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ORIGINAL ARTICLE

Natural Anti-Hepatitis B Virus Flavones Isolated from *schimperi* Vatke Growing in Saudi Arabia: Cell Culture and Molecular Docking Study

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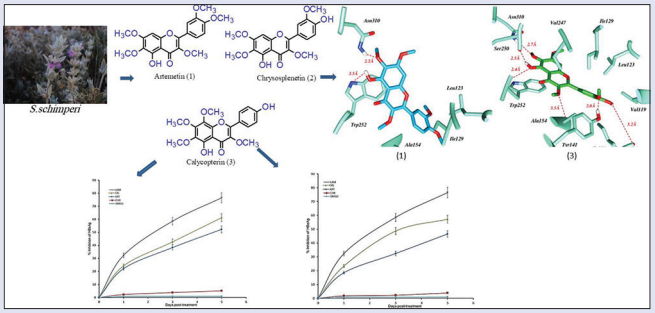
ABSTRACT

Background: *Stachys schimperi* Vatke has been previously reported for its analgesic, antipyretic, antioxidant, antimicrobial and cardioprotective properties. **Objectives:** Phytochemical analysis and assessment of anti-hepatitis B virus (anti-HBV) activity of *S. schimperi*. **Materials and Methods:** Surface extraction was performed to isolate the phytoconstituents using chromatographic techniques, including HPLC. The isolates were identified by a 1D and 2D NMR spectroscopic data. Further, the isolates were tested for cytotoxicity using an MTT assay. Non-cytotoxic doses of the isolates were assessed for their antiviral potential on cultured HepG2.2.15 cells. To rationalize the plausible mechanisms of the tested anti-HBV active compounds, molecular docking studies were carried out using HBV polymerase (Pol) enzyme. **Results:** The NMR data proved the structure of isolates as artemetin [5-hydroxy, 3, 3′,4′,6,7-penta methoxy flavone] (1), chrysosplenetin [5,4′-dihydroxy, 3,3′, 6,7-tetra methoxy flavone] (2) and calycopterin [5,4′-dihydroxy, 3, 6,7,8-tetra methoxy flavone] (3). Notably, this is the first report on the isolation of these three compounds from *S. schimperi* as well as artemetin and calycopterin from the genus *Stachys*. Further antiviral assessment of the non-cytotoxic dose showed marked inhibitions of HBV antigens (HBsAg/HBeAg) by artemetin (52.28%/46.52%) and calycopterin (61.24%/57.26%) in HepG2.2.15 cells. Chrysosplenetin, however, did not show any anti-HBV activity. Artemetin and calycopterin exhibited anti-HBV activity, possibly through inhibition of HBV-Pol as revealed by molecular docking. **Conclusion:** We report the identification of anti-HBV active flavones artemetin and Calycopterin from *S. shimperi*. Our data strongly warrant further molecular and pharmacological studies on artemetin and calycopterin toward developing potential anti-HBV therapeutics.

Key words: Anti-HBV, artemetin, calycopterin, chrysosplenetin, molecular docking, *S. schimperi*

SUMMARY

- Surface extraction was performed on freshly collected aerial parts of *S. schimperi* to isolate the compounds.
- Three structurally-related polymethoxylated flavones artemetin, chrysosplenetin, and calycopterin are reported.
- This is the first report on isolation of these three compounds from *S. schimperi* as well as artemetin and calycopterin from the genus *Stachys*.
- Artemetin and calycopterin showed anti-HBV activity *in vitro* at non-toxic dose.
- Molecular docking studies strongly support the *in vitro* studies.



Abbreviations used: HPLC: High-performance liquid chromatography; CTLC: Centrifugal thin layer chromatography; NMR: Nuclear magnetic resonance; HBV: Hepatitis B Virus; HBsAg: HBV surface antigen; HBeAg: HBV e-antigen; NA: nucleoside analogue; Pol: Polymerase; ADME: Absorption, Distribution, Metabolism and Excretion.

