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# Greater Mekong Subregion Medical Journal (GMSMJ)

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## Publication Ethics

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Greater Mekong Subregion Medical Journal presents articles in the field of basic and advanced clinical research in medicine and related health sciences, medical education as well as community medicine in Thailand and international, especially in countries of Greater Mekong Subregion.

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## Content


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Original Article

GMSMJ

Greater Mekong Subregion Medical Journal

# Exploring Potential of Phytochemicals from Houttuynia cordata Thunb. as Angiogenesis Inhibitors in Melanoma Treatment: A Molecular Docking Study

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## Abstract:

**Background:** *Houttuynia cordata* Thunb. extract has shown programmed cell death induction in melanoma. Antagonism of the VEGF receptors (VEGFR) has been suggested as a potential mechanism of action due to its role in the progression of melanoma. Given the downsides of the current anti-VEGFR drugs, including lack of selectivity and unwanted side effects, the phytochemical constituents of *Houttuynia cordata* Thunb. were investigated for their inhibition of VEGFR using molecular docking simulations.

**Objective:** To investigate and identify the efficacy of potential orally-compatible phytochemical constituents that bind and inhibit the ATP binding sites of VEGFR1 and VEGFR2 using molecular docking simulations.

**Materials and Method:** The X-ray crystal structures of VEGFR1 and VEGFR2 were downloaded and prepared. A total of 74 phytochemical compounds in *Houttuynia cordata* Thunb. were constructed and energy minimized in 3D format and docked to the ATP binding sites of VEGFR1 and VEGFR2. Drug-like properties were calculated. This is followed by analysis of the binding modes, calculated docking scores and oral pharmacokinetics of potential candidates.

**Results:** Five compounds, luteolin, quercetin, isorhamnetin, apigenin, and kaempferol, were identified to have acceptable oral pharmacokinetics and docking scores, and were predicted *in silico* to have adequate VEGFR inhibition. Notably, apigenin and quercetin were predicted to have the best inhibitory action against VEGFR1 and VEGFR2, respectively, i.e., apigenin scored -9.148 kcal/mol against VEGFR1, and quercetin scored -9.945 kcal/mol against VEGFR2.

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**Conclusion:** Luteolin, quercetin, isorhamnetin, apigenin, and kaempferol could serve as potential candidates for effective inhibition of the ATP binding site of VEGFR. In this light, these phytochemical constituents of *Houttuynia cordata* Thunb. are suggested as potential therapeutics for the treatment of melanoma through direct inhibition of VEGFR at the ATP binding site. Specifically, apigenin and quercetin were predicted to be the strongest VEGFR1 and VEGFR2 inhibitors and are suggested for in *vitro* and in *vivo* drug tests.

**Keywords:** Melanoma, *Houttuynia cordata*, Quercetin, Apigenin, VEGFR, Angiogenesis, Molecular docking

## Introduction

Melanoma, a highly malignant form of skin cancer, is characterized by its aggressive nature and propensity for metastasis, rendering it resistant to conventional therapeutic modalities. In melanoma, angiogenesis is indispensable for the growth and progression of the tumor. Traditionally, angiogenesis is thought to be driven by hypoxia, which occurs when the tumor outgrows its blood supply, leading to low oxygen levels within the cancer.[1] However, recent studies have shown that angiogenesis can occur independently of hypoxia, driven by various signaling pathways such as BRAF V600E, PI3 kinase, ET-1, reactive oxygen species (ROS), NF-κB, MITF, NRAS (with GAB2), ILK, and NRF2.[2] These findings highlight the complex and multifaceted nature of angiogenesis in melanoma, underscoring the need for targeted therapies that can effectively inhibit this process.

Targeted therapies that inhibit angiogenesis, such as the anti-VEGF antibody bevacizumab, have shown promise in clinical trials. Furthermore, combining antiangiogenic therapies with immune checkpoint inhibitors has improved survival outcomes in patients with metastatic melanoma, suggesting a potential synergistic effect. Over the years, the development of angiogenesis inhibitors has become a focal point in the fight against cancer, including melanoma.[3-5]

*Houttuynia cordata*, commonly known as the Chameleon plant, is a perennial herb for use as a regimen in traditional medicine across Asia. It is renowned for its diverse therapeutic properties which include anti-inflammatory, antimicrobial, antioxidant, promotion of immunity and anticancer activities.[6] Modulation of various molecular pathways by the phytochemical constituents were reported mainly for abundant phytochemical groups which include flavonoids, phenolic acids and polysaccharides. Specifically, polysaccharides have shown promotion of macrophage function and quenching of superoxide radicals, and cytotoxic activities and induction of apoptosis were reported for flavonoids against various cancer cell lines.[6] Given the presence of these phytochemical groups in many dietary supplements, there is a notion that the chemical scaffolds of these phytochemical constituents are viable options to be used for anticancer drug development in the future. This can replace the more cytotoxic drugs that are currently in clinical use, which contain stronger and often, unbearable side effects. However, the role of these phytochemicals as angiogenic inhibitors, particularly for the treatment of melanoma remained largely unexplored.

In treating melanoma, the Vascular Endothelial Growth Factor (VEGF) and its pathway are pivotal targets for inhibiting angiogenesis.[7] VEGF, a primary stimulator

of angiogenesis, plays a critical role in developing new blood vessels within tumors. It is chiefly produced by cancer cells and is instrumental in mediating vascular permeability and facilitating tube formation. In particular, the VEGF receptors (VEGFR) which has a tyrosine kinase domain has been a successful anti-angiogenic drug target for treatment of various cancers.[8] Targeting VEGF pathways has been clinically effective at suppressing melanoma growth and progression, especially in metastatic cases.

Our previous *in vitro* study demonstrated that *Houttuynia cordata* Thunb. extract induces programmed cell death in melanoma by activating the caspase-dependent pathway and p38 phosphorylation associated with HMGB1 reduction.[9] In this study, we conducted a virtual screen by employing molecular docking simulations to study and analyze the interactions between 74 phytochemical constituents from *Houttuynia cordata* Thunb. and the ATP binding site of VEGFR; the ATP binding site has been a target site for mainly phytochemicals and small molecule drugs. This approach allows prediction of the binding affinities and mode of interactions between the phytochemicals and VEGFR, providing insights into their potential efficacies as angiogenesis inhibitors for melanoma therapy. Through this *in silico* analysis, we aim to identify promising orally compatible candidates for development of new therapeutic agents against melanoma.

## Materials and method

### Retrieval and preparation of ligands for molecular docking

A comprehensive list of 74 phytochemical compounds in *Houttuynia cordata* Thunb. were obtained from Kumar *et al*.,[10] as shown in Table S1 (supplementary materials); the volatile oils were excluded from the selection as these are large structures, which plausibly do not interact with the ATP binding site, and oils in general have been reported to show no direct or weak kinase inhibition, rather a binding site specified for lipids.[11] The structures of seventy-four phytochemical compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[12, 13] in SDF file format. Furthermore, three drugs sorafenib (CID: 216239), axitinib (CID: 6450551) and pazopanib (CID: 10113978) were downloaded from the same database. The ligand molecules were then prepared using the Open Babel tool (v 2.4.1)[14] of PyRx software (v1.1)[15] by minimizing their energies and following conversion into a PDBQT file format for use in the molecular docking study.

### Retrieval and preparation of target proteins for molecular docking

The 3D protein structures were obtained from the RCSB Protein Data Bank (RCSB PDB)[16] in 3D SDF file format: VEGFR1 kinase domain (PDB ID: 3HNG) and VEGFR2 kinase domain (PDB ID: 2XIR). The target receptors were first prepared by removing the solvent molecules and co-crystallized ligands, addition of hydrogen atoms, partial charge adjustments, 3D protonation, and energy minimization using Discovery Studio Visualizer (version 21.1.0.20290).

### Molecular Docking Study.

The PyRx with Vina Wizard was utilized in molecular docking experiments to determine the docking scores, ligand binding modes and ligand-protein interactions. The Vina Wizard is a user-friendly interface for running molecular docking simulations with Autodock Vina[17] version 1.2.5 as the molecular docking engine. The prepared structures of target proteins were imported into PyRx and converted into PDBQT file format. An

exhaustiveness value of 20 for the experiment was selected and the best docked conformations were characterized by the lowest docking scores. The Discovery Studio Visualizer was used to visualize the interactions and binding modes between the ligands and receptor proteins.

**Results and discussion**

The protocol was first verified by removing and re-docking the co-crystallized ligands (compounds 78 and 79) to the VEGFRs at different exhaustiveness values (Table S2 in the supplementary materials). The RMSD values were low for the two co-crystallized ligands at exhaustiveness values at 10, 15 and 20 indicating reproducibility.

Antagonistic potential of the phytochemical constituents in *Houttuynia cordata* Thunb. as VEGFR inhibitors were investigated using molecular docking simulations. The docking scores represent the predicted binding affinities of the compounds are as shown in Table S3 (supplementary materials) and the histogram in Figure 1. The scores range from -4.857 to -10.620 kcal/mol for VEGFR1, and -4.669 to -10.051 kcal/mol for VEGFR2. Hesperidin (compound 14) was predicted to be the strongest inhibitor, whereas 5-methoxy-1-methylpyrrolidin-2-one (compound 61) was the predicted to be the weakest. The docking scores of hesperidin to VEGFR1 are -10.620 kcal/mol, and -10.051 kcal/mol for VEGFR2. However, the structure of the compound is large and is likely orally incompatible. The physicochemical properties of hesperidin (Table 1) were calculated using the DruLiTo web server (http://pitools.niper.ac.in/DruLiToWeb/ DruLiTo_index.html) that showed it to be incompatible for oral drug administration since hesperidin do not adhere to the Lipinski's Rule of Five.[18] Following this, the docking scores of the clinically approved VEGFR inhibitors were evaluated, which include sorafenib, axitinib, and pazopanib. Against VEGFR1, sorafenib, axitinib, and pazopatinib exhibited docking scores of -11.650, -10.498 and -10.398 kcal/mol, respectively. Whereas against VEGFR2, sorafenib, axitinib and pazopatinib exhibited scores of -10.492, -11.267 and -10.496 kcal/mol, respectively. It is apparent that the docking scores of the phytochemical constituents are lower than the clinically approved drugs. This is reasonable as the drugs have a larger van der Waal's surface, *i.e.*, greater hydrophobic and van der Waal's contacts with the surrounding amino acid residues. Secondly, the drugs have been optimized for their pharmacological and clinical effectiveness through multiple stages of development. A threshold value of -9 kcal/mol was used as the cut-off to identify 11 potentially active compounds (Table 1), *i.e.*, 11 compounds with scores of less than -9 kcal/mol were selected as orally compatible VEGFR inhibitor candidates. Amongst the 11 compounds, only 5 adhered to the Lipinski's Rule of Five and were predicted to have acceptable oral bioavailability: luteolin, quercetin, isorhamnetin, apigenin, and kaempferol. From these 5 compounds, apigenin was predicted to have the strongest inhibition against VEGFR1 (-9.148 kcal/mol), and quercetin as the strongest inhibitor against VEGFR2 (-9.945 kcal/mol).

Literature reports have implicated apigenin as an anticancer agent and a suppressor of angiogenesis in human lung cancers by reducing HIF-1α expression, and decrease in endothelial and pericyte motility,[19, 20] in which our findings suggest a new anti-angiogenic mechanism for apigenin, *i.e.*, direct inhibition of the VEGFR kinase domain. On the other hand, quercetin has been reported to reduce VEGFR2 expression in hepatocellular carcinoma,[21] and colorectal cancer[22], decrease migration of VEGF-induced primate choroid-retinal endothelial cells,[23] and suppression of

VEGF induced phosphorylation of VEGFR2 and their downstream protein kinases AKT, mTOR, and ribosomal protein S6 kinase in human umbilical vein endothelial cells.[24] As mentioned, the anti-angiogenic properties of apigenin and quercetin are clearly established. Regardless, none reported direct inhibition of the ATP binding site of VEGFR at the kinase domain, which suggest that our findings are new, and suggest that apigenin and quercetin can potentially inhibit VEGFRs directly contributing to their anti-angiogenic properties. The docking conformations of quercetin and apigenin will be further analyzed for their interactions with the kinase domain of VEGFR, and compared to reference drugs, sorafenib, axitinib, pazopanib, and the re-docked co-crystallized ligands present in VEGFR crystal structures (Figures 2 and 3).

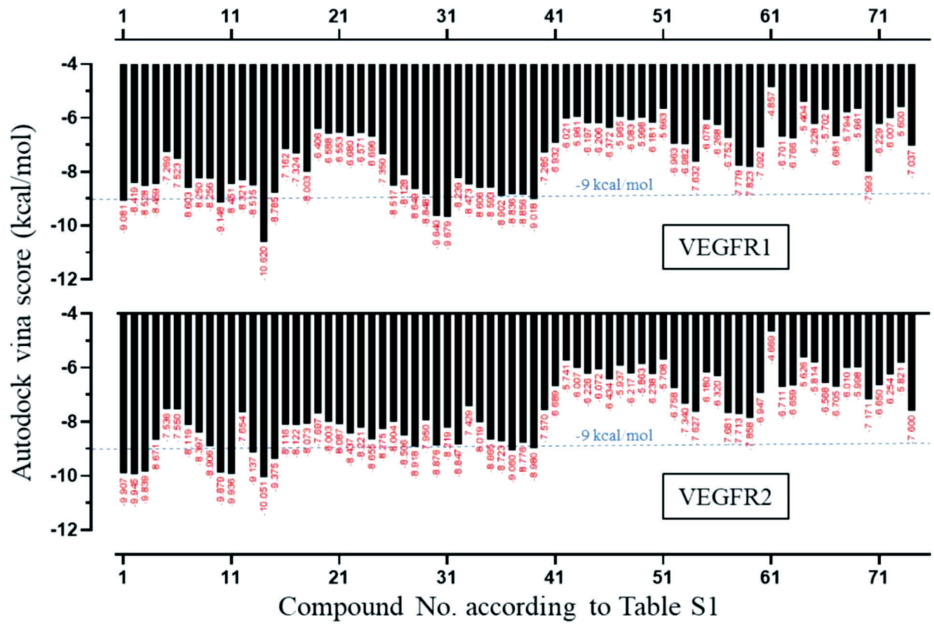


**Figure 1** The docking scores calculated using Autodock Vina for the 74 phytochemical compounds from *Houttuynia cordata* Thunb., targeting VEGFR1 and VEGFR2.

