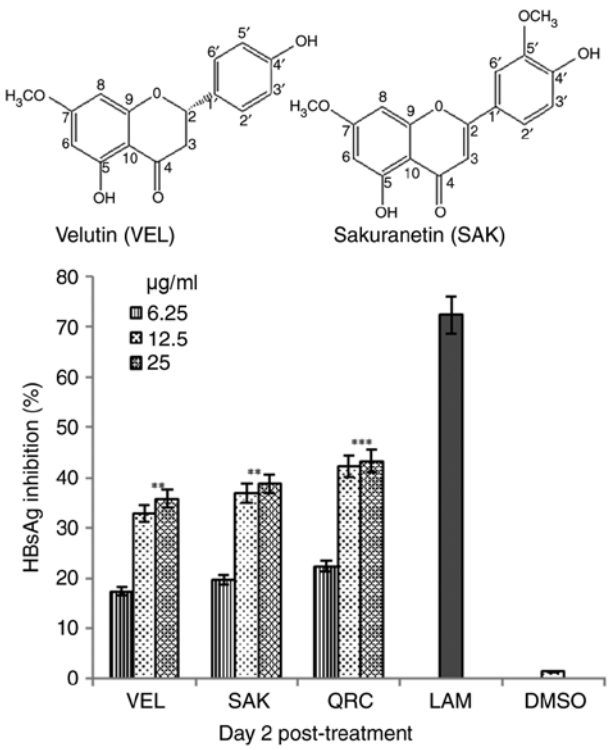

Figure 1. *Rhus retinorrhoea*-derived flavonoids VEL and SAK (upper panel), showing dose-dependent (6.25, 12.5 and 25 μg/ml) inhibition of HBV surface or HBsAg synthesis in treated HepG2.2.15 cells at day 2 (lower panel). QRC; 12.5 μg/ml and LAM; 2 μM served as positive controls while DMSO (0.1%) acted as negative or vehicle control. Data are presented as the mean ± standard error of the mean (n=3). **P<0.01, ***P<0.001 vs. LAM. VEL, velutin; SAK, sakuranetin; HBV, hepatitis B virus; sAg, 's' antigen; QRC, quercetin; LAM, lamivudine.

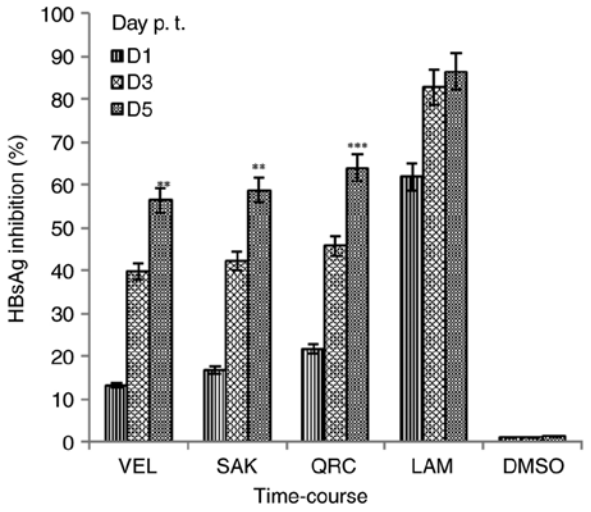

Figure 2. Time-course inhibitions of HBV surface or HBsAg by *Rhus retinorrhoea*-derived VEL and SAK at the optimal selected dose (12.5 μg/ml, each) at day 1, 3 and 5 p. t. in HepG2.2.15 cells. QRC; 12.5 μg/ml and LAM; 2 μM served as positive controls while DMSO (0.1%) acted as negative or vehicle control. Data are presented as the mean ± standard error of the mean (n=3). **P<0.01, ***P<0.001 vs. LAM. HBV, hepatitis B virus; sAg, 's' antigen; VEL, velutin; SAK, sakuranetin; p. t., post-treatment; QRC, quercetin; LAM, lamivudine; D, day.

time-points (day 1, 3 and 5), the maximal inhibition rate in HBeAg production was ~55.5% by SAK and ~52.4% by VEL

Table II. Estimated docking energies (kcal M^{-1}) of anti-hepatitis B virus active flavonoids and [a]standards.