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INTRODUCTION

The drugs came from plants to cure a variety of ailments used by human civilization in all the cultures since ancient times.[1] In primitive culture, people learned by trial and error to differentiate valuable plants having remedial properties from those plants that were inactive or had toxicity. They were pioneers regarding the combination of drugs or processing techniques being used to achieve consistency as well as best or optimum results.[2] A well-defined herbal pharmacopeia was developed by tribal people based on the methodically collected information. The information of plant-derived drugs came into existence gradually and was transferred from one individual to another, thereby laying the basis of the foundation of many systems of traditional medicine worldwide.[2,3]

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Hepatitis B virus (HBV) is a hepatotropic pathogen that causes acute and chronic hepatitis in about one-third of the world's population.[4] Of these, nearly 360 million individuals have chronic hepatitis B (CHB) who remain at risk for developing fulminant liver failure, cirrhosis or hepatocellular carcinoma.[4] Notably, approximately two-thirds of the world population lives in highly endemic regions in Asia, the Middle East, and Africa. Currently, nucleoside analogue (NA)-based drugs (e.g. lamivudine, adefovir and entecavir, etc.) are used to effectively treat CHB.[5] However, prolonged treatment with such drugs generally leads to the emergence of drug-resistant HBV polymerase (Pol) gene mutants.[6] To counter this issue, several herbal formulations or natural products have become globally popular in treating CHB without any sign of drug resistance.[6] In continuation of this, we have recently identified several medicinal plants and isolated anti-HBV active compounds using the HBV-reporter cell culture model.[7-12]

In the present scenario, therefore, naturally occurring compounds with molecular complexity and diversity offer a great opportunity to find new anti-HBV lead compounds.[13]

Genus *Stachys* is one of the largest genera of the family Lamiaceae which includes about 300 species distributed in Asia, America, southern Africa, and also temperate regions of the Mediterranean.[14] For centuries, herbs and shrubs of this genus have been used in folk medicine to treat genital tumors, sclerosis of the spleen, inflammatory diseases, coughs and ulcers.[15] The phytoconstituents analyses of several *Stachys* species revealed the presence of flavonoids,[16] phenylethanoid glycosides,[17,18] diterpenoids,[19,20] saponins.[21]

Saudi Arabia hosts five species of this genus including *Stachys schimperi* Vatke.[22] Pharmacologically methanol, chloroform, hexane and acetonitrile extractives of *S. schimperi* have been examined for analgesic and antipyretic effects in male Swiss albino mice.[23] In another study, methanol extract of *S. schimperi* have been investigated for antioxidant activity and cardioprotective activity against doxorubicin-induced cardiotoxicity.[15] Methanol extract of *S. schimperi* has also been examined for antimicrobial activity.[24] The qualitative phytochemical analysis of *S. schimperi* studied by Abdel-Sattar *et al.*[15] on High-Performance Liquid Chromatography (HPLC) equipment., exposed the presence of flavonoids: luteolin, rutin, isorhamnetin, kaempferol, hypersonic, and isoflavonoid daidzein; phenolic acids: (*E*)-hydroxyl cinnamic acid, hydroxy-4-phenylbutanoic acid, syringic acid, vanillic acid, ferulic acid and *p*-coumaric acid. In addition, a major flavonoid glycoside compound, Isoscutellarein 7-*O*-[2″-*O*- (6‴ -acetyl)-β-D-allopyranosy]-β-D-glucop yranoside have also been reported, showing significant antioxidant activity.

The present investigation reports the isolation and structure elucidation of three flavones: artemetin (1), chrysosplenetin (2) and calycopterin (3), including assessment of their *in vitro* anti-HBV activities supported by molecular docking analysis.

MATERIALS AND METHODS

General procedure

The ^{1}H and ^{13}C NMR spectra were acquired in deuterated DMSO-d_6 on a Bruker Avance spectrometer (Switzerland), operational at 700 MHz for ^{1}H and 175 MHz for ^{13}C. Chemical shift values are reported in δ (ppm), relative to the residual solvent peak; coupling constants (*J*) are in hertz (Hz). Centrifugal thin layer chromatography (CTLC) was achieved on Chromatotron (Harrison Research, Ser. No. 5153, Made in the USA). TLC profiling was monitored on analytical purpose TLC plates coated with UV_{254} fluorescence indicator (Merck, Germany). Compounds were visualized under UV radiation in CAMAG UV cabinet (Germany) of dual-wavelength, 254/366 nm and also by spraying with *p*-anisaldehyde (Loba Chemie, India)/sulphuric acid (Merck, Germany) reagent followed by heating with a heat gun (Master heat gun, HG 501A, Master Appliance Corp., USA) to visualize spots. Semi preparative HPLC was performed on a Shimadzu system (Kyoto, Japan), consisting of two LC-6AD semi-preparative solvent delivery pumps, bus module CBM-20A, a multi-wavelength photo-diode array detector (SPD-M20A), columns shim-pack PREP-ODS (H) Kit (A) 250 mm × 4.6 mm I.D. with 5 μm particles (B) 250 mm × 20 mm I.D. 5 μm.