**Table 1** The docking scores of eleven best scoring candidates and the calculated physicochemical properties.

| No. | Name | Docking score (kcal/mol) | | Lipinski's rule of five | | | |
|---|---|---|---|---|---|---|---|
| | | VEGFR1 | VEGFR2 | MW | Log P | HBD | HBA |
| **1** | **Luteolin** | -9.081 | -9.907 | 286.25 | 1.486 | 4 | 6 |
| **2** | **Quercetin** | -8.419 | -9.945 | 302.24 | 1.834 | 5 | 7 |
| **3** | **Isorhamnetin** | -8.528 | -9.839 | 316.26 | 1.726 | 4 | 7 |
| **10** | **Apigenin** | -9.148 | -9.879 | 270.24 | 1.138 | 3 | 5 |
| **11** | **Kaempferol** | -8.451 | -9.936 | 286.24 | 1.486 | 4 | 6 |
| **14** | **Hesperidin** | -10.620 | -10.051 | 610.62 | -1.110 | 8 | 15 |

The predicted binding mode of apigenin with the ATP binding site of VEGFR1 was analyzed (Figure 2A). It was found that the phenol group was able to form hydrogen bonds with a Cys912, the ketone with Lys861, and a hydroxyl group on the bicyclic ring to residue Glu878. In comparison the docking simulations of the known inhibitors, sorafenib (Figure 2B) and axitinib (Figure 2C) exhibited hydrogen bond formations with Glu878 and Cys912, whereas pazopanib (Figure 2D) and compound 78 (Figure 2E) displayed hydrogen bonds with Glu878. It was established that a pharmacophoric feature of inhibitors of VEGFR1 at the ATP binding site is hydrogen bond formations with residues Cys912 and Glu878, which was seen for the reported inhibitors in the literature.[25-27] These key interactions are important contributions for explaining its prominent docking scores compared to the other compounds. Additionally, hydrophobic interactions were seen for sigma interactions with the π-delocalized system were observed with residues Leu833, Val892, Val909 and Leu1029, whereas alkyl-π interactions were formed with residues Val841, Ala859 and Cys1039. These residues were seen to form π-interactions with the reference drugs used and compound 78. In Figure 2A, it can be seen that the phenol ring of apigenin was inserted into the deep hydrophobic gorge of the ATP binding site, which is consistent with the binding modes of sorafenib, axitinib, pazopanib and compound 78, as the gorge is specific for occupation of hydrophobic aromatic rings. Here, hydrophobic interactions with leucine clusters were observed. Apigenin is mainly hydrophobic and thus, its binding affinity to VEGFR1 can be partly accounted for its non-polar van der Waal's interactions, which were formed with mainly hydrophobic residues: Gly915, Phe1041, Leu882, Val907, Val860 and Tyr911. Additionally, the side chains of residues charged polar residues including Asp1040 and Glu910 were also seen to form the van der Waal's interactions. Residues Asp1040 and Phe1041 are part of the DFG motif, which regulates structural conformational change of VEGFR1, *i.e.*, interactions with these two residues are known to hamper VEGFR1 activity.

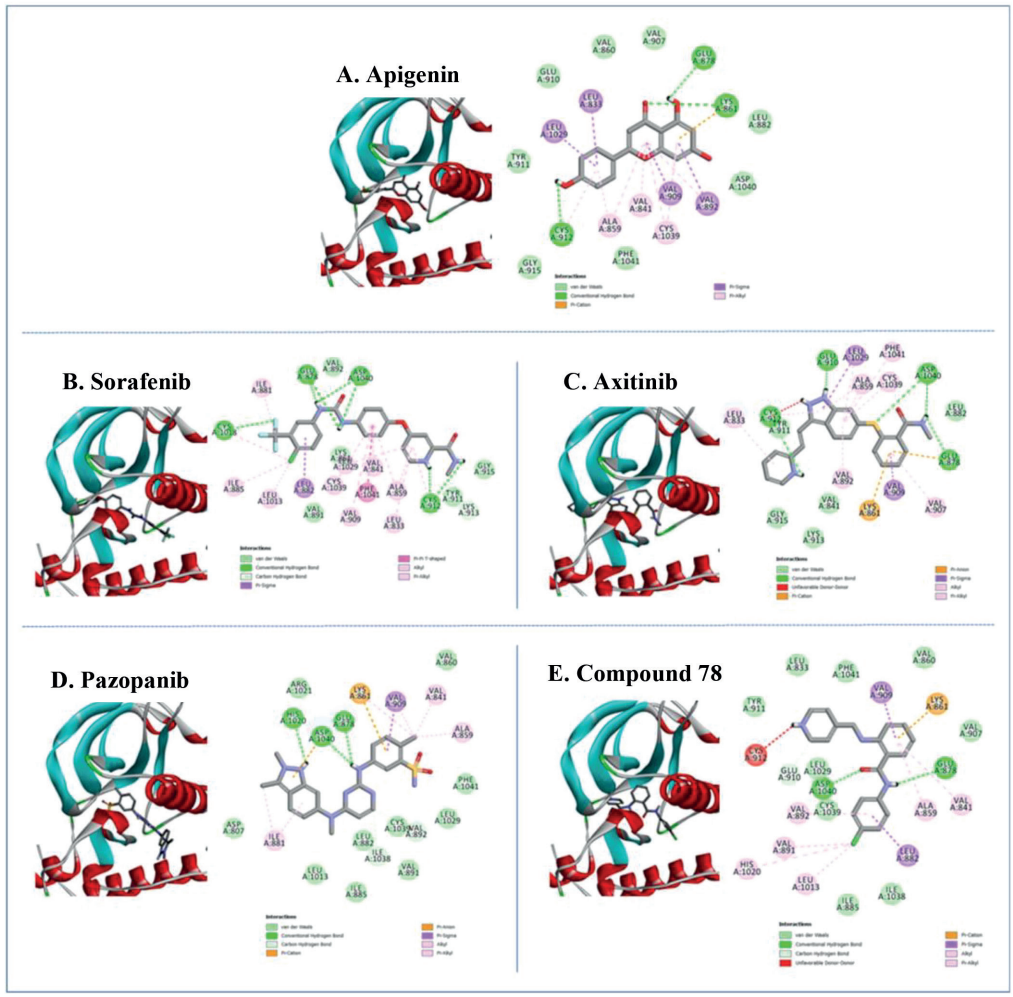


**Figure 2** Docked conformations of (A) apigenin, (B) sorafenib, (C) axitinib, (D) pazopanib, and (E) compound 78 (*N*-(4-Chlorophenyl)-2-((pyridin-4-ylmethyl)amino) benzamide) (PDB ID: 3HNG) to the VEGFR1 kinase domain, accompanied by their 2D interaction diagrams.

The predicted binding mode of quercetin with the ATP binding site of VEGFR2 was analyzed (Figure 3A). The structure of the VEGFR2 subtype is very similar and closely related to the VEGFR1 subtype. Hydrogen bonds were formed between quercetin and key pharmacophoric residues Cys919, Asp1046 and Glu917, *i.e.*, interactions with these residues were reported in literature to be important for VEGFR2 inhibition.[28-30] Residue Asp1046 is part of the VEGFR2 DFG motif. Thus, by forming a hydrogen bond interaction with Asp1046, quercetin is able to disrupt the activation mechanism of the receptor. Comparing to the docked conformations of the reference controls, axitinib and compound 79, clearly formed these interactions. Quercetin showed hydrophobic π-interactions; π-sigma between the bicyclic aromatic ring with residues Leu1035 and Leu840, and π-alkyl interactions between phenol ring and residues Ala866, Cys1045, Val916 and Val848. In comparison to the interactions with the reference controls, these amino acid residues were seen to form π-interactions with the controls. The bicyclic ring of quercetin was seen to insert into the hydrophobic gorge of the binding site where hydrophobic interactions and van der Waal's attractions were predicted. Quercetin is mainly hydrophobic. Thus, the binding strength of quercetin to VEGFR2

is accountable for its non -polar and hydrophobic interactions. Non-polar van der Waal's forces were observed between quercetin and mainly hydrophobic residues including Val867, Val899, Phe918, Gly922, Asn923 and Phe1047; Phe1047 is part of the DFG motif in VEGFR2, which recognizes this as a crucial interaction for VEGFR2 inhibition.[29] Other polar residues that contribute to van der Waal's attractions to quercetin include Lys920, Cys1045 and Lys868. These non-polar attractions were observed in the reference controls.

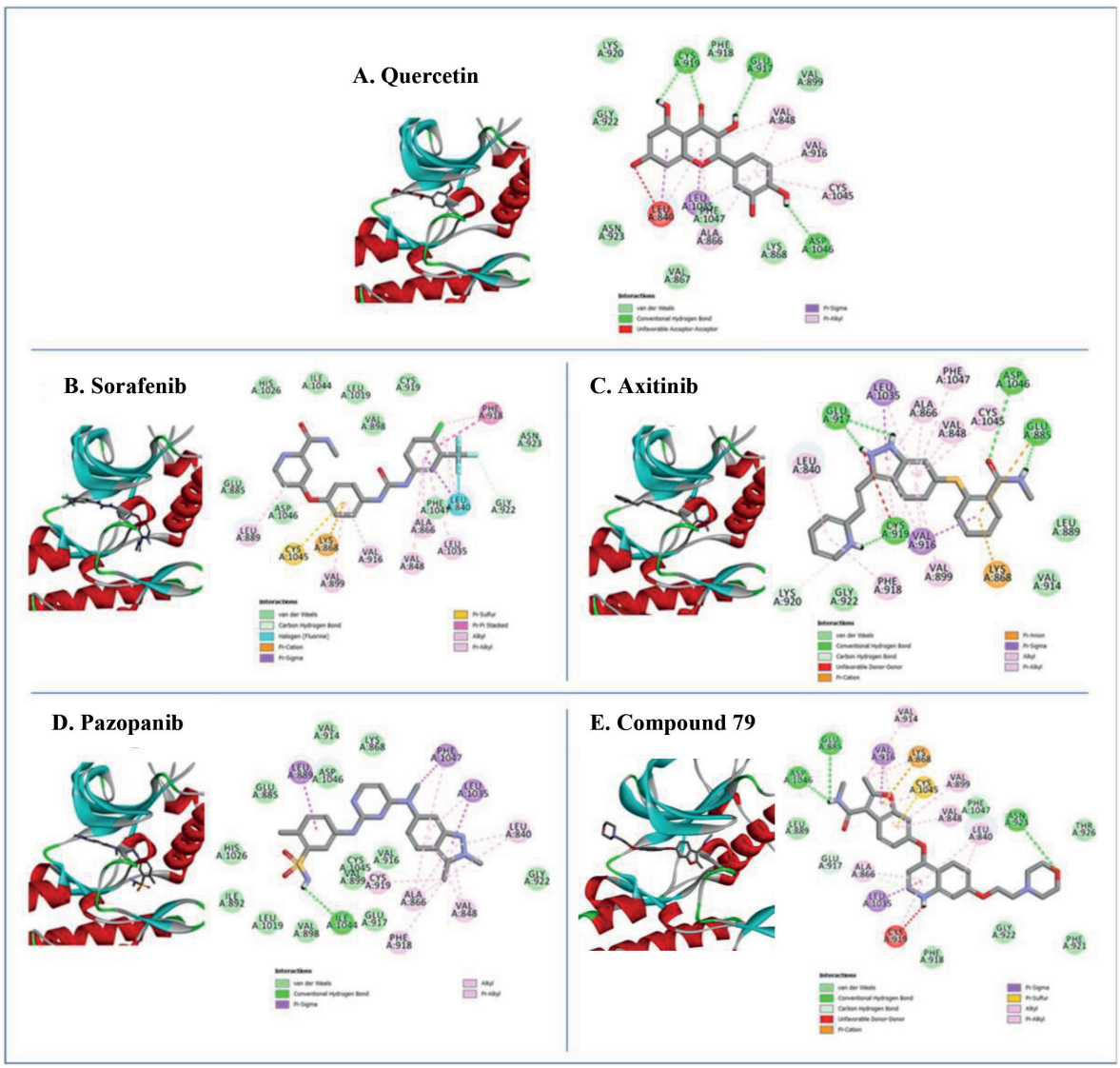


**Figure 3** Docked conformations of (A) quercetin, (B) sorafenib, (C) axitinib, (D) pazopanib, and (E) compound 79 (PF-0033721) (PDB ID: 2XIR) to the VEGFR2 kinase domain, accompanied by their 2D interaction diagram.

## Conclusion

This study highlights the medicinal potential of five phytochemical constituents namely, luteolin, quercetin, isorhamnetin, apigenin, and kaempferol, from *Houttuynia cordata* Thunb. as anti-angiogenic agents and VEGFR inhibitors with acceptable oral pharmacokinetics for the treatment of melanoma, which were identified using virtual screening. Specifically, apigenin and quercetin were predicted to be the strongest

VEGFR1 and VEGFR2 inhibitors, respectively. In this study, it is proposed that the compounds inhibit VEGFR through an unreported mechanism of action; direct inhibition of VEGFR at the ATP binding site. Apigenin and quercetin were proposed for further *in vitro* verification for VEGFR inhibition such as VEGFR protein-based inhibition assays, and further *in vivo* test such as evaluation of toxicity and oral pharmacokinetics in mice. Additionally, examining the synergistic effects of these phytochemicals with existing VEGFR inhibitors could provide a basis for developing combination therapies to enhance efficacies against melanoma.