	Hepatitis B virus target proteins	
Ligands	Polymerase	Capsid
Velutin	-8.092	-9.079
Sakuranetin	-7.502	-8.526
Lamuvidine triphosphate[a]	-9.245	
Heteroaryldihydropyrimidine[a]		-8.876

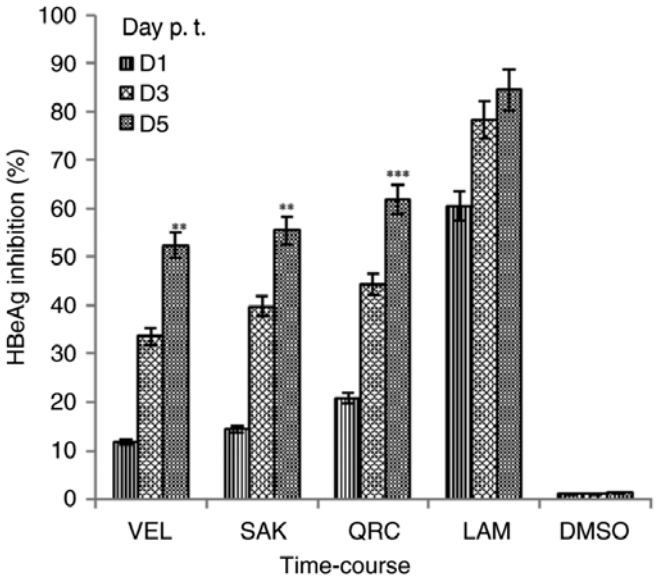

Figure 3. Time-course inhibitions of HBV pre-core or HBeAg by *Rhus retinorrhoea*-derived VEL and SAK at the optimal selected dose (12.5 μg/ml, each) at day 1, 3 and 5 p. t. in HepG2.2.15 cells. QRC; 12.5 μg/ml and LAM; 2 μM served as positive controls while DMSO (0.1%) acted as negative or vehicle control. Data are presented as the mean ± standard error of the mean (n=3). **P<0.01, ***P<0.001 vs. LAM. HBV, hepatitis B virus; eAg, 'e' antigen; VEL, velutin; SAK, sakuranetin; p. t., post-treatment; QRC, quercetin; LAM, lamivudine; D, day.

on day 5 (Fig. 3). Comparatively, LAM and QRC suppressed HBeAg generation by ~64 and ~62%, respectively. As aforementioned, since flavonoids could promote cell overgrowth and apoptotic death, the assay was performed on day 5.

Structure-based interactions of the isolated flavonoids with HBV proteins. The two isolated anti-HBV active flavonoids, VEL and SAK, were virtually docked into the binding pocket of POL and CORE proteins. The results revealed good re-alignments of the ligands. Docking of LAM and HAP generated complexes with good docking energies and orientations, thus indicating a good docking protocol (Fig. 4; Table II). Owing to their common flavonoid structure, VEL and SEK acquired relatively similar alignment and orientations inside the binding site of POL (Fig. 5; Table II) and CORE (Fig. 6; Table II). In addition, both flavonoids shared interactions with key active residues of the target proteins.