Chemicals

The solvents (acetone, hexane, dichloromethane and methanol) used for extraction and fractionation purposes were of analytical grade and acetonitrile and water used in HPLC to isolate the compounds were of HPLC grade. All the solvents were purchased from Sigma Aldrich, Germany or Fisher Scientific, UK. The solvents used in the extraction and fractionation process were distilled before use. Deuterated solvent, DMSO-d_6 (Chemical Dynamic Corporation, New Jersey, USA) was used for NMR.

Plant material

The aerial parts of *Stachys schimperi* Vatke were collected from Al-Hada, Saudi Arabia and identified by an expert taxonomist, Dr M. Yousuf. A voucher specimen (no. 15260) was assigned at the Herbarium of the College of Pharmacy, King Saud University, Riyadh.

Extraction, fractionation and isolation

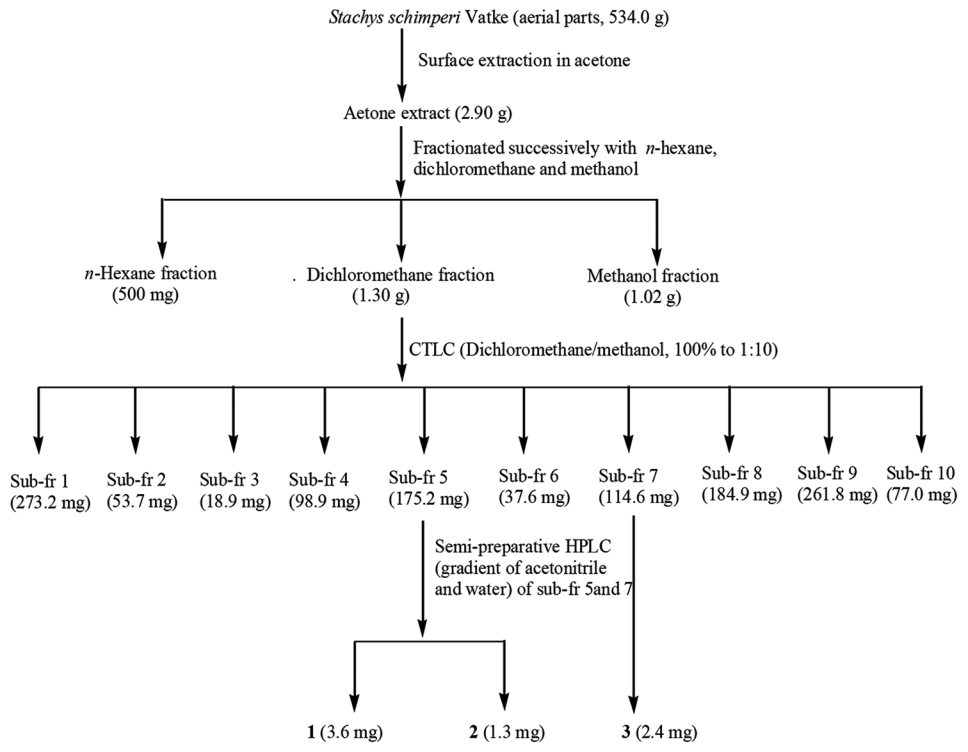
Surface extraction of clean and freshly collected aerial parts (534.0 g) of *S. schimperi* was achieved with acetone at room temperature. The extract was filtered through Whatman No. 1 filter paper. The solvent was evaporated to dryness at 40°C *in vacuo* using Buchi Rotavapor (Model R-210, Switzerland) which left a syrupy mass of acetone extracts. The acetone extract was fractionated successively with *n*-hexane, dichloromethane and methanol to give *n*-hexane, dichloromethane and methanol soluble fractions. In each case, solvent was evaporated to dryness using Buchi Rotavapor. Based on complexity in TLC profile and HPLC chromatogram dichloromethane (1.30 g) was subjected for further fractionation through CTLC which yielded ten subfractions (sub-fr 1–10). The sub-fr 5 and 7 were considered for further chemical investigation because of less complications in HPLC chromatogram as compared to others. We can isolate compounds 1 and 2 from sub-fr 5 and compound 3 from sub-fr 7. The protocol adopted from extraction to isolation of pure compounds is depicted in detail in the below scheme 1.