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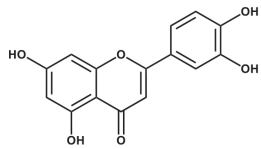
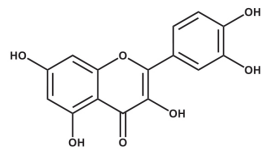
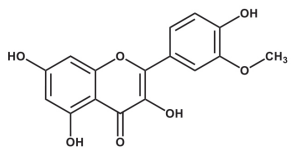
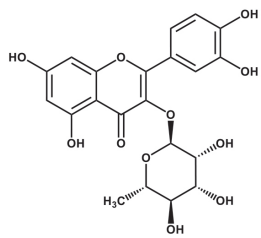
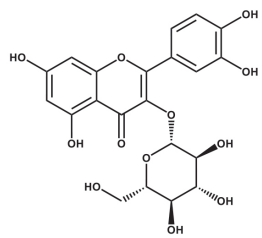
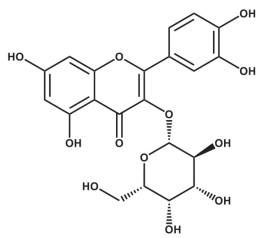
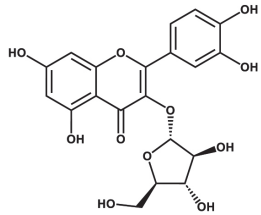
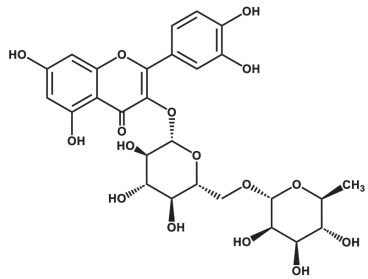
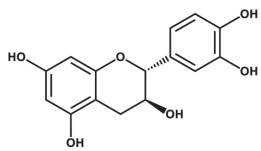
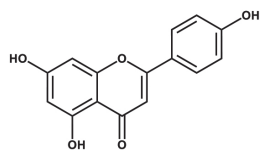
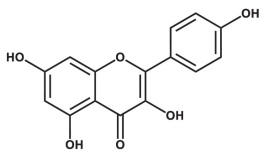
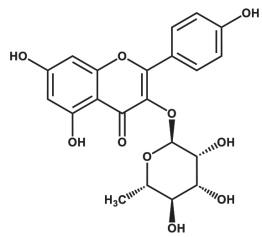
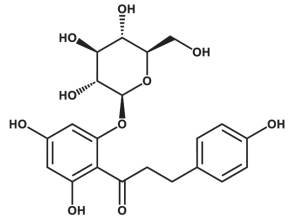
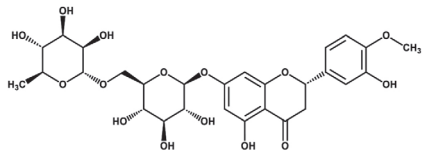
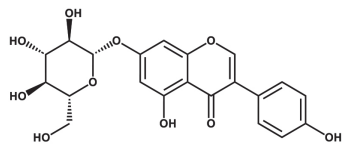
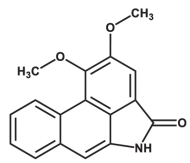
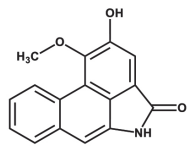
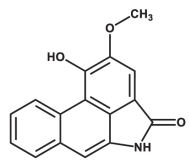
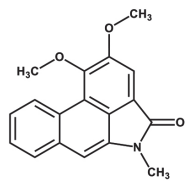
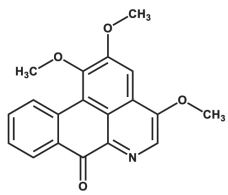
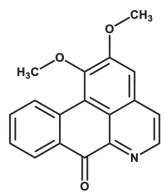
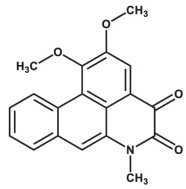
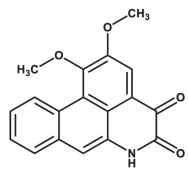
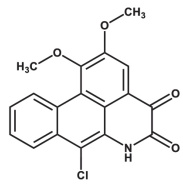
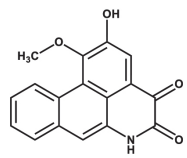
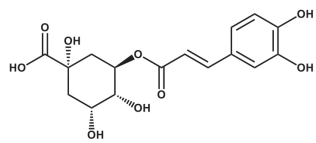
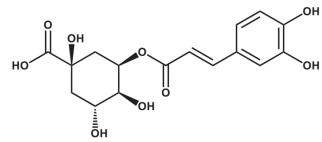
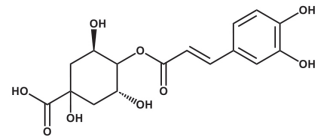
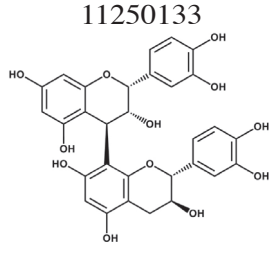
## Supplementary Materials

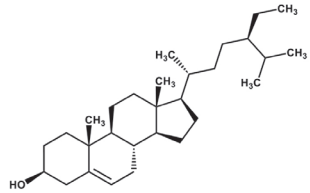
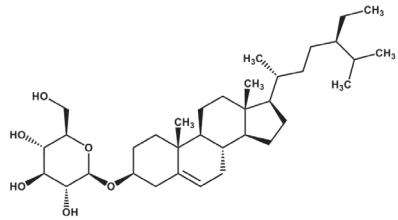
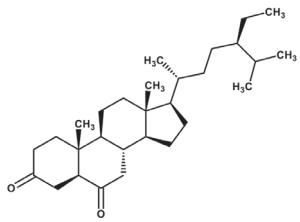
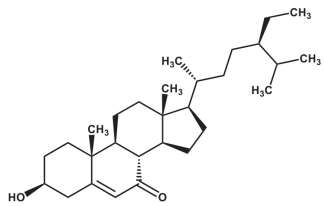
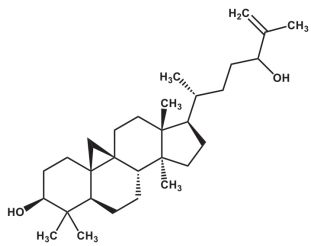
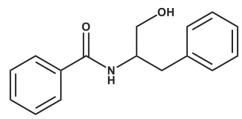
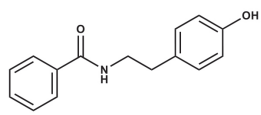
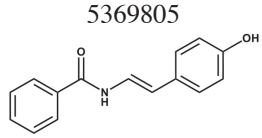
**Table S1** List of phytochemicals in Houttuynia cordata Thunb., and VEGFR inhibitors.

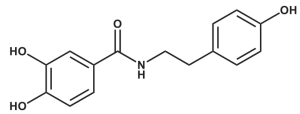
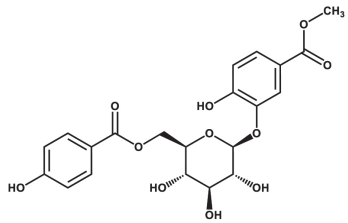
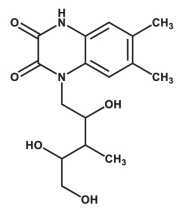
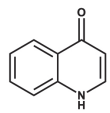
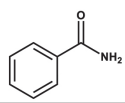
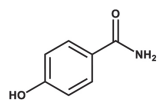
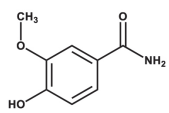
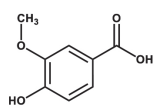
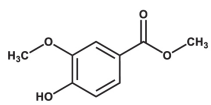
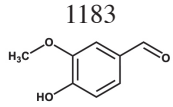
| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 1 | Luteolin<br>3′,4′,5,7-Tetrahydroxyflavone | 5280445<br>  |
| 2 | Quercetin<br>3,3′,4′,5,7-Pentahydroxyflavone | 5280343<br>  |
| 3 | Isorhamnetin / 3-Methylquercetin /<br>Quercetin 3’-methyl ether<br><br>3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one | 5281654<br>  |
| 4 | Quercitrin /<br>Quercetin 3-rhamnoside<br><br>3′,4′,5,7-Tetrahydroxy-3-(α-L-rhamnopyranosyloxy) flavone | 5280459<br>  |
| 5 | Isoquercitrin<br><br>3-(β-D-Glucopyranosyloxy)-3′,4′,5,7-tetrahydroxyflavone | 5280804<br>  |
| 6 | Hyperin / Hyperoside /<br>Quercetin 3-galactoside<br><br>3-(β-D-Galactopyranosyloxy)-3′,4′,5,7-tetrahydroxyflavone | 5281643<br>  |

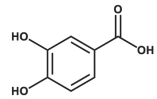
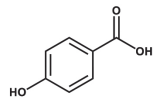
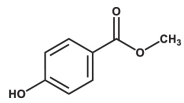
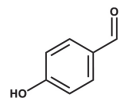
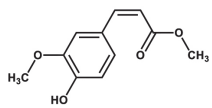
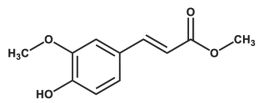
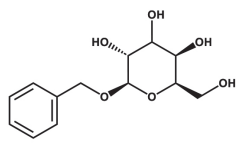
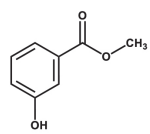
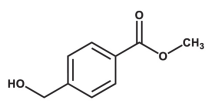
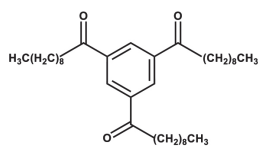
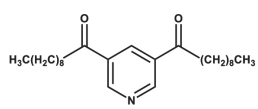
| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 7 | Avicularin<br><br>3-(((2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)oxy)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one | 5490064  |
| 8 | Rutin / Quercetin 3-rutinoside<br><br>3′,4′,5,7-Tetrahydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavone | 5280805  |
| 9 | Catechin<br><br>(2R,3S)-2-(3,4-dihydroxyphenyl)chromane-3,5,7-triol | 9064  |
| 10 | Apigenin<br><br>4′,5,7-Trihydroxyflavone | 5280443  |
| 11 | Kaempferol<br><br>3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one | 5280863  |
| 12 | Afzelin / Kaempferol 3-rhamnoside<br><br>4′,5,7-Trihydroxy-3-(α-D-rhamnopyranosyloxy)flavone | 5316673  |
| 13 | Phlorizin | 6072  |

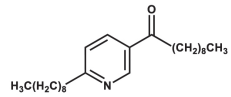
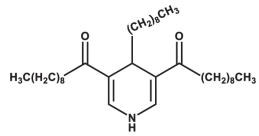
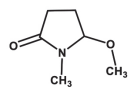
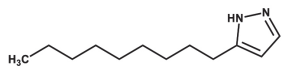
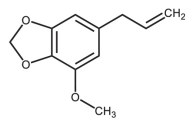
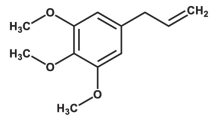
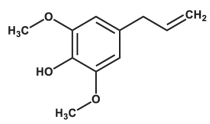
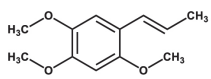
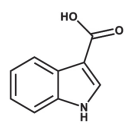
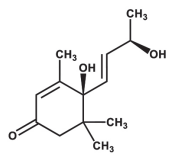
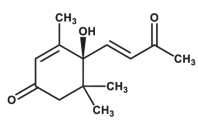
| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 14 | Hesperidin<br><br>(2S)-3′,5-Dihydroxy-4′-methoxy-7-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavan-4-one | 10621  |
| 15 | Genistin | 5281377  |
| 16 | Aristolactam BII / Cepharanone B<br><br>1,2-dimethoxydibenzo[cd,f]indol-4(5H)-one | 162739  |
| 17 | Aristolactam AII<br><br>2-hydroxy-1-methoxydibenzo[cd,f]indol-4(5H)-one | 148657  |
| 18 | Piperolactam A / Aristolactam F1<br><br>1-hydroxy-2-methoxydibenzo[cd,f]indol-4(5H)-one | 3081016  |
| 19 | Caldensine<br><br>1,2-dimethoxy-5-methyldibenzo[cd,f]indol-4(5H)-one | 21680139  |
| 20 | Splendidine<br><br>1,2,4-trimethoxy-7H-dibenzo[de,g]quinolin-7-one | 196452  |
| 21 | Lysicamine / Oxonuciferine<br><br>1,2-dimethoxy-7H-dibenzo[de,g]quinolin-7-one | 122691  |

| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 22 | Cepharadione B<br><br>1,2-dimethoxy-6-methyl-4H-dibenzo[de,g]quinoline-4,5(6H)-dione | 189151  |
| 23 | Norcepharadione B<br><br>1,2-dimethoxy-4H-dibenzo[de,g]quinoline-4,5(6H)-dione | 189168  |
| 24 | 7-Chloro-6-demethylcepharadione B<br><br>7-chloro-1,2-dimethoxy-4H-dibenzo[de,g]quinoline-4,5(6H)-dione | 131752718  |
| 25 | Noraristolodione<br><br>2-hydroxy-1-methoxy-4H-dibenzo[de,g]quinoline-4,5(6H)-dione | 10108434  |
| 26 | Chlorogenic Acid<br><br>(1S,3R,4R,5R)-3-(((E)-3-(3,4-dihydroxyphenyl) acryloyl)oxy)-1,4,5-trihydroxycyclohexane-1-carboxylic acid | 1794427  |
| 27 | Neochlorogenic acid<br><br>(1R,3R,4S,5R)-3-(((E)-3-(3,4-dihydroxyphenyl) acryloyl)oxy)-1,4,5-trihydroxycyclohexane-1-carboxylic acid | 5280633  |
| 28 | Cryptochlorogenic acid<br><br>(3R,5R)-4-(((E)-3-(3,4-dihydroxyphenyl) acryloyl)oxy)-1,3,5-trihydroxycyclohexane-1-carboxylic acid | 9798666  |
| 29 | Procyanidin B1<br><br>(2R,2'R,3R,3'S,4R)-2,2'-bis(3,4-dihydroxyphenyl)-[4,8'-bichromane]-3,3',5,5',7,7'-hexaol | 11250133  |

| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 30 | β-Sitosterol / Stigmast-5-en-3β-ol | 222284  |
| 31 | β-Sitosteryl glucoside | 5742590  |
| 32 | 5-α-Stigmastane-3,6-dione | 13992092  |
| 33 | 3-Hydroxy-β-sitost-5-en-7-one | 160608  |
| 34 | Cycloart-25-ene-3,24-diol | 11419367  |
| 35 | N-(1-hydroxy-3-phenylpropan-2-yl)benzamide | 100005  |
| 36 | N-(4-hydroxyphenylethyl)benzamide | 433864354  |
| 37 | trans-N-(4-hydroxystyryl)benzamide | 5369805  |

| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 38 | Houttuynamide A | 44521377  |
| 39 | Houttuynoside A | 44521323  |
| 40 | 6,7-dimethyl-1-(2,4,5-trihydroxy-3-methylpentyl)-1,4-dihydroquinoxaline-2,3-dione | 605462  |
| 41 | 4-Hydroxyquinoline | 69141  |
| 42 | Benzamide / Phenylcarboxyamide | 2331  |
| 43 | 4-Hydroxybenzamide | 65052  |
| 44 | 4-Hydroxy-3-methoxybenzamide | 354088  |
| 45 | Vanillic acid | 8468  |
| 46 | Methyl vanillate | 19844  |
| 47 | Vanillin | 1183  |

| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 48 | Protocatehuic acid<br>3,4-dihydroxybenzoic acid | 72  |
| 49 | 4-Hydroxybenzoic acid | 135  |
| 50 | Methylparaben | 7456  |
| 51 | p-Hydroxybenzaldehyde | 126  |
| 52 | Methyl cis-ferulate | 10176654  |
| 53 | Methyl trans-ferulate | 5357283  |
| 54 | Benzyl-β-D-glucopyranoside | 13254166  |
| 55 | Methyl 3-hydroxybenzoate | 88068  |
| 56 | Methyl 4-(hydroxymethyl) benzoate | 81325  |
| 57 | 1,3,5-Tridecanoylbenzene | 86173717  |
| 58 | 3,5-Didecanoylpyridine | 85697557  |

| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 59 | 5-Decanoyl-2-nonylpyridine | 85697559  |
| 60 | 3,5-didecanoyl-4-nonyl-1,4-dihydropyridine | 129711227  |
| 61 | 5-Methoxy-1-methylpyrrolidin-2-one | 11423602  |
| 62 | 3-Nonyl-1H-pyrazole | 24844218  |
| 63 | Myristicin | 4276  |
| 64 | Elemicin | 10248  |
| 65 | 4-allyl-2,6-dimethoxyphenol | 226486  |
| 66 | α-Asarone | 636822  |
| 67 | Indole-3-carboxylic acid | 69867  |
| 68 | Vomifoliol | 5280462  |
| 69 | Dehydrovomifoliol | 688492  |

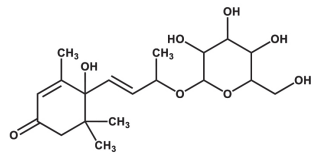
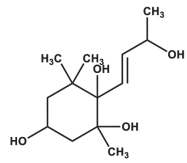
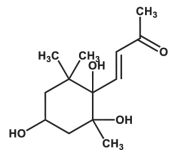
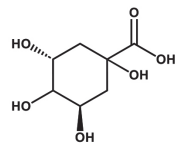
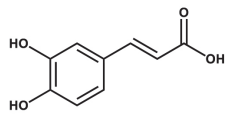
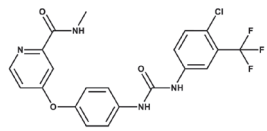
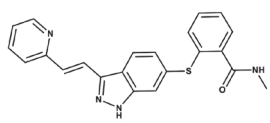
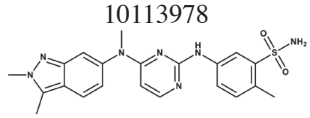
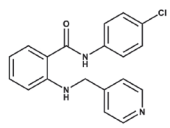
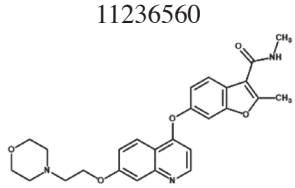
| No. | Name | PubChem CID Chemical structure |
|---|---|---|
| 70 | Roseoside | 73815023  |
| 71 | (E)-1-(3-hydroxybut-1-en-1-yl)-2,6,6-trimethylcyclohexane-1,2,4-triol | 72751004  |
| 72 | (E)-4-(1,2,4-trihydroxy-2,6,6-trimethylcyclohexyl) but-3-en-2-one | 51136538  |
| 73 | Quinic acid | 6508  |
| 74 | Caffeic Acid | 689043  |
| 75 | Sorafenib | 216239  |
| 76 | Axitinib | 6450551  |
| 77 | Pazopanib | 10113978  |
| 78 | N-(4-Chlorophenyl)-2-((pyridin-4-ylmethyl)amino)benzamide<br><br>(VEGFR tyrosine kinase inhibitor)<br>(Native ligand in PBD ID: 3HNG) | 9797919  |
| 79 | PF-00337210<br><br>(VEGFR2 tyrosine kinase inhibitor)<br>(Native ligand in PBD ID: 2XIR) | 11236560  |

**Table S2** The root mean square deviations of superimposed docked compounds to the VEGFRs. The compounds were docked at different exhaustiveness values.

| Target | Compound | Exhaustiveness superimposition[b] | RMSD[d] (Å) |
|---|---|---|---|
| | | | |
| VEGFR1 | Apigenin | 10 & 15 | 0.019 |
| | | 10 & 20 | 0.013 |
| | | 15 & 20 | 0.013 |
| | Quercetin | 10 & 15 | 0.009 |
| | | 10 & 20 | 0.005 |
| | | 15 & 20 | 0.010 |
| | Compound 78[a] | 10 & 15 | 0.408 |
| | | 10 & 20 | 0.353 |
| | | 15 & 20 | 0.134 |
| | | Co-crystallized conformation[c] & 20 | 0.787 |
| | | | |
| VEGFR2 | Apigenin | 10 & 15 | 6.884 |
| | | 10 & 20 | 6.881 |
| | | 15 & 20 | 0.013 |
| | Quercetin | 10 & 15 | 0.028 |
| | | 10 & 20 | 0.014 |
| | Compound 79[a] | 15 & 20 | 0.029 |
| | | 10 & 15 | 0.692 |
| | | 10 & 20 | 2.089 |
| | | 15 & 20 | 2.015 |
| | | Co-crystallized conformation[c] & 20 | 2.727 |

[a]Co-crystallized ligands; compounds 78 and 79 are N-(4-Chlorophenyl)-2-((pyridin-4-ylmethyl)amino)benzamide, and PF-00337210, respectively.
[b]Superimposition of docked conformations, in which the exhaustiveness values of 10, 15 and 20 were used.
[c]The conformations of the co-crystallized ligand was used.
[d]The DockRMSD web server was used to calculate the RMSDs (*J. Cheminform*. 2019, 11, 40).

**Table S3** Binding energies (kcal/mol) of compounds docked with VEGFR1 and VEGFR2.

| No. | Binding energy | |
|---|---|---|
| | VEGFR1 | VEGFR2 |
| 1 | -9.081 | -9.907 |
| 2 | -8.419 | -9.945 |
| 3 | -8.528 | -9.839 |
| 4 | -8.459 | -8.671 |
| 5 | -7.269 | -7.536 |
| 6 | -7.523 | -7.550 |
| 7 | -8.603 | -8.119 |
| 8 | -8.250 | -8.397 |
| 9 | -8.256 | -8.906 |
| 10 | -9.148 | -9.879 |
| 11 | -8.451 | -9.936 |
| 12 | -8.321 | -7.654 |
| 13 | -8.515 | -9.137 |
| 14 | -10.620 | -10.051 |
| 15 | -8.785 | -9.375 |
| 16 | -7.162 | -8.116 |
| 17 | -7.324 | -8.122 |
| 18 | -8.003 | -8.073 |
| 19 | -6.406 | -7.697 |
| 20 | -6.588 | -8.003 |
| 21 | -6.553 | -8.087 |
| 22 | -6.680 | -8.437 |
| 23 | -6.571 | -8.221 |
| 24 | -6.696 | -8.655 |
| 25 | -7.350 | -8.275 |
| 26 | -8.517 | -8.004 |
| 27 | -8.128 | -8.506 |
| 28 | -8.649 | -8.918 |
| 29 | -8.848 | -7.950 |
| 30 | -9.640 | -8.876 |
| 31 | -9.679 | -8.219 |
| 32 | -8.239 | -8.847 |
| 33 | -8.473 | -7.429 |
| 34 | -8.606 | -8.019 |
| 35 | -8.593 | -8.665 |
| 36 | -8.902 | -8.723 |
| 37 | -8.836 | -9.060 |
| 38 | -8.856 | -8.776 |
| 39 | -9.018 | -8.980 |
| 40 | -7.285 | -7.570 |
| 41 | -6.932 | -6.689 |
| 42 | -6.021 | -5.741 |
| 43 | -5.961 | -6.007 |
| 44 | -6.197 | -6.226 |
| 45 | -6.206 | -6.072 |
| 46 | -6.372 | -6.434 |
| 47 | -5.965 | -5.937 |
| 48 | -6.083 | -6.217 |
| 49 | -5.998 | -5.863 |
| 50 | -6.181 | -6.238 |
| 51 | -5.663 | -5.708 |
| 52 | -6.963 | -6.758 |
| 53 | -6.982 | -7.340 |
| 54 | -7.632 | -7.627 |
| 55 | -6.078 | -6.180 |
| 56 | -6.268 | -6.320 |
| 57 | -6.752 | -7.681 |
| 58 | -7.779 | -7.713 |
| 59 | -7.823 | -7.858 |
| 60 | -7.092 | -6.947 |
| 61 | -4.857 | -4.669 |
| 62 | -6.701 | -6.711 |
| 63 | -6.766 | -6.659 |
| 64 | -5.404 | -5.626 |
| 65 | -6.228 | -5.814 |
| 66 | -5.702 | -6.568 |
| 67 | -6.681 | -6.705 |
| 68 | -5.794 | -6.010 |
| 69 | -5.661 | -5.998 |
| 70 | -7.993 | -7.171 |
| 71 | -6.229 | -6.65 |
| 72 | -6.007 | -6.254 |
| 73 | -5.600 | -5.821 |
| 74 | -7.037 | -7.600 |
| 75 | -9.51 | -8.946 |
| 76 | -11.414 | -10.065 |
| 77 | -11.650 | -10.492 |
| 78 | -10.736 | -9.477 |
| 79 | -10.427 | -11.168 |

Note: Compounds number according in Table S1


สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
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# Body Mass Index Has Significant Moderate Positive Correlation with High Sensitivity C-Reactive Protein in Overweight and Obese Thai Adults

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## Abstract:

**Background:** Thailand's population is becoming more sedentary, while obesity, another major health issue, is rising worldwide. This trend increases non-communicable disease risk, especially cardiovascular disease. Chronic low-grade systemic inflammation caused by adipose tissue inflammation can be recognized by a rise in hs-CRP, a biomarker of cardiovascular disease risk. According to earlier studies, hs-CRP is significantly greater in obese people and related to lesser physical activity, but obesity may influence the results.

**Objectives:** This study aimed to study the association between total physical activity, screen time, and hs-CRP in overweight and obese adults. This study also examines screen time, a sedentary behavior indicator.

**Materials and Method:** This study was a cross-sectional study conducted in 21 healthy, normal-weight, overweight, and obese Thai adults aged between 20 and 40 years old. Subjects' body weight and height were measured, and they were interviewed to answer the GPAQ questionnaire and screen time questionnaire.

**Results:** Using the Pearson correlation coefficient, total physical activity and screen time had no significant correlation with hs-CRP ($p > 0.05$), while there was a significant, moderate positive correlation between BMI and hs-CRP ($r = 0.462$, $p = 0.035$).

**Conclusion:** Total physical activity and screen time had no significant correlation with hs-CRP in overweight and obese adults. However, BMI had a significant moderate positive correlation with hs-CRP.

**Keywords:** hs-CRP; Physical Activity; Overweight; Obesity

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## Introduction

Nowadays, the Thai population is starting to shift to a more sedentary lifestyle. The amount of time spent on physical activity is replaced by sedentary behavior, leading to a more negative health outcome.[1] Being sedentary, such as watching television, playing video games, using computers or tablets, and sitting or lying down, is one of the risk factors of many non-communicable diseases, including obesity.[2] Screen time, one of the measures for sedentary behavior, is also associated with a higher risk of overweight and obesity.[3] Overweight and obesity are conditions with abnormal or excessive fat accumulation that may impair health.[4] Overweight is defined as a BMI greater than or equal to 25 kg/m$^2$, and obesity is defined as a BMI greater than or equal to 30 kg/m$^2$ by the World Health Organization.[4] There is another classification from the WHO Western Pacific Region (WPRO) standard, which is more appropriate for Asians due to different body fat percentage and body composition from Caucasians. The WPRO criteria classified overweight with a BMI between 23.0 and 24.9 kg/m$^2$ and obese with a BMI greater than or equal to 25.0 kg/m$^2$.[5] While in Thailand, overweight is when the BMI is between 23.0-24.9 kg/m$^2$, and obesity is divided into several levels including (a) obesity level 1a BMI is between 25.0-29.9 kg/m$^2$, (b) obesity level 1b BMI is between 30.0-34.9 kg/m$^2$, (c) obesity level 2 BMI is between 35.0-39.9 kg/m$^2$, and (d) obesity level 3 BMI is greater than or equal to 40 kg/m$^2$ (National Health Security Office [NHSO], 2010).[6] The criteria used in this research is the WPRO standard, which is widely used in Asians.