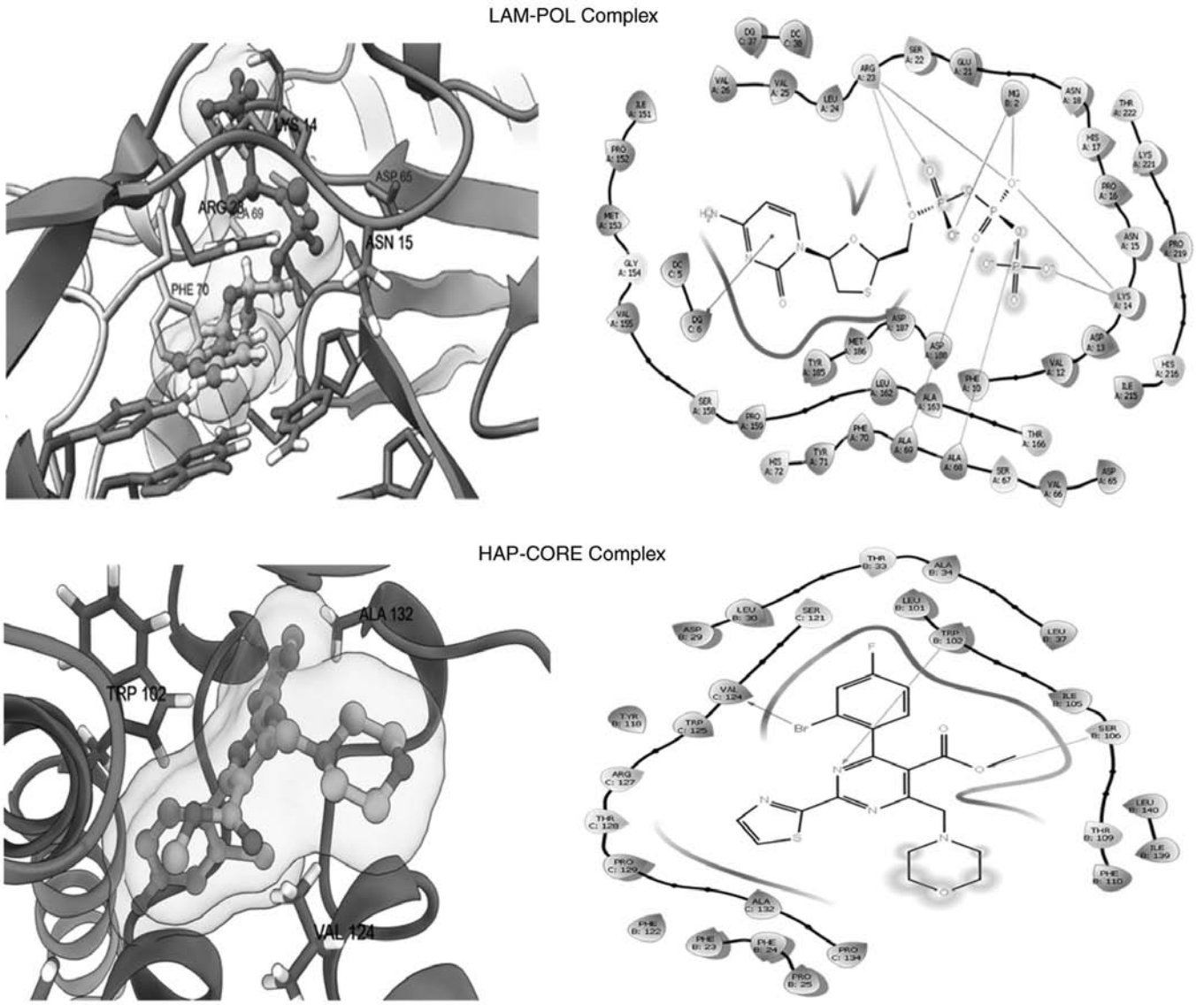

Figure 4. Molecular docking analysis showing 3D and 2D interactions of LAM (standard; control ligand) with POL (upper panel) as well as HAP (standard; control ligand) with CORE (lower panel). LAM, lamivudine; HBV, hepatitis B virus; POL, HBV polymerase; HAP, heteroaryldihydropyrimidine; CORE, HBV capsid.

Notably, similar to the negative charges of the triphosphate group, which significantly interacted with positive charged residues at Arg23 and Lys14 in the LAM-POL complex, and in coordination with Mg^{+2} (Fig. 4; upper panel), the oxygen atoms of VEL and SEK showed the same interactions in the VEL-POL and SAK-POL complexes, respectively (Fig. 5). The VEL-POL complex was further stabilized by π-cation with Lys14 and π-stacking with Phe70 (Fig. 5; upper panel). In addition to the POL catalytic 'Tyr-Met-Asp-Asp' motif residues, other surrounding residues, such as those at Ser67 and Ala68, could also be involved in the stability of the VEL-POL and SAK-POL complexes. Nonetheless, LAM (standard) showed a more potent binding affinity compared with both VEL and SAK, which could be due to its more efficient electrostatic interactions (Table II).

Regarding docking with the HBV-CORE protein, both VEL and SAK formed complexes with very close poses (Fig. 6), while VEL displayed a higher binding affinity compared with SEK (Table II). The two ligands shared H-bonding with Ala132 and π-stacking with Trp102. Notably, HAP (standard) could also interact with Trp102 through H-bonds. Other surrounding residues, such as those at Val124 and Ser106 could also contribute to the VEL-HAP and SAK-HAP complex stabilities. Taken together, the molecular docking data suggested that the activities of VEL and SEK against HBV could be mediated by the inhibition of the viral POL and CORE proteins.

Discussion

Flavonoids are a class of phytochemical polyphenols, which are further subclassified into flavonols, flavanones, flavones, chalcones, anthocyanidins and catechins (40). In addition to their known health benefits, it has been reported that several flavonoids exhibit therapeutic potentials against several viruses (41,42), including HBV (24,25,27-30). In the current study, the anti-HBV activities of the two structurally-similar *R. retinorrhoea*-derived flavonoids, namely SEK and VEL, were evaluated using a HBV-reporter cell culture model.