Cell culture and drugs

The human hepatoma cell line HepG2 and its HBV-reporter derivative HepG2.2.15 (kind gift of Dr S. Jameel, International Center for Genetic Engineering and Biotechnology, New Delhi, India) were maintained in RPMI-1640 medium, supplemented with 10% fetal bovine serum (Gibco, USA), 1× penicillin-streptomycin mix (Invitrogen, USA), and 1× sodium pyruvate (Gibco, USA) at 37°C with 5% CO_2 supply. The approved anti-HBV drug, lamivudine (Sigma-Aldrich, Germany) was used as standard or positive control whereas DMSO (1%; Sigma-Aldrich, Germany) acted as a vehicle or negative control.

Cytotoxicity assay of *S. schimperi*–derived compounds

HepG2 cells (0.5 × 10^{5}/well) were seeded in a 96-well culture plate (Corning, USA), and grown overnight. The next day, the cells were treated with freshly constituted compounds (artemetin, chrysosplenetin



Scheme 1: Isolation protocol compounds 1-3

and calycopterin), which were prepared in DMSO (<0.1% final) and culture media at different doses (6.25, 12.5, 25 and 50 µg/ml) or controls. After 48 h of incubation, the cells were subjected to 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (TACS MTT Cell Proliferation Assay Kit, Tervigen, USA) as per the manufacturer's protocol to determine their cytotoxic effect, if any. All samples were tested in triplicate and repeated.

Assay of HBV surface antigen (HBsAg) inhibition by *S. schimperi*–derived compounds

HepG2.2.15 cells (0.5×10^5/well) were seeded in a 96-well plate and incubated overnight at 37°C. The next day, cells were treated with artemetin, chrysosplenetin and calycopterin (50 µg/ml, each) or controls, and incubated until 5 days with the periodic change of media containing compounds. Culture supernatants collected on day 1, 3, and 5 were stored at −20°C, and analyzed for the inhibition of viral HBsAg synthesis, using the commercial diagnostic Elisa kit (Monolisa HBsAg ULTRA, BioRad, USA) as per the manufacturer's manual. All samples were tested in triplicate and repeated. The absorbance was recorded (450 nm) (Microplate reader; BioTek, ELx800) and non-linear regression analysis was performed.

Assay of HBV pre-core antigen (HBeAg) inhibition by *S. schimperi*–derived compounds

HBeAg, the processed form of viral pre-core protein, is a diagnostic gold marker of active viral DNA replication. Therefore, all treated culture supernatants collected on day 1, 3, and 5 after incubation at 37°C were further subjected to HBeAg analysis using the commercial diagnostic kit (HBeAg/Anti-HBe Elisa Kit; DIASource, Belgium) as per the manufacturer's manual. All samples were tested in triplicate and repeated. The absorbance was recorded (450 nm) (Microplate reader; BioTek, ELx 800) and non-linear regression analysis was performed.

Statistical analysis

The triplicated data were presented as mean ± SE and interpreted using a one-way analysis of variance (ANOVA). Comparisons of differences between two groups were done using student's *t* test ($P < 0.05$). All statistical analyses were performed with SPSS software (version 25; IBM, USA).

Homology modelling

Since no crystal structure of HBV-Pol has been reported yet, homology modelling was utilized to predict the 3D structure of HBV-pol. The primary sequence of HBV-Pol was retrieved from GenBank (accession code AGA95798.1) and subjected to Robetta (https://robetta.bakerlab.org/) for structure prediction using PDB ID 1RTD as a template. The generated model was optimized by energy minimization using molecular mechanics force fields. Further, the stereochemical quality of the optimized model was measured by Ramachandran Plot, Verify 3D, and Errata Plot. Based on stereochemical parameters, the modelled structure of HBV-Pol was utilized for molecular docking studies.

Molecular docking studies

The modelled HBV-Pol with the best stereochemical parameters was used to establish the binding mode of *S. schimperi*–derived artemetin, calycopterin, including the reference drug lamivudine. The binding site was identified by the Site-Finder module implemented in MOE, which recognizes the possible binding sites from the 3D atomic coordinates of the protein. The residues of the identified site were assigned as dummy atoms and used as grid spacing during docking simulation. For the HBV-Pol target proteins, Triangular Matcher was used as a primary placement method with London dG and GBVI/WSA dG as primary

placement scoring and rescoring functions, respectively. For each ligand, thirty poses were generated and the top-ranked poses were visually analyzed to characterize their binding pattern.