An increase in BMI can lead to noncommunicable diseases such as (a) cardiovascular diseases, especially heart disease and stroke, and diabetes type 2; (b) musculoskeletal disorders, especially osteoarthritis;[4] (c) Cancers, including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon;[4] (d) gallstones and gallbladder disease; and (e) breathing difficulties, such as asthma and sleep apnea,[7] respectively. While the BMI increases, the risk for these noncommunicable diseases also increases.[4] Obesity is a serious health problem in Thailand and has been increasing worldwide.[8] About 17.1% and 23.8% of Thai adults aged 19 and over are overweight and obese, respectively, with the 40-59-year-old group found to have the highest prevalence, based on the Western Pacific Region of the World Health Organization (WPRO) criteria for obesity.[5] An increase in BMI can lead to many health consequences, such as cardiovascular disease, musculoskeletal disorders, breathing difficulties, and even cancer.[4,7]

Inflammation of adipose tissue in individuals with overweight and obesity can lead to chronic low-grade systemic inflammation,[9] detectable by elevated levels of high-sensitivity C-reactive protein (hs-CRP), an acute-phase protein synthesized by the liver in response to proinflammatory cytokines.[10] High-sensitivity C-reactive protein (hs-CRP) serves as a biomarker for predicting cardiovascular disease risk and may be raised in various circumstances, including acute infections, inflammation, and trauma.[11] Previous studies revealed that hs-CRP is significantly higher in obese individuals,[12] indicating a higher risk of cardiovascular disease. Another factor that could be affecting the level of hs-CRP is physical activity. Previous studies on the association between hs-CRP and physical activity showed that people with lower physical activity had higher levels of hs-CRP; however, these levels may be affected by the level of adiposity of the participants.[13,14] Moreover, longer television screen time was significantly associated with higher C-reactive protein levels, while computer

and reading time had no association.[15] Thus, this study aimed to investigate the association between BMI and total physical activity adequacy in overweight and obese Thai adults.

## Materials and Method

***Participants:***

Healthy Thai males and females, aged 20 to 40 years, with a BMI of 18.5 kg/m$^2$ or higher. The study was conducted at Mae Fah Luang University Hospital in Bangkok, Thailand. The number of participants was decided based on data from a similar study, "Disparate Habitual Physical Activity and Dietary Intake Profiles of Elderly Men with Low and Elevated Systemic Inflammation" by Dimitrios Draganidis, because there wasn't enough information about physical activity and hs-CRP from earlier research.[16] We enrolled 21 subjects in this study.

*Inclusion Criteria:*

(a) Male and female participants were aged between 20 and 40 years old.

(b) BMI equal to or more than 18.5 kg/m$^2$

(c) Non-smokers or those who quit smoking for more than 20 years.

(d) Participants are healthy and not on any supplements or medication.

(e) Willingness to sign the informed consent and follow the instructions given.

Participants agreed to examine blood samples.

*Exclusion Criteria:*

(a) Pregnant and breastfeeding women

(b) Participants with other inflammatory conditions, such as autoimmune diseases, inflammatory bowel diseases, inflammatory joint diseases, and cancer.

(c) Participants with recent infections (within 2 weeks)

(d) Participants with recent traumas (within 2 weeks)

*Discontinuation Criteria:*

(a) Participants who want to drop out of the study

(b) Participants who cannot comply with the instructions

(c) Participants who suffer from serious illness during the study

(d) Participants with hs-CRP higher than 10 mg/L.

This study was conducted in strict accordance with Good Clinical Practice (GCP) criteria. This guideline encompasses the protection of human rights in clinical trials, the assurance of subjects' safety and well-being, and the requirements for conducting clinical studies. The Mae Fah Luang University Ethics Committee on Human Research, No. EC 23166-20, gave its consent for this study.

***Questionnaires:***

GPAQ (Global Physical Activity Questionnaire) includes 16 Questions, 3 Domains (Activity at work, Travel to and from places, Recreational activities). Total Physical Activity (TPA) calculated by the equation: TPA (MET-minutes/week) = summation of the total MET-minutes of activity computed for each domain (days of activity per week * amount of time * intensity of activity) (* = multiplication). MET Value Used for the Calculation of a Person's Overall Energy Expenditure Using GPAQ Data is shown below:

**Table 1** MET Value Used for the Calculation of a Person's Overall Energy Expenditure

| Domain | MET Value |
|---|---|
| Work | Moderate MET value = 4.0<br>Vigorous MET value = 8.0 |
| Transport | Cycling and walking MET value = 4.0 |
| Recreation | Moderate MET value = 4.0<br>Vigorous MET value = 8.0 |

***Physical Activity cut-off value:*** Score of less than 600 MET minutes per week do not meet WHO recommendations on physical activity for health

***Physical Examination***

Weight was measured by digital scale, done in light clothing and without shoes. Height was measured by the stadiometer without shoes. The body mass index (BMI) was calculated by dividing weight in kilograms (kg) by height in centimeters (cm) squared. Blood pressure and heart rate were measured by a digital sphygmomanometer. In Thailand, overweight is defined as a BMI between 23.0 and 24.9 kg/m$^2$, with obesity categorized into several levels, including (a) obesity level 1a, where the BMI is between 25.0 and 29.9 kg/m$^2$; (b) obesity level 1b, where the BMI is between 30.0 and 34.9 kg/m$^2$; (c) obesity level 2, where the BMI is between 35.0 and 39.9 kg/m$^2$; and (d) obesity level 3, where the BMI is greater than or equal to 40 kg/m$^2$.[6] The criteria used in this research is the WPRO standard, which is widely used in Asians, as shown below:

**Table 2** Obesity Classification by WPRO Criteria

| Classification | BMI (kg/m$^2$) |
|---|---|
| Underweight | < 18.5 |
| Normal | 18.5 - 22.9 |
| Overweight | 23.0 - 24.9 |
| Obesity Class I | 25.0 - 29.9 |
| Obesity Class II | ≥ 30 |

***Statistical Analysis***

The documentation of the medical records and the outcome of this study are recorded and analyzed by Microsoft Excel and SPSS software version 29.0 (IBM Corp., 2023). Qualitative data, such as gender, were analyzed and presented as numeric data and percentages, while other data, such as weight, height, and BMI, were analyzed and presented as the mean, median, standard deviation, and interquartile range. The chi-square test was used to assess the association between the classification of body mass index and total physical activity adequacy, calculating the odds ratio (OR) and 95% confidence intervals (CI). A two-sided P-value less than 0.05 is considered statistically significant.

## Results

We conducted this study to investigate the relationship between hs-CRP, total physical activity, and screen time in adults who are overweight or obese. It was conducted on 21 healthy volunteers aged between 20 and 40 with a BMI of more than 18.5 kg/m$^2$ and no underlying diseases and concurrent use of any medication or supplements. General characteristics, total physical activity, and screen time were collected by a questionnaire, and the participants' body weight, height, and serum hs-CRP were measured. We found that 15 individuals were female and 6 were male. The mean age was 34.90 ± 5.34 years old, the mean height was 162.43 ± 7.86 cm, and the mean weight was 60.41 ± 11.51 kg. The mean body mass index (BMI) was 22.77 ± 3.20 kg/m$^2$. 10 subjects were normal weight, followed by 6 subjects who were obese and 5 overweight subjects. Most of the participants were office workers (16 subjects), followed by medical personnel (4 subjects) and one housekeeper. Total physical activity had a median value of 1,120 (IQR 200, 1,615) MET-minutes/week. A total of 14 subjects, accounting for 66.7%, demonstrated adequate physical activity. The mean screen time score was 11.42 ± 2.50. The median hs-CRP level was 0.92 (IQR 0.41, 1.86) mg/L. The majority had a low risk of cardiovascular disease (13 subjects), followed by intermediate risk (7 subjects) and high risk (1 subject) (Table 3).

**Table 3** Demographic data

| Demographic data | n = 21 |
|---|---|
| Sex, n (%) | |
| Male | 6 (28.6) |
| Female | 15 (71.4) |
| Age, n (%) | |
| 21-30 | 5 (23.8) |
| 31-40 | 16 (76.2) |
| Height (cm), mean ± SD (min-max) | 162.43 ± 7.86 (150-183) |
| Weight (kg), mean ± SD (min-max) | 60.41 ± 11.51 (45-83) |
| Body mass index (kg/m2), mean ± SD (min-max) | 22.77 ± 3.20 (18.73-27.48) |
| Total physical activity (MET-minutes/week), median (IQR) | 1,120 (200, 1,615) |
| Total physical activity level, n (%) | |
| Adequate physical activity | 14 (66.7) |
| Inadequate physical activity | 7 (33.3) |

It was found that 70% of the participants with normal weight had adequate physical activity and 30% had inadequate physical activity, while 63.6% of those who were overweight or obese had adequate physical activity and 36.4% had inadequate physical activity. The relationship between BMI classification and total physical activity

adequacy was not statistically significant ($p = 0.758$). However, considering the odds ratio value of 0.75 (95% CI 0.12, 4.66), it can be said that the odds of overweight or obese participants who had adequate physical activity are 0.75 times lower than normal-weight participants (Table 4).

**Table 4** The Association Between BMI and Total Physical Activity Adequacy

| BMI classification | Total Physical Activity Adequacy | | Odds ratio (95%CI) | *p*-value |
|---|---|---|---|---|
| | Adequate (n = 14) | Inadequate (n = 7) | | |
| Normal | 7 (70.0) | 3 (30.0) | Reference | |
| Overweight/ Obese I | 7 (63.6) | 4 (36.4) | 0.75 (0.12, 4.66) | 0.757 |

Note: Data were analyzed with Chi-square test.

## Discussion

This study intended to investigate the correlation between BMI and overall physical activity in overweight and obese people. The study involved 21 healthy adults aged 20 to 40 years, with a BMI of 18.5 kg/m$^2$ or more. Total physical activity was collected by questionnaires. Low physical activity and high screen time usage represent a sedentary lifestyle, which could lead to a higher risk of non-communicable diseases, including obesity and cardiovascular diseases.[2] The benefits of this study were to identify the increased risk of developing cardiovascular disease in overweight and obese adults and to use it as reference data for future studies. Previous studies demonstrated that BMI was moderately positively correlated with hs-CRP in children and adults.[17-19] A recent study indicated that those with elevated physical activity exhibited lower hs-CRP levels compared to those with diminished physical activity; however, these findings may be influenced by a higher prevalence of overweight and obese participants in the low physical activity cohort.[14]

Obesity is more prevalent in people from lower socioeconomic classes in developing countries without food scarcity, presumably from high-fat diets, which are more affordable. On the other hand, in low-income countries with food scarcity, the rich are more susceptible to obesity because of more accessibility to excess food and less involvement in labor work.[20] A study in Ghana and Nigeria, which are low-to-middle-income countries, showed that older adults in urban areas with higher income and education were associated with a higher chance of obesity.[21] The prevalence of overweight and obesity has been increasing worldwide.[8] It is also considered a serious health problem in Thailand. From a study in 2018, the prevalence of overweight and obesity in Thai adults aged 19 and over was 17.1% and 23.8%, respectively, based on the WRPO criteria, but only 19.0% and 4.8% based on the WHO criteria. According to the WPRO criteria, adults in the 40–59-year-old group were found to have the highest prevalence, and the population in Bangkok had the highest prevalence of obesity.[5] The main cause of obesity and being overweight is when there is an energy imbalance between calories taken in and calories expended, leading to excess weight gain and abnormal accumulation of fat in

the body.[4,20] Many factors contribute to obesity, including genetics, individual factors, and environmental factors.[20] Different genetic contributing mechanisms can classify obesity into three groups: monogenic, polygenic, or syndromic. Monogenic obesity, which involves chromosomal deletion or single gene defects, contributes to rare and early-onset obesity; for example, gene defects that are related to leptin deficiency, proopiomelanocortin (POMC) deficiency, and melanocortin-4 receptor (MC4R) deficiency.[22]

Polygenic obesity, which involves hundreds of polymorphisms with small effects, is more common.[22] Individuals possessing a genetic predisposition are at an elevated risk of developing obesity. Genome-wide association studies (GWAS) have identified over 300 single nucleotide polymorphisms (SNPs) linked to obesity-related traits, such as BMI and waist-to-hip ratio, along with more than 500 genetic loci associated with obesity.[24] SNPs in FTO (fat mass and obesity-associated gene) are found in multiple populations, and studies indicated that FTO is associated with appetite and feeding behavior; however, the mechanism is not well understood.[25] The FTO gene can cause an increase in hunger level, caloric intake, body fat storage, and tendency to a sedentary lifestyle. Moreover, it can reduce satiety and lead to overeating.[26] Syndromic obesity is a rare genetic condition that is present from birth and often comes with other health issues, like problems with thinking, unusual physical features, or organ problems. This includes Prader-Willi syndrome, Bardet-Biedl syndrome, and Cohen syndrome.[26]

An imbalance between caloric intake and energy expenditure causes body bodyweight change. When caloric consumption surpasses energy expenditure, a positive energy balance results in an increase in body mass, typically in the form of body fat. Conversely, when energy expenditure surpasses energy intake, a negative energy balance results in a reduction of body mass.[27] Individuals with obesity face a heightened risk of numerous diseases and health complications in comparison to those of normal weight.[7] Obesity affects many dimensions, including physical health, mental health, and social factors, among others.[18] Individual factors that can affect obesity are the factors that play an important role in energy expenditure. These include the basal metabolic rate (BMR), the energy used in breaking down food, and the energy used in physical activity.[20] Also, excessive energy from calorie intake can contribute to fat accumulation, especially from high-energy-dense food.[28] High energy-dense diets, which are high in energy and fat but low in fruits, vegetables, and fiber,[29] are positively correlated with increased weight gain and excess adiposity.[30] Obesity elevates the likelihood of impairments and age-associated ailments, including cardiovascular disease, diabetes, osteoarthritis, and cancer. Furthermore, research suggests that obesity shortens life expectancy and influences the aging process in cells. According to previous studies, obesity is negatively associated with telomere length, which may be due to oxidative stress and inflammation.[31] Some diseases or endocrine disorders are related to obesity, for example, Cushing's disease, hypothyroidism, and polycystic ovary syndrome.[20] A study on medication-induced weight gain revealed that antipsychotics, antidepressants, antihyperglycemics, antihypertensives, and corticosteroids include drugs significantly linked to weight increase.[32] Being obese may create stigma, and obesity discrimination may lead to some mental disorders.[18] Previous studies have linked obesity to depression, eating disorders, anxiety, substance abuse, sexual abuse,

and other issues. They also affect an individual's self-esteem, their body image dissatisfaction, and a decreased quality of life.[33] Obesity in childhood and adolescence is associated with an increased risk of premature morbidity and mortality, specifically cardio-metabolic morbidity.[18] They also have an increased risk of fractures, metabolic syndrome, and breathing problems in the future.[4]