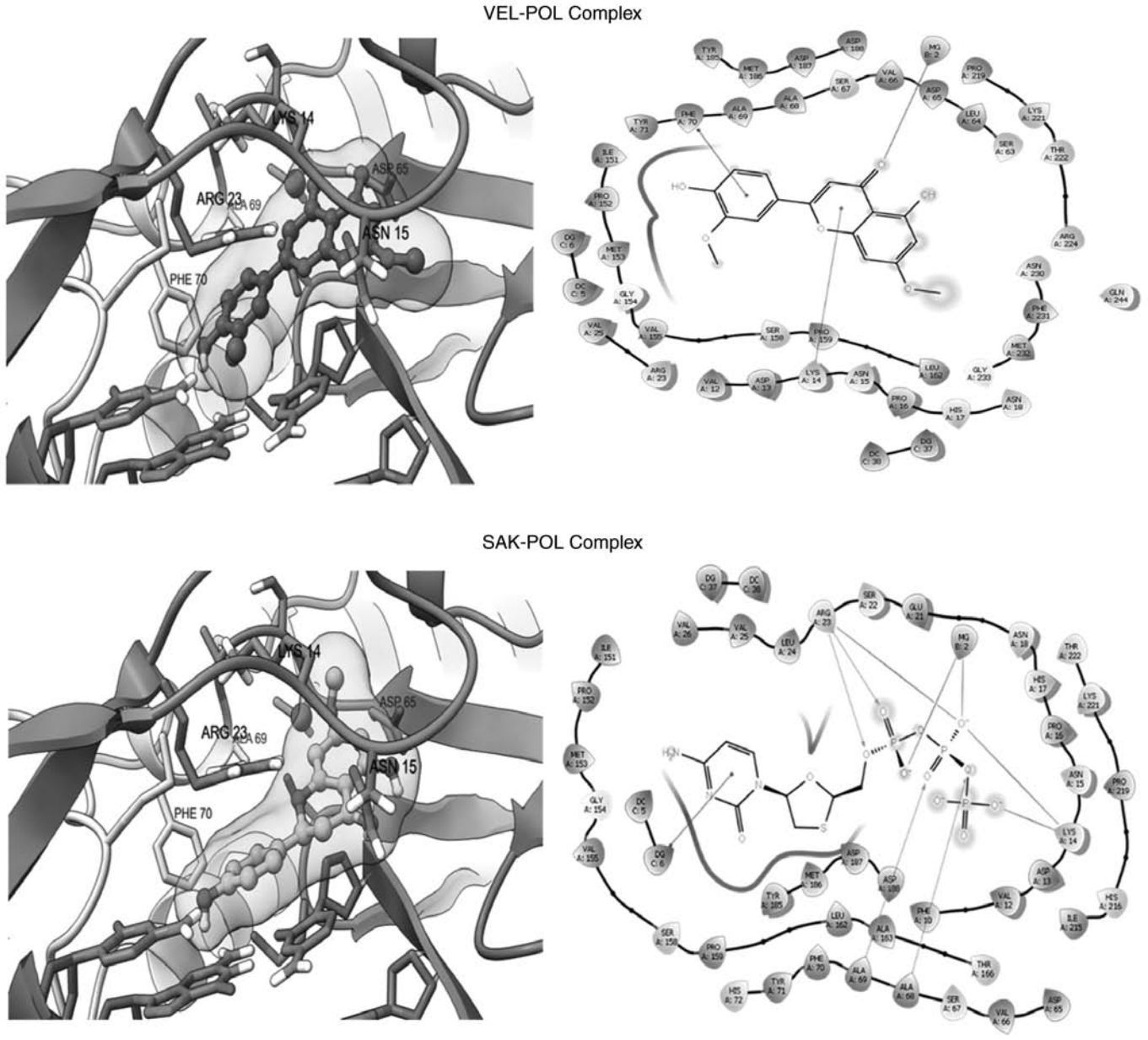

Figure 5. Molecular docking analysis showing 3D and 2D interactions of POL with *Rhus retinorrhoea*-derived VEL (upper panel) and SAK (lower panel). HBV, hepatitis B virus; POL, HBV polymerase; VEL, velutin; SAK, sakuranetin.

Notably, since several therapeutic plant products or isolated compounds can cause liver toxicity (43), prior to anti-HBV assays, both SEK and VEL were first assessed for hepatotoxicity.