ADME analysis

SwissADME (http://www.swissadme.ch/), an online web server was used for the assessment of physico-chemical, pharmacokinetics, drug-likeness and medicinal chemistry friendliness properties of two anti-HBV active compounds (artemetin and calycopterin) from *S. schimperi*. SMILES notation of potential anti-HBV artemetin and calycopterin were retrieved from PubChem and subjected to submission webpage of SwissADME for the estimation of aforesaid properties.

RESULTS

Phytochemical analysis

The phytochemical investigations on aerial parts of *S. schimperi* led to the isolation of three compounds (1–3), shown in Figure 1. The structures of isolated compounds were elucidated using 1D and 2D NMR spectroscopic data. ^{1}H and ^{13}C NMR values are depicted in Table 1 and the full 1D- and 2D NMR spectra are provided in the supplementary file. Interpretation and analysis of 1D and 2D NMR data of isolates were in comparison with the literature values. Compounds were identified as artemetin [5-hydroxy, 3, 3′,4′,6,7-penta methoxy flavone] (1),[25] chrysosplenetin [5,4′-dihydroxy, 3,3′, 6,7-tetra methoxy flavone] (2),[26] and calycopterin [5,4′-dihydroxy, 3, 6,7,8-tetra methoxy flavone] (3).[27,28] Notably, this is the first report on the isolation of these three compounds from *S. schimperi* as well as artemetin and calycopterin from the genus *Stachys*. Conversely, chrysosplenetin has been previously reported from *S. ionica* Halácsy, S. *lavandulifolia* Vahl. and *S. aegyptiaca* Persl.[29]

Effect of *S. schimperi*–derived flavonoids on cell viability

Our MTT assay showed no cytotoxicity by artemetin, chrysosplenetin and calycopterin even at 50 µg/ml, the maximal concentration (data not shown).

Anti-HBV activities of *S. schimperi*–derived flavonoids

The three flavonoids artemetin, chrysosplenetin and calycopterin were tested for anti-HBV activity at the maximal safe dose (50 µg/ml). As compared to days 1 and 3, HBsAg production on day 5 was maximally inhibited by artemetin (52.28%) and calycopterin (61.24%), except chrysosplenetin about untreated control [Figure 2]. Similarly, both artemetin (46.52%) and calycopterin (57.26%) suppressed HBeAg production maximally on day 5 [Figure 3], reflecting the downregulation of HBV DNA replication.

Homology modeling

The generated homology model of HBV-Pol from the Robetta server was subjected to minimization to correct the geometry, to remove the bad clashes, and to allow the loop regions to attain stability. The Ramachandran plot for the optimized model was drawn to check the quality of the predicted model. The plot showed that 79.3% of residues were in the most favored region. Similarly, 16.6% and 2.5% of residues were inside the additionally allowed and generously allowed regions, respectively, while only 1.7% of residues were inside the disallowed region. Further, to check the compatibility of the 3D model with the 1D amino acid sequence was evaluated by plotting Verify 3D. The plot showed that 75.68% of the residues had averaged 3D-1D score ≥0.2. Furthermore, the Errata plot showed the a quality factor of 79.43 for the optimized homology model, which suggested that the predicted model was accurate enough to be used for docking.

Molecular docking studies

The HBV-Pol participates in virus replication, and therefore, has been an important target for developing anti-HBV agents. Herein, molecular docking studies were carried out to demonstrate the observed anti-HBV activity of isolated flavones artemetin and calycopterin. The top-ranked binding poses are presented in Figure 4. It was interesting to note that artemetin and calycopterin interacted more tightly with the binding affinity of – 8.1 and – 7.6 kcal/mol, respectively in comparison to that of lamivudine, –5.9 kcal/mol. As evident from Figure 4a, the pyrimidine ring of lamivudine mediate π-alkyl and π-π interactions with Pro249 and Trp252. Similarly, nitrogen in the pyrimidine ring establishes two hydrogen bonds with Asn310 and Arg368 while the methyl hydroxyl group establishes two hydrogen bonds with Ser250 and Ser274. The aromatic rings of artemetin stacked against Leu123, Ile129, Ala154, and Trp252 by mediating π-alkyl and π-π interactions [Figure 4b]. The carbonyl and hydroxyl group of artemetin established hydrogen bonds with Trp252 and Asn310, respectively. The three methoxy groups at C-6, C-7, and C-8 and aromatic rings helped to accommodate the compound calycopterin to reside firmly at the binding site of HBV polymerase by mediating hydrophobic interactions with Ile110, Val119, Leu123, Ile129, Ala154, Val247 and Trp252 [Figure 4c]. In addition, hydroxyl groups were observed to mediate hydrogen bonds with Ile110, Tyr141, Ala154, Ser250, Trp252 and Asn310.