Workplace environments can also affect obesity. Long working hours can lead to increased sitting time and reduced time for exercise and other physical activities, resulting in an increase in BMI. Also, it can affect the meal by processed food or fast food instead of healthier homemade food.[20] Nowadays, the improvement in labor-saving technology, such as communication devices and internet media platforms, is related to a decrease in work-related energy expenditure and weight gain.[34] Neighborhoods also have an effect on obesity. A study in Canada found that high neighborhood walkability is associated with decreased prevalence of overweight and obesity.[35] Neighborhood deprivation or a neighborhood with crime is associated with an increased probability of being overweight.[34] Recently, an improvement in socioeconomic status leads to a more sedentary lifestyle, which contributes to an increase in noncommunicable diseases, including obesity. Studies indicate that physical inactivity is associated with weight gain and sedentary behavior is associated with abdominal obesity. However, the association between screen time and obesity is still inconclusive.[36]

According to this study, there was no significant association between BMI and physical activity. However, there are still some other sedentary behaviors that could bestudied including hs-CRP, screen time, and time spent sitting or lying down. The amount and intensity of physical activity might have an influence on hs-CRP, while too little physical activity could not affect hs-CRP. Apart from hs-CRP, there are other markers related to inflammation and cardiovascular diseases that could be studied, for example, arterial stiffness, and inflammatory markers like erythrocyte sedimentation rate (ESR), fibrinogen, and interleukin-6 (IL-6). The limitation of this study was the data collection method. Questionnaires were used to record total physical activity. Therefore, the participants need to recall their memory of their daily physical activity throughout the week, which could possibly lead to inaccurate answers and results.

## Conclusion

There was no significant connection between total physical activity and screen time with hs-CRP levels in overweight and obese subjects. Nonetheless, BMI exhibited a notable moderate positive connection with hs-CRP. The quantity and intensity of physical exercise may affect hs-CRP, but insufficient physical activity may not impact hs-CRP.

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**Author Contributions:** Conceptualization: Suchanart Tangchitnob and Vitoon Jularattanaporn; methodology: Suchanart Tangchitnob and Vitoon Jularattanaporn; software: Suchanart Tangchitnob; validation: Vitoon Jularattanaporn and Phakkharawat Sittiprapaporn; formal analysis: Suchanart Tangchitnob, Vitoon Jularattanaporn, Phakkharawat Sittiprapaporn; investigation:

Vitoon Jularattanaporn; data curation: Suchanart Tangchitnob; writing—original draft preparation: Phakkharawat Sittiprapaporn; writing—review and editing: Phakkharawat Sittiprapaporn; visualization: Vitoon Jularattanaporn; supervision: Vitoon Jularattanaporn; project administration: Vitoon Jularattanaporn; funding acquisition: Suchanart Tangchitnob. All authors have read and agreed to the published version of the manuscript.

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# Knowledge, Awareness and Attitude about Human Papilloma Virus (HPV) Infection and HPV Vaccination among Adolescents in Chiang Rai, Thailand

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## Abstract:

**Background:** Human Papilloma Virus (HPV) is a significant cause of cervical cancer and other HPV-related diseases. The HPV vaccine is an effective method for preventing these conditions, particularly when administered during adolescence.

**Objectives:** To evaluate the knowledge, awareness, and attitudes of adolescents in Chiang Rai, Thailand, with the aim of identifying key factors that influence the need for HPV vaccination.

**Materials and Method:** A total of 426 participants were recruited from the secondary schools in Chiang Rai, Thailand. Data were collected through online questionnaires, which included sections on baseline characteristics, knowledge, awareness, attitudes toward HPV infection and vaccination, and the perceived need for HPV vaccination.

**Results:** The proportions of participants with high levels of knowledge, awareness, and attitudes about HPV were 72.30%, 14.31%, and 62.68%, respectively. Additionally, 64.08% expressed the need for HPV vaccination. Being female (OR=2.20, $p < 0.001$), having a high level of awareness (OR=2.96, p=0.007) and attitude toward HPV infection and vaccination (OR=3.35, $p = 0.038$) were significantly associated with the perceived need for HPV vaccination.

**Conclusion:** Health promotion about HPV is vital for adolescents, with an emphasis on both females and males. Initiatives to improve awareness and attitude of HPV infection should be implemented to increase vaccination rates and reduce the prevalence of HPV.

**Keywords:** HPV, Vaccine, Knowledge, Awareness, Attitude, Adolescents

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## Introduction

Cervical cancer is the fourth most common cancer among women globally, with approximately 660,000 new cases and 350,000 deaths reported in 2022.[1] In Thailand, it ranks as the third most frequent cancer among women and the second most common among those aged 15-44.[2] Most cases of cervical cancer are caused by human papillomavirus (HPV), particularly types 16 and 18.[3]

The World Health Organization (WHO) recommends HPV vaccination as a key preventive measure against cervical cancer and other HPV-related diseases. Six prophylactic vaccines provide protection against HPV types 16 and 18, which are responsible for over 70% of cervical cancers. Research has shown these vaccines to be safe and highly effective. Vaccination is recommended for both males and females, ideally between the ages of 9 and 14, before the onset of sexual activity.[4] In Thailand, the quadrivalent (HPV types 6, 11, 16 and 18) and 9-valent (HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58) vaccines are available in public and private sectors. Since 2023, the National Health Security Office (NHSO) has implemented a school-based HPV vaccination program for Grade 5 girls, aiming for full nationwide coverage by 2025. Early data show high effectiveness in preventing HPV infection and precancerous cervical lesions.

Previous studies have identified multiple factors influencing HPV vaccine acceptance among adolescents and young adults. These include gender, level of knowledge and awareness about HPV, perceived susceptibility and severity of infection, perceived benefits of vaccination, social norms, and recommendations from healthcare providers. Cultural beliefs, parental influence, and accessibility of vaccination services have also been shown to affect vaccine uptake. For instance, perceived benefits and social influences were significant predictors of HPV vaccination intentions among young women[6], while higher knowledge and positive attitudes were associated with increased willingness to receive the vaccine in studies conducted among adolescents in Ethiopia and Italy.[7,11] Collectively, these findings highlight the need to understand these multidimensional factors within specific cultural contexts, such as northern Thailand, to design effective public health strategies.

Despite the availability of effective vaccines, gaps in knowledge, awareness, and attitudes toward HPV vaccination remain, particularly among adolescents, the primary target group for vaccination programs. This study aims to assess the levels of knowledge, awareness, and attitudes about HPV and its vaccine among adolescents, with the goal of identifying key factors influencing vaccine uptake and informing targeted public health strategies.

## Method

This cross-sectional study was conducted from November 1, 2023, to January 31, 2024, at a large public secondary school in Chiang Rai Province.

### Study population and samples

Adolescents aged 12-20 years enrolled at a large public secondary school in Chiang Rai Province in the 2023 academic year were included in the study. Participants who were unable to complete the questionnaire due to absence, underlying pathology, or physical abnormalities were excluded from the study. This included individuals with significant physical or mental disabilities, serious underlying medical conditions, or a history of HPV-related pathology.

The required sample size was estimated based on a previous study, which reported

53.39% awareness of HPV infection among women.[5] A minimum sample size of 426 participants was calculated to achieve a 0.05 margin of error and acceptable differences in apparent and adjusted R-squared values. Stratified proportional (based on number of students in each grade) random sampling was applied according to educational level, with 213 participants recruited from middle school (grades 6-8) and 213 participants from high school (grades 9-12), a total of 426 participants.

**Assessment of knowledge, awareness, attitude about HPV infection and vaccination and interested in receiving the HPV vaccine**

Data were collected using an online questionnaire via Google Forms. The questionnaire consisted of four sections to assess participants' interest in receiving the HPV vaccine, specifically the quadrivalent (HPV types 6, 11, 16 and 18) and the 9-valent (HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58) vaccines, as well as their knowledge[6], awareness[7], and attitude[5] about HPV infection and vaccination. Items were adapted from a previous study and translated into Thai. The content validity of the questionnaire after translation was 0.85 which was evaluated by 2 community and family medicine physicians and 1 gynecologist.

Levels of knowledge, awareness, and attitude about HPV infection and vaccination were categorized into three groups based on total score, low or negative (< 60%), moderate or neutral (60-79%), and high or positive (80-100%), following on Bloom's cutoff criteria.[8]

**Statistical analysis**

Data analysis was conducted using Stata 17 (StataCorp, College Station, TX, USA). A p-value of < 0.05 was considered statistically significant. Categorical variables were described using frequencies and percentages, while numerical variables were summarized as mean and standard deviation (SD) or median and interquartile range (IQR), depending on their distribution. Fisher's exact test was used to compare categorical variables. Student's t-test or the Mann-Whitney U test was applied for numerical variables. Univariable and multivariable logistic regression were employed to assess associations between baseline characteristics, knowledge, awareness, attitude levels, and interest in receiving the HPV vaccine. There was no missing data in this study.

## Results

**Participant's Characteristics**

This study recruited 426 participants, with females comprising 55.63%. The average age was 15 years. High levels of knowledge, positive awareness, and positive attitudes about HPV were reported by 72.30%, 14.31%, and 62.68% of participants, respectively. Furthermore, 64.08% expressed a need for HPV vaccination. Ninety-seven participants (22.77%) reported having received the HPV vaccination before being recruited into this study.

**Baseline Characteristics Between Groups Interested and Not Interested in Receiving the HPV Vaccine**

The results showed a significant difference in gender, age, level of education, awareness, and attitude about HPV infection ($p < 0.05$) between the groups interested and not interested in receiving the HPV vaccine (table 1). Female participants showed greater willingness to receive the HPV vaccine. In contrast, Male participants showed reduced willingness vaccine acceptance.

**Table 1** Comparison of Baseline Characteristics Between Groups Interested and Not Interested in Receiving the HPV Vaccine

| | **Interested in receiving the HPV vaccine** (n = 273) n (%) | **Not interested in receiving the HPV vaccine** (n = 153) n (%) | **p-value** |
|---|---|---|---|
| **Gender** | | | |
| • Male | 97 (35.53%) | 92 (60.13%) | < 0.001 |
| • Female | 176 (64.47%) | 61 (39.87%) | |
| **Age** (years)* | 15 (14-16) | 15 (14-16) | 0.036 † |
| **Level of education** | | | |
| • Middle school (grades 6 to 8) | 124 (45.42%) | 89 (58.17%) | 0.015 |
| • High school (grades 9 to 12) | 149 (54.58%) | 64 (41.83%) | |
| **Level of knowledge** | | | |
| • Low (0-4 points) | 18 (6.59%) | 19 (12.42%) | 0.120 |
| • Moderate (5-6 points) | 52 (19.05%) | 29 (18.95%) | |
| • High (7-8 points) | 203 (74.36%) | 105 (68.63%) | |
| **Level of awareness** | | | |
| • Negative (0-2 points) | 145 (53.11%) | 106 (69.28%) | < 0.001 |
| • Neutral (3 points) | 76 (27.84%) | 38 (24.84%) | |
| • Positive (4 points) | 52 (19.05%) | 9 (5.88%) | |
| **Level of attitude** | | | |
| • Negative (0-4 points) | 6 (2.20%) | 10 (6.54%) | 0.001 |
| • Neutral (5-6 points) | 80 (29.30%) | 63 (41.18%) | |
| • Positive (7-8 points) | 187 (68.50%) | 80 (52.29%) | |

*Median (IQR), † Mann-Whitney U test

### Knowledge about HPV infection and HPV vaccination

The total score of knowledge was 10 points. The mean knowledge score about HPV infection and HPV vaccination for the groups interested and not interested in receiving the HPV vaccine were 9 (2-10) and 8 (2-10) points, respectively. Questions 10, 7, and 5 had the highest correct answer rates (89.67%, 88.73%, and 87.32%, respectively), while questions 1, 3, and 6 had the lowest correct answer rates (71.36%, 73.47%, and 76.29%, respectively) (table 2).

**Table 2** Knowledge of students about HPV infection and HPV vaccination

| Question | Correct N (%) | Wrong N (%) |
|---|---|---|
| **Q.1** There is only one type of HPV infection. | 304 (71.36%) | 122 (28.64%) |
| **Q.2** HPV causes genital herpes. | 343 (80.52%) | 83 (19.48%) |
| **Q.3** HPV causes genital warts. | 313 (73.47%) | 113 (26.53%) |
| **Q.4** HPV infection can cause cancer in both men and women. | 331 (77.70%) | 95 (22.30%) |
| **Q.5** Both men and women can get the HPV vaccine. | 372 (87.32%) | 54 (12.68%) |
| **Q.6** Age 11-12 years is the most suitable age to receive the HPV vaccine. | 325 (76.29%) | 101 (23.71%) |
| **Q.7** Adults can get the HPV vaccine. | 378 (88.73%) | 48 (11.27%) |
| **Q.8** If you have received the HPV vaccine, you can have unprotected sex. | 330 (77.46%) | 96 (22.54%) |
| **Q.9** Prevention of HPV infection can be done by using condoms during sex. | 362 (84.98%) | 64 (15.02%) |
| **Q.10** Most HPV infections are transmitted through sexual contact or through body fluids. | 382 (89.67%) | 44 (10.33%) |

**Awareness about HPV infection and HPV vaccination**

The total score of awareness was 4 points. Question no. 3, "Have you ever heard of cervical cancer?" had the highest "Yes" answer rate (92.72%), while question no. 1, "Have you received the HPV vaccine?" had the lowest "Yes" answer rate (22.77%) (table 3).