SEK is one of the best characterized and most studied flavanones, which is also the derivative of naringenin. In SEK, the hydroxy group at C7 is swapped by a methoxy group (44). It has been reported that SEK has several pharmacological properties, including antioxidant, anti-inflammatory and chemopreventive activities (44-49). Notably, a study revealed that SEK derived from *Sorbus commixta* exerted a marked activity against influenza B virus (IBV) in MDCK cells, at the non-cytotoxic concentration of 100 μg/ml (50). Additionally, a dose of 100 μg/ml SEK isolated from *S. commixta* could inhibit the replication of human rhinovirus (RV3) in cultured HeLa cells, with no cytotoxicity (51). Consistent with the above findings, the results showed that the optimal concentration of *R. retinorrhoea*-derived SEK against HBV activity was at 12.5 μg/ml, which was comparatively 1/8 of that used against IBV and RV3.

VEL is a dimethoxyflavone, where the hydroxy groups at C7 and C3' are swapped by methoxy groups (52). VEL has several pharmacological activities, such as antioxidant, anti-allergic, anti-inflammatory and anti-microbial properties (52,53). A previous study demonstrated that VEL derived from marine seaweeds displayed enhanced anti-microbial and anti-protozoal activities *in vitro* (54). To the best of the authors' knowledge, there is currently no published data on the antiviral activity of VEL. However, a previous *in silico* study suggested that mushroom-derived VEL could significantly inhibit the main protease of SARS-CoV-2 (55). The present study demonstrated that the optimal inhibitory activity of *R. retinorrhoea*-derived VEL against HBV was at 12.5 μg/ml, which was comparatively 1/8 of the structurally-similar SEK, which was used against IBV and RV3.

Figure 6. Molecular docking analysis showing 3D and 2D interactions of CORE with *Rhus retinorrhoea*-derived VEL (upper panel) and SAK (lower panel). CORE, hepatitis B viruscapsid; VEL, velutin; SAK, sakuranetin.

Structure-based docking is a widely used computational tool in drug research. It is most commonly applied to more accurately predict how a small molecule could interact with a macromolecule to form a stable complex via evaluating their potential energies through a scoring tool. To further uncover the supportive mechanism of the *in vitro* observed anti-HBV activities of VEL and SEK, the aforementioned flavonoids were docked against viral POL and CORE. The HBV non-structural protein POL remains the most favored antiviral target. It has been reported that its inhibition can block its DNA replication (23). By contrast, the HBV CORE protein, a structural protein that has been recently emerged as a potential anti-HBV target, can destabilize or disrupt the formation of viral nucleocapsid (34). In the present study, both VEL and SEK formed stable complexes with HBV POL, as well as with CORE, with very good docking scores. Notably, owing to their structural similarity, both flavonoids exhibited very similar alignments and orientations inside the active sites of the HBV target proteins. Blocking or inhibition of HBV POL activity leads to downregulation or cessation of viral sub-genomic (sg) RNA and mRNA transcriptions resulting in suppressions of HBV proteins syntheses. Therefore, in HBV infected individuals or polymerase inhibitor-treated patients, serological test (quantitative) for HBsAg and HBeAg levels is a routine and 'indirect' diagnostic method to monitor HBV replication. Further, both the CORE (HBcAg) and pre-core (HBeAg) proteins are synthesized from a common bicistronic mRNA, and its downregulated transcription due to 'direct-acting' polymerase-inhibitors leads to 'indirect' inhibitions of HBcAg and HBeAg production. In addition, there are limited anti-HBV molecules (e.g., HAP) that 'directly' interfere with CORE assembly and capsid formation with HBV DNA in experimental settings. This 'direct' interference leads to failing to capsid maturation, virus morphogenesis and production of

infectious virions or HBV DNA replication. In view of this, the 'indirect' anti-HBV activity measured by suppressions of both HBsAg and HBeAg in cell culture models is a well and universally accepted assay for evaluating the 'direct-acting' POL or CORE inhibitors. Since the two isolated flavonoids showed inhibitions of both HBsAg and HBeAg in cell culture, the *in silico* molecular docking was performed against viral polymerase or core proteins. The strong binding of the flavonoids with both proteins further supports our *in vitro* data, suggesting their possible mechanism of antiviral activities. In conclusion, the *in silico* data of the current study strongly endorsed the *in vitro* anti-HBV activities of VEL and SEK.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SA collected and extracted the plant material, isolated compounds and participated in structural analysis and manuscript writing. MKP conceived, designed and supervised the research, performed *in vitro* assays, collected and analyzed data, and wrote the manuscript. MSA statistically analyzed the data and participated in manuscript writing. MASA performed molecular docking and data analysis. TAA and AJR participated in plant collection, structural analysis of compounds, and manuscript review. SA and MKP confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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