In silico ADME analysis

Optimization of structural, physico-chemical, and biochemical properties in the early stage of the drug development process reduces the late-stage failure of many drugs. Thus, *in silico* assessment of physico-chemical, pharmacokinetics, and drug-likeness properties of potential anti-HBV compound artemetin and calycopterin were carried out using SwissADME. For optimization of oral bioavailability

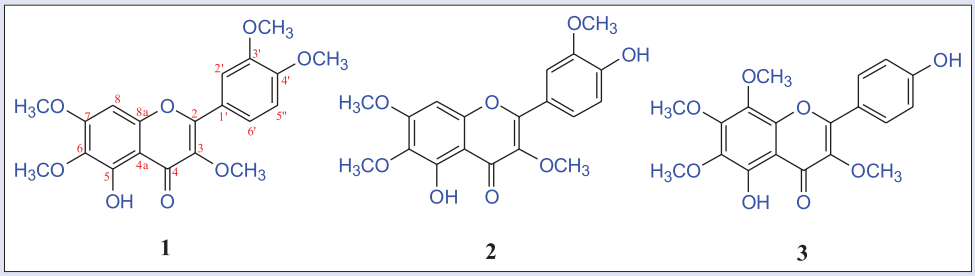


Figure 1: Structure of compounds 1–3

of artemetin and calycopterin, a radar plot presenting six physicochemical properties (lipophilicity, size, polarity, insolubility, flexibility and unsaturation) was plotted [Figure 5]. The physiochemical range for both compounds entirely falls in the pink area except unsaturation of calycopterin which indicated the good oral bioavailability for both compounds. Similarly, both compounds showed high gastrointestinal absorption with the estimated water/octanol coefficient value of 3. Taken together the isolated compounds from *S. schimperi* were in good agreement with the given criteria to be considered as drug-like.

DISCUSSION

Despite the availability of several potential NA-based drugs, CHB is still a global public health concern due to the emergence of drug-resistant HBV mutants. In recent decades, several classes of natural phytochemicals, notably flavonoids and their aglycone, glycoside and methylated derivatives have been recognized with potential antiviral properties with no known resistance.[6,30–35] Of these, wogonin isolated from *Scutellaria radix*,[36] hyperoside from *Abelmoschus manihot*,[37] luteolin and isovitexin from *Swertia yunnanensis*,[38] and isoorientin

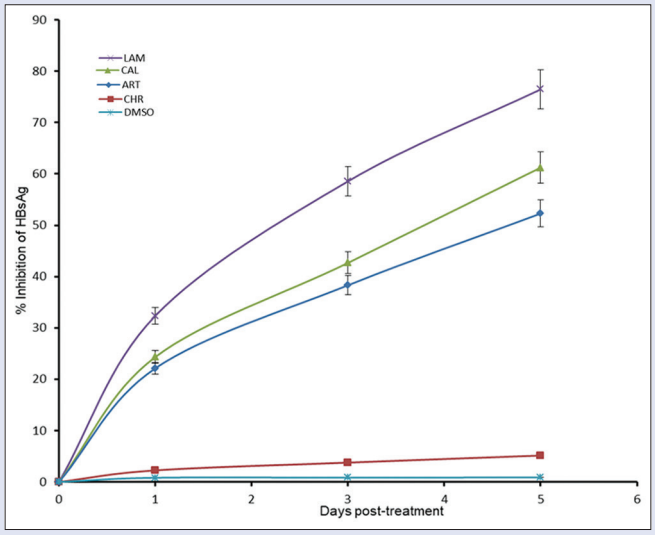


Figure 2: Time-course analysis of HBV surface antigen (HBsAg) inhibition by S. schimperi–derived flavonoids (50 µg/ml): artemetin (ART), chrysosplenetin (CHR) and calycopterin (CAL) relative to untreated control in HepG2.2.15 cells. Lamivudine (LAM; 2 µM) and DMSO (0.1%) served as positive and negative controls, respectively. Values on Y-axis are means of three determinations