**Table 3** Awareness of students about HPV infection and HPV vaccination

| Question | Yes N (%) | No N (%) |
|---|---|---|
| **Q.1** Have you received the HPV vaccine? | 97 (22.77%) | 329 (77.23%) |
| **Q.2** Have you ever heard of the HPV vaccine? | 182 (42.72%) | 244 (57.28%) |
| **Q.3** Have you ever heard of cervical cancer? | 395 (92.72%) | 31 (7.28%) |
| **Q.4** Have you ever heard of cervical cancer screening (Pap smear)? | 320 (75.12%) | 106 (24.88%) |

**Attitude about HPV infection and HPV vaccination**

The total score of attitudes was 8 points. Quote no.4, 5 and 8 had the highest median attitude score, while quote no.2, "I think I may have been exposed to or contracted HPV", had the lowest median attitude score (table 4).

**Table 4** Attitude of students about HPV infection and HPV vaccination

| Quote | Attitude Score N (%) | | | | | Median (IQR) |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| **Q.1** HPV can cause a number of serious diseases. | 6 (1.41%) | 5 (1.17%) | 72 (16.90%) | 151 (35.45%) | 192 (45.07%) | 4 (4-5) |
| **Q.2** I think I may have been exposed to or contracted HPV. | 126 (29.58%) | 85 (19.95%) | 100 (23.47%) | 51 (11.97%) | 64 (15.02%) | 3 (1-4) |
| **Q.3** It is helpful to talk about HPV infection or STDs in the home. | 11 (2.58%) | 12 (2.82%) | 83 (19.48%) | 112 (26.29%) | 208 (48.83%) | 4 (4-5) |
| **Q.4** Talking about HPV infection and sexually transmitted diseases in schools can be helpful. | 10 (2.35%) | 5 (1.17%) | 67 (15.73%) | 124 (29.11%) | 220 (51.64%) | 5 (4-5) |
| **Q.5** It is helpful to talk to your doctor or healthcare professional about HPV infection or sexually transmitted diseases. | 10 (2.35%) | 1 (0.23%) | 52 (12.21) | 101 (23.71) | 262 (61.50%) | 5 (4-5) |
| **Q.6** The HPV vaccine can prevent cervical cancer and genital warts. | 11 (2.58%) | 7 (1.64%) | 67 (15.73%) | 134 (31.46%) | 207 (48.59%) | 4 (4-5) |
| **Q.7** The HPV vaccine is not harmful. | 9 (2.11%) | 20 (4.69%) | 104 (24.41%) | 145 (34.04%) | 148 (34.74%) | 4 (3-5) |
| **Q.8** Both teenage girls and boys need to get the HPV vaccine. | 10 (2.35%) | 7 (1.64%) | 70 (16.43%) | 108 (25.35%) | 231 (54.23%) | 5 (4-5) |

**Factors Affecting Participants' Interest in Receiving the HPV Vaccine**

From the multivariable logistic regression analysis, being female, having a high awareness and attitude level toward HPV infection and vaccination were significant factors affecting participants' interest in receiving the HPV vaccine (mOR = 2.20, 2.96 and 3.35, respectively) (table 5).

**Table 5** Factors Affecting Participants' Interest in Receiving the HPV Vaccine

| | OR | p-value | aOR | 95%CI | p-value |
|---|---|---|---|---|---|
| **Female** | 2.74 | < 0.001 | 2.20 | 1.42-3.42 | < 0.001 |
| **Age ≥ 15 years** | 1.51 | 0.716 | 0.96 | 0.50-1.85 | 0.911 |
| **High School level** | 1.67 | 0.012 | 1.38 | 0.73-2.60 | 0.326 |
| **Level of knowledge** | | | | | |
| • Low | Ref. | | | | |
| • Moderate | 1.89 | 0.113 | 1.36 | 0.72-2.43 | 0.473 |
| • High | 2.04 | 0.042 | 1.14 | 0.36-2.43 | 0.716 |
| **Level of awareness** | | | | | |
| • Negative | Ref. | | | | |
| • Neutral | 1.46 | 0.110 | 1.22 | 0.74-1.98 | 0.435 |
| • Positive | 4.22 | < 0.001 | 2.96 | 1.35-6.48 | 0.007 |
| **Level of attitude** | | | | | |
| • Negative | Ref. | | | | |
| • Neutral | 2.12 | 0.168 | 2.03 | 0.63-6.53 | 0.231 |
| • Positive | 3.89 | 0.011 | 3.35 | 1.06-10.55 | 0.038 |

OR = Crude odds ratio, aOR = Adjusted odds ratio, CI = Confidence interval, Ref.=Reference

## Discussion

The findings of this study highlight significant gaps in awareness and attitudes toward HPV vaccination among adolescents in Chiang Rai, Thailand, despite high levels of knowledge. This discrepancy underscores the need for targeted health promotion strategies to bridge the gap between knowledge, awareness and attitude in preventing HPV-related diseases. In this study, only 22.77% of participants reported receiving HPV vaccination, which is consistent with previous findings indicating suboptimal uptake among Thai adolescents.

In December 2024, the inclusion of HPV vaccination as a benefit under Thailand's National Health Security System for Grade 5 girls by 2025 represents a crucial step toward improving vaccination rates.[9] Evidence from countries that have implemented similar national programs shows a substantial reduction in HPV infections and related diseases. For instance, a meta-analysis by Drolet et al. (2019) demonstrated a significant decline in HPV prevalence and cervical pre-cancers following the introduction of national HPV immunization programs globally.[10] The focus on early vaccination aligns with WHO recommendations for vaccinating girls aged 9-14 before sexual activity begins to maximize vaccine efficacy.[4]

In this study, 72.30% of participants demonstrated high knowledge about HPV, only 14.31% reported positive awareness, and 62.68% had a positive attitude toward vaccination. In addition, positive awareness

and positive attitude are significant factors to perceiving the HPV vaccine. These findings suggest that knowledge alone may not be sufficient to drive vaccine uptake. Studies have shown that awareness and positive attitudes are critical predictors of vaccine acceptance. For example, Gerend and Shepherd (2012) found that perceived benefits of vaccination and social influences significantly impact vaccine intentions.[11]

To improve HPV vaccination rates and reduce the prevalence of HPV-related diseases, several strategies are recommended. Nationwide awareness campaigns should be implemented to emphasize the importance of HPV vaccination for both males and females. Culturally sensitive and age-appropriate education programs should be introduced in schools to enhance understanding and acceptance among adolescents. Healthcare providers should be actively engaged as trusted sources of information to address concerns and misconceptions about the vaccine. Additionally, it is essential to monitor vaccine coverage regularly and focus on addressing access disparities, particularly in rural and underserved areas, to ensure equitable immunization efforts.

This study has notable strengths and limitations. One strength is that the participants are adolescents, the primary target group for HPV vaccination. Additionally, the study evaluates three key dimensions (knowledge, awareness, and attitude) which are essential for effective health promotion. However, the study has some limitations. First, as a cross-sectional study, its findings may not account for future changes in vaccine technology or national policies, which could influence participants' knowledge, awareness, attitudes, and interest in HPV vaccination. Second, the study was conducted in northern Thailand, where cultural and societal perspectives may differ from other regions. As a result, the findings may not fully represent the knowledge, awareness, attitudes, or interests of adolescents across the region or country. Furthermore, some factors that may influence vaccine acceptance, such as vaccine cost, family income, religion, and ethnicity, were not included in this study. In addition, this study did not directly assess vaccine hesitancy for example, concerns about safety, side effects, or misunderstanding about the need for vaccination. Future research should explore these social and psychological factors to better understand barriers to HPV vaccine uptake among adolescents.

## Conclusion

Health care providers and policy makers must focus on translating knowledge into awareness and positive attitudes to achieve widespread acceptance. Targeted health promotion and community-based strategies are essential to maximize the impact of national vaccination initiatives and reduce the burden of HPV-related diseases.

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## Conflict of interests

There was no conflict of interest to declare.

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สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
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# Antimicrobial Activity of *Scutellaria Baicalensis* Extract with Different Solvents against *Escherichia coli*

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## Abstract:

**Background:** Huang Qin (*Scutellaria baicalensis, S. baicalensis*) has been used to treat various diseases such as fever, diarrhea, and dysentery. *Escherichia coli* (*E. coli*) is a standard part of intestinal microbiota and the common cause of several diseases that come with diarrhea, stomach cramps, and fever.

**Objectives:** This paper aimed to determine the antimicrobial activity of *S. baicalensis* extract with different solvents (water, ethanol, and ethyl acetate) against *E. coli*.

**Materials and Method:** *S. baicalensis* is ground into powder and macerated in solvents until exhausted and extracted via evaporation under vacuum. The water extract *S. baicalensis* (WHQ), ethanol extract *S. baicalensis* (EtOHHQ), and Ethyl Acetate extract *S. baicalensis* (EtOAcHQ) dissolved in dimethyl sulfoxide (DMSO). The agar wall diffusion method was used to test antimicrobial activity. The microdilution method determined the minimum inhibitory concentration (MIC) of the extractions. The extractions' minimum bactericidal concentration (MBC) was evaluated from the agar plate, and no microbial growth area was observed.

**Results:** The average inhibition zone in MIC of EtOHHQ was 7.33 ± 0.58 mm, EtOAcHQ was 7.67 ± 0.58 mm, Baicalein was 7.67 ± 0.58 mm, while Gentamicin showed 16.33 ± 0.58 mm. MIC of EtOHHQ, EtOAcHQ, and Baicalein were > 2,000 μg/ml, while MIC of Gentamicin was 6.25 μg/ml. MBC of EtOHHQ, EtOAcHQ, and Baicalein were > 2,000 μg/ml, while MBC of Gentamicin was 6.25 μg/ml. WHQ and Baicalin did not show antimicrobial activity against *E. coli*.

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**Conclusion:** *S. baicelensis* extraction can inhibit the growth of *E. coli* with a high concentration.

**Keywords:** *Scutellaria Baicalensis, Escherichia coli*, Ethanol, Ethyl acetate, Anti-microbial

## Introduction

*S. baicalensis* is a flowering plant in the Lamiaceae family and is widely used in traditional Chinese medicine (TCM). It is mainly distributed in East Asia, Europe, and America; China remains the primary producer for medical purposes.[1] In TCM, *S. baicalensis* is known for clearing heat, removing dampness, and calming the fetus. It is important in various TCM formulas, such as Xiao Chai Hu decoction, Ban Xia Xie Xin decoction, and Huang Lian Jie Du decoction. Clinical applications mainly use the plant's roots as a medicine to treat diseases related to diarrhea, inflammation, and respiratory infections.[2]

*S. baicalensis* has been isolated and identified using various methods to find the compounds' content. The Chemical constituents of *S. baicalensis* are divided into five categories: flavonoids, volatile oils, terpenoids, polysaccharides, and other components. The major compounds of *S. baicalensis* are flavonoids and glycosides.[3] 126 small molecule compounds and six polysaccharides have been isolated from *S. baicalensis*, with baicalein and baicalin being the main active compounds of *S. baicalensis*.[4]

*S. baicalensis* and its major compounds exhibit significant anti-microbial activities. The water extract of S. baicalensis could inhibit a broad spectrum of oral bacteria (MIC, 15.7-62.5 MBC, 20-125 mg/ml), including *Streptococcus sanguis II*, *S. salivarius*, *Actinomyces viscosus*, *A. naeslundi*, *A. odontolyticus*, two strains of *Capnocytophaga*, *Bacteroides melaninogenicus ss intermedius*, *B. gingivalis*, *Fusobacterium nucleatum*, and *Actinobacillus actinomycetemcomitans*.[5] It could also inhibit the growth of *Candida albicans* at a concentration of 5 mg/ml and 2.5 mg/ml.[6] *S. baicalensis* extracts have shown substantial antibacterial effects against *Bacillus*, *cereus*, *E. coli*, *Listeria monocytogenes*, *Salmonella anatum*, and *Staphylococcus aureus* in a previous study.[7]

*E. coli* bacteria typically reside in the intestines of humans and animals.[8] Most types of *E. coli* are harmless, but a few strains of *E. coli* can cause severe gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common symptoms include abdominal cramps, diarrhea with blood, and vomiting.[9] Most people get well within 5 to 7 days, but some can become severe or life-threatening. Approximately 5 to 10% of people who are diagnosed with *Shiga toxin-producing E. coli* (STEC) infection develop a potentially life-threatening complication known as hemolytic uremic syndrome.

Consumption of fresh vegetables can expose individuals to enteric pathogens, which include *Salmonella, Shigella, Listeria monocytogenes,* and pathogenic *E. coli*.[10,11] In Thailand, the dietary habit of consuming uncooked vegetables might be a risk to people's *E. coli* infection. An investigation by Leelaporn et al. reported that Enterotoxigenic *E. coli* (ETEC) was 11.6% and STEC was 2.2% among 181 isolated pathogens.[12] Another study by Chomvarin et al. reported 140 *E. coli* obtained from 186 food samples from various categories in Khon Kaen province, Thailand, and reported the occurrence of 140 *E. coli*.[13] Nawattanapaibool et al. found *E. coli* and STEC in 11.00% and 9.67% of 300 fruits and vegetables samples in Bangkok.[14]

This research aimed to determine the antimicrobial activity of *S. baicalensis* extract with different solvents against *E. coli* and provide a choice of natural medicine to inhibit the growth of *E. coli*. The research gaps of this study are comparing the antimicrobial activity differences between three types of *S. baicalensis* extracts.

## Materials and Method

### *S. Baicalensis* preparation

The root of *S. baicalensis* will be collected from the Chinese herbal store "Tong Hua" in Chiangrai, Thailand, and authenticated by the Chinese Medicine expert from Chengdu University of Traditional Chinese Medicine Affiliated Hospital. Ethanol and ethyl acetate were purchased from Union Science Co., Ltd. Baicalin and Baicalein were purchased from Life Science AP Co., Ltd. All materials were deposited at the School of Integrative Medicine TCM laboratory at Mae Fah Luang University.

The root of *S. baicalensis* was washed and dried in a hot air oven at 45°C.[15] Once dried, it is ground into powders and macerated in distilled water, ethanol, and ethyl acetate until exhausted, using a ratio of 100g of powder with 400g of solvent (1:4). The resulting extract evaporates under vacuum. The yield was weighted, recorded, and stored at -20°C.

### Antimicrobial activity

The *S. baicalensis* extract dissolved in dimethyl sulfoxide (DMSO). The agar well diffusion method was used to test antimicrobial activities.[16] Laboratory strain *E. coli* (Code: dmst4212) was grown on Mueller Hinton agar (for bacteria) and then incubated at 37°C for 24 hours. The turbidity of the culture was modulated to about 0.5 McFarland standard and suspended in 0.85% sodium chloride. The assay was performed using the double agar layer technique. One hundred of the suspension was added to 3 ml of sterile seeds agar, and then, it was poured on sterile base agar and offered all plates dried at room temperature. A sterile cork borer (6mm) was applied to punch holes on agar plates. Added 20 µl of *S. baicalensis* extracts (200 mg/ml), 20 w DMSO as a negative control, and 10 µl of Gentamicin (10 mg/ml) as positive controls in each well, respectively. Incubated for 24 hours. The diameters of the inhibition zone were measured. Each sample was tested in triplicate.

### Minimum inhibitory concentration and minimum bactericidal concentration.

MIC was determined by the micro-dilution method in 96 microtiter plates.[17] All test solutions, including extracts and controls, were analyzed in triplicate for reproducibility. Serial two-fold dilutions of the test extract or positive control were prepared across wells in columns 1 to 10. Column 11 was negative control, while column 12 contained only broth media as sterility control. Each well was filled with 50 µl of tested solutions in broth and 50 µl microbial suspended in broth and incubated at 37°C for 24 hours. MIC was recorded at the last well, which showed a clear solution. Streaked clear inoculate broth on Mueller Hinton agar (for bacteria), then incubated the agar plate at 37°C for 24 hours. The determination of MBC was evaluated from the agar plate, which showed no microbial growth. All tested solutions were analyzed in triplicate. In the agar plate, the inhibition zone is the area that is not entirely clear of microbial growth but clearer than the areas of the plate with uninterrupted microbial growth. When considering the anti-microbial results, the killing zone was counted as the anti-microbial substance to kill the microbial entirely and not only reduce it.

### High performance liquid chromatography (HPLC)

WHQ, EtOHHQ, and EtOAcHQ compounds were determined using versatile high-performance liquid chromatography (Agilent 1260 infinity II, USA). Baicalin and Baicalein were determined as standard compounds. The column (particle size 5 µm, 150 mm x 4.6 mm) was used. The flow rate was 1.0 ml/min at 35 °C. The mobile phase comprised acetonitrile and phosphoric acid (20:80, v/v).[18]

## Results

### Antimicrobial activity

Figure 1 shows the agar plate for inhibiting *S. baicalensis* against *E. coli*. Gentamicin (G) showed a clear kill zone of Gentamicin. At the same time, EtOHHQ (T1), EtOAcHQ (T2), and Baicalein (B2) showed an inhibition zone against *E. coli*. WHQ (T3) and Baicalin (B1) did not show a kill zone and inhibition zone against *E. coli*.

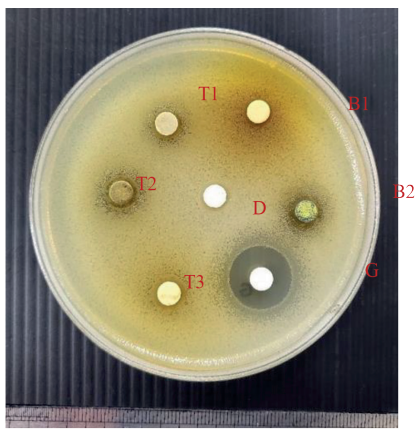



**Figure 1** Inhibition zones against *Escherichia coli (T1:EtOHHQ,T2:EtOAcHQ, T3: WHQ, B1: Baicalin, B2: Baicalein, G: Gentamicin, D: DMSO)*

The inhibition effects of *S. baicalensis* extracts on *E. coli* are shown in Table 1 and Figure 2. EtOHHQ, EtOAcHQ, and baicalein showed the inhibition effects on *E. coli*, but WHQ and baicalin did not show the effects on *E. coli* inhibition and clearing. EtOHHQ had an average 7.33 ± 0.58 mm inhibition zone, and EtOAcHQ and baicalein had an average 7.67 ± 0.58 mm inhibition zone.

**Table 1** Inhibition zones against Escherichia coli using agar diffusion method

| | Tested substance | Inhibition zone (mm) (Mean ± SD) |
|---|---|---|
| T1 | EtOHHQ (100 mg/ml) | 7.33 ± 0.58 |
| T2 | EtOAcHQ (100 mg/ml) | 7.67 ± 0.58 |
| T3 | WHQ (100 mg/ml) | N/A |
| B1 | Baicalin (100 mg/ml) | N/A |
| B2 | Baicalein (100 mg/ml) | 7.67 ± 0.58 |
| G | Gentamicin (1 mg/ml) | 16.33 ± 0.58 |
| D | DMSO | N/A |

*Mean ± SD, Ø 6 mm of disc, N/A = no activity

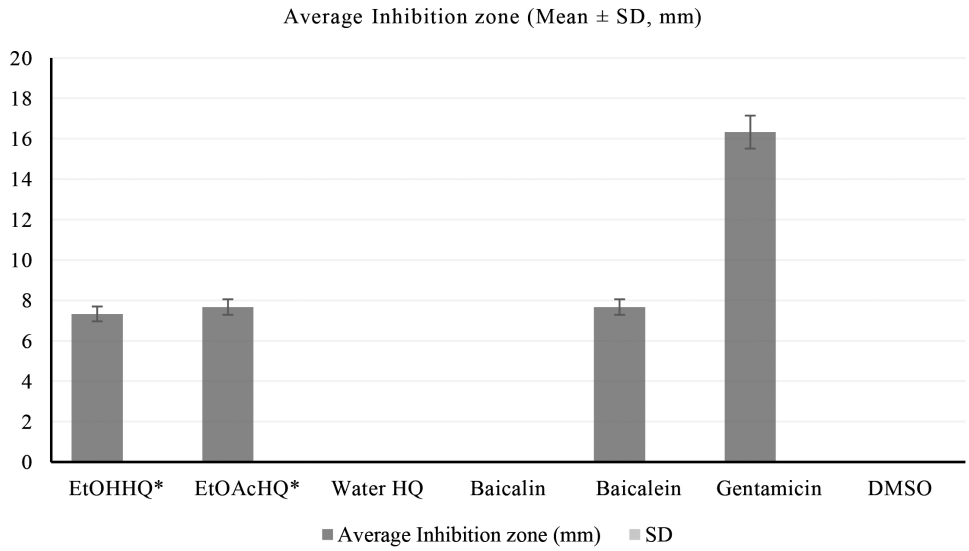



**Figure 2** Inhibition zones of EtOHHQ, EtOAcHQ, WHQ, Baicalin, Baicalein, Gentamicin, and DMSO on *E. coli*

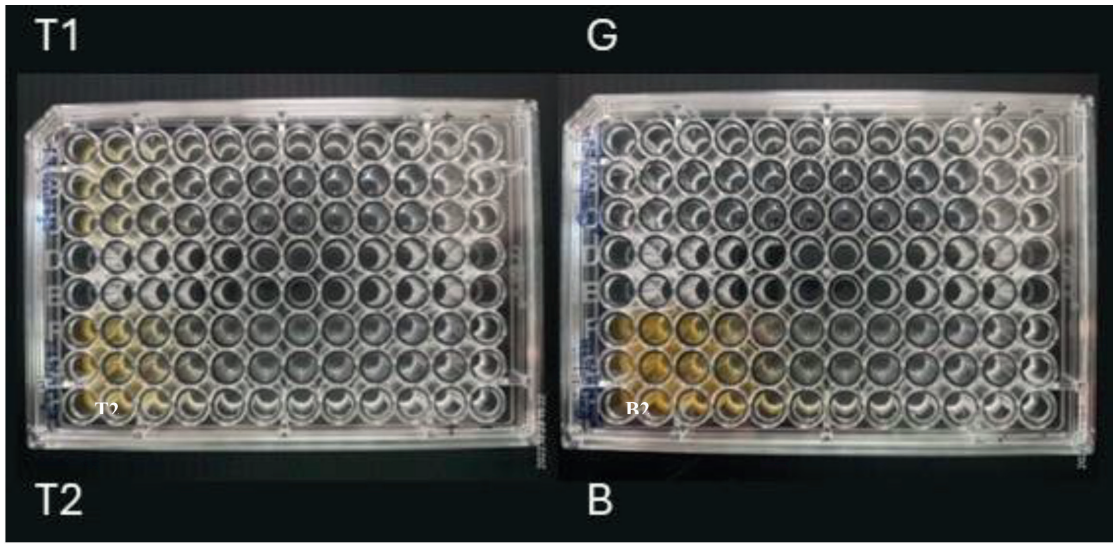
**Minimum Inhibition Concentration**

The reduction in anti-microbial effects with lower concentrations of the herbal infusion was tested. Table 2, Figure 3, and Figure 4 show the MIC and MBC of *S. baicalensis* extracts against *E. coli*. MIC and MBC of EtOHHQ, EtOAcHQ, and Baicalein were > 2,000 µg/ml. MIC and MBC of Gentamicin were 6.25 µg/ml. Meanwhile, the research did not show MIC and MBC of WHQ and Baicalin.

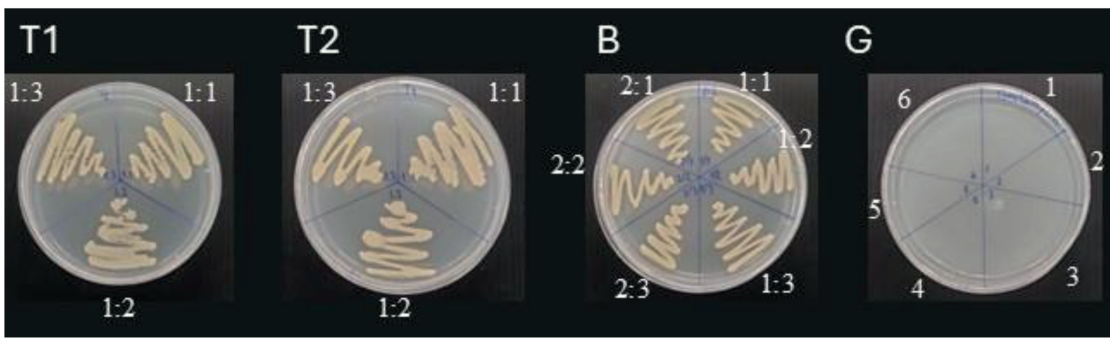
**Table 2** Antimicrobial activity against *Escherichia coli* using broth microdilution method

| Tested substance | MIC | MBC |
|---|---|---|
| EtOHHQ | > 2,000 µg/ml | > 2,000 µg/ml |
| EtOAcHQ | > 2,000 µg/ml | > 2,000 µg/ml |
| WHQ | N/A | N/A |
| Baicalin | N/A | N/A |
| Baicalein | > 2,000 µg/ml | > 2,000 µg/ml |
| Gentamicin | 6.25 µg/ml | 6.25 µg/ml |
| DMSO | N/A | N/A |

* N/A = no activity




T1 = EtOHHQ, T2 = EtOAcHQ, B = Baicalein, G = Gentamicin
**Figure 3** Antimicrobial activity against *Escherichia coli* (MIC)




T1 = EtOHHQ, T2 = EtOAc HQ, B = Baicalein, G = Gentamicin
**Figure 4** Antimicrobial activity against *Escherichia coli* (MBC)

## HPLC

The contents of Baicalin and Baicalein of WHQ, EtOHHQ, and ETOAcHQ were tested. Table 3 shows the content of Baicalin and Baicalein in WHQ, EtOHHQ, and EtOAcHQ. WHQ has more Baicalin compounds than EtOHHQ and EtOAcHQ. EtOHHQ has more Baicalein compounds than WHQ and EtOACHQ.

**Table 3** Baicalin and Baicalein in S. Baicalensis extracts analyzed by HPLC.

| Baicalin (% w/w) | Baicalein (% w/w) | MBC |
|---|---|---|
| WHQ | 18.46 ± 0.06 | 0.93 ± 0.01 |
| EtOHHQ | 1.70 ± 0.01 | 13.80 ± 0.06 |
| EtOAcHQ | 2.64 ± 0.1 | 3.07 ± 0.05 |

## Discussion

EtOH and EtOAc extracts of *S. baicalensis* showed inhibition activity against *E. coli*, significantly different from WHQ. The area of baicalein showed the inhibition zone of *E. coli*. However, the area around baicalin did not show the inhibition area, which means that the active compound that inhibits the growth of *E. coli* in *S. baicalensis* was Baicalein. The results of MIC showed that EtOHHQ and EtOAcHQ required a high concentration to produce a reduction zone of *E. coli*. The results suggest that *S. baicalensis* affects *E. coli* growth inhibition but needs to be with high concentration.

The inhibition and antimicrobial activity of *E. coli* by *S. baicalensis* extraction had been shown in another study.[19] The research showed that *S. baicalensis* extraction brought an average 5mm killed zone, which proved the antimicrobial and inhibition activity of *S. baicalensis* extraction on *E. coli*. Research showed that Baicalin had an inhibitory effect on *E. coli* in vitro; the MIC of Baicalin against *E. coli* isolated from mastitis in dairy cattle was 4000 μg/ml, and antimicrobials such as streptomycin, ciprofloxacin, and ampicillin had synergistic effects in combination with Baicalin. The combination could significantly increase the susceptibility to *E. coli*.[20,21]

However, Wang X. K. found that the active compound that effected antimicrobial activity of the root of *S. baicalensis* was Baicalein.[22] Baicalein can reduce the pathogenic bacteria such as *S. aureus* and *E. coli* by disrupting the cell wall integrity, reducing