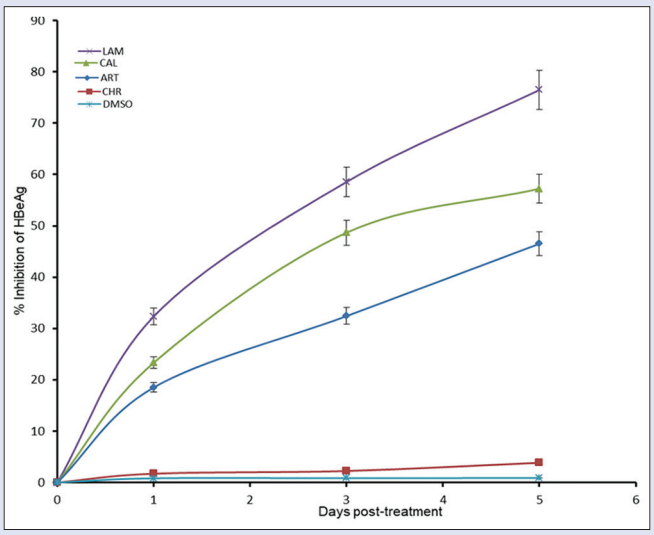


Figure 3: Time-course analysis of HBV pre-core antigen (HBsAg) inhibition by S. schimperi derived flavonoids (50 µg/ml): artemetin (ART), chrysosplenetin (CHR) and calycopterin (CAL) relative to untreated control in HepG2.2.15 cells. Lamivudine (LAM; 2 µM) and DMSO (0.1%) served as positive and negative controls, respectively. Values on Y-axis are means of three determinations

Table 1: ^{1}H and ^{13}C NMR spectroscopic data of compounds 1-3 in DMSO-d_6

Position	1		2		3	
	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C
2	-	155.9	-	156.3	-	156.8
3	-	138.6	-	138.3	-	138.1
4	-	178.8	-	178.7	-	179.1
4a	-	106.1	-	106.1	-	107.3
5	-	152.3	-	152.2	-	148.7
6	-	132.1	-	132.0	-	136.0
7	-	159.2	-	159.2	-	152.8
8	6.96 s	92.0	6.95 s	92.0	-	133.0
8a	-	152.1	-	152.1	-	144.9
1′	-	122.5	-	121.3	-	121.0
2′	7.76 dd (7.0, 1.4)	122.5	7.66 d (7.0)	122.8	7.99 d (7.0)	130.7
3′	7.17 d (7.0)	112.0	6.99 d (7.0)	116.1	7.0 d (7.0)	116.3
4′	-	151.8	-	150.5	-	161.1
5′	-	148.9	-	148.0	7.0 d (7.0)	116.3
6′	7.68 d (1.4)	111.7	7.69 s	112.5	7.99 d (7.0)	130.7
OCH_3-3	3.84 s	60.2	3.83 s	60.1	3.81 s	60.1
OCH_3-6	3.74 s	60.6	3.75 s	60.6	3.83 s	61.2
OCH_3-7	3.94 s	57.0	3.95 s	57.0	4.03 s	62.0
OCH_3-8	-	-	-	-	3.90 s	62.3
OCH_3-4′	3.88 s	56.1	-	-	-	-
OCH_3-3′	3.88 s	56.1	3.89 s	56.3	-	-
OHs	12.6 s (OH-5)		12.6 s (OH-5), 9.99 s (OH-4')		12.5 s (OH-5), 10.4 s (OH-4')	-

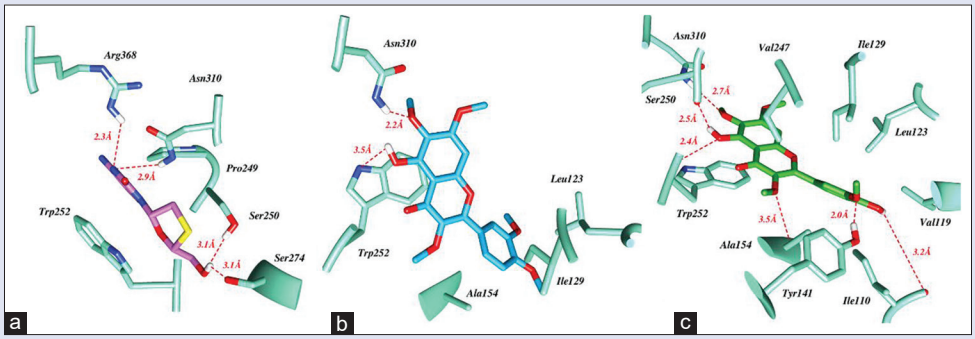


Figure 4: Binding mode of (a) lamivudine, (b) artemetin, and (c) calycopterin in the binding site of HBV polymerase enzyme. The hydrogen bonds are presented as red dashed lines

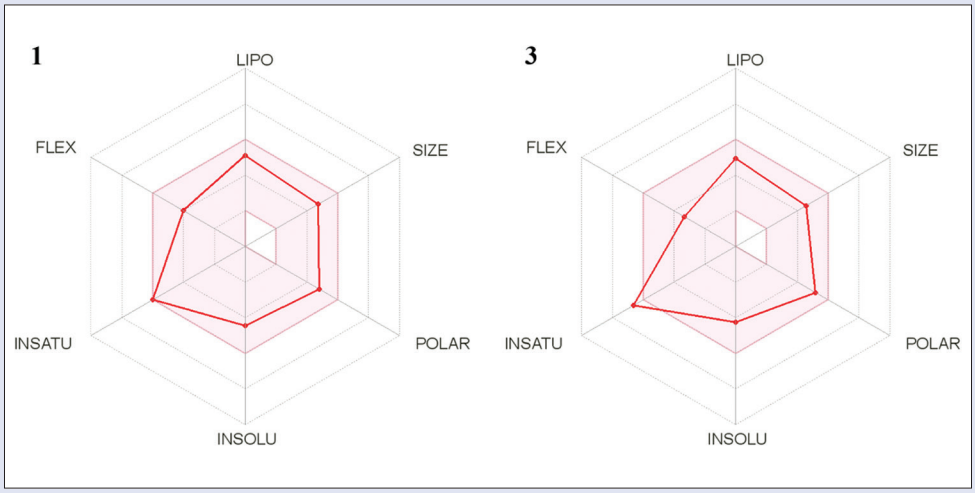


Figure 5: Bioavailability radar of compound artemetin (1) and calycopterin (3). Pink region represents the acceptable range for six physiochemical properties

from *Swertia mussolini*,[39] etc., have been shown to effectively suppress the secretion of both HBsAg and HBeAg in HepG2.2.15 cells. In line with this, we have also identified quercetin, rutin, hesperidin and myricetin-3-*O*-rhamnoside as potential inhibitors of HBsAg and HBeAg in HepG2.2.15 cells.[9,40]

In the present study, we have assessed the anti-HBV activities of *S. schimperi* derived three structurally-close polymethoxylated flavones: artemetin, chrysosplenetin and calycopterin. When used at an optimally tested non-toxic concentration (50 µg/ml), artemetin and calycopterin showed promising inhibition of HBsAg and HBeAg, except chrysosplenetin. Notably, our most effective dose (50 µg/ml) is very much comparable to the previous reports.[9,36–40] Interestingly, to the best of our knowledge, there is no published report on the antiviral activity of artemetin and calycopterin. However, chrysosplenetin isolated from *Laggera pterodonta* as well as obtained from commercial sources has been shown to efficiently inhibit enterovirus 71 in cultured cells and mice model.[41,42] In addition, our molecular docking analyses have inferred that artemetin and calycopterin could potentially bound to the HBV-Pol through significant hydrogen bonds and hydrophobic interactions. Therein, both compounds are bound at the same pocket where lamivudine got complexed but with a different binding mode due to their chemical diversity. These potential inter-molecular interactions between the two tested compounds and crucial residues of HBV-Pol may account for suppression of HBsAg and HBeAg productions through inhibition of HBV replication in HepG2.2.15 cells. Because there is no purified HBV-Pol available, the *in vitro* biochemical validation of this interaction cannot be achieved. *In silico* prediction of pharmacokinetic properties of compounds is an alternative to experimental validation in the early stage of drug discovery, which significantly reduces the late-stage failure of clinical trials. Given this, our ADME analysis revealed that artemetin and calycopterin possessed drug-like favorable oral bioavailability and physiochemical properties. Taken together, this is the first report on *in vitro* anti-HBV activity of artemetin and calycopterin, supported by *in silico* data.

CONCLUSION

We report identification of three flavones artemetin, chrysosplenetin, and calycopterin from *S. schimperi*. Of these, artemetin and calycopterin have exhibited *in vitro* anti-HBV activities, possibly through inhibition of HBV-Pol enzyme as revealed by molecular docking. Our data strongly warrants further molecular and pharmacological studies on artemetin and calycopterin towards developing potential anti-HBV therapeutics.

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Authors contribution

SA, isolated the compounds, obtained and interpreted spectroscopic data, wrote the first draft of the manuscript; AJA, contributed to plant collection overall structural elucidation and writing the manuscript; MKP and MSA contributed to *in vitro* assay and data analysis, KZ and ZH contributed to molecular docking studies. MNA contributed to data analysis and manuscript writing.

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Conflicts of interest

There are no conflicts of interest.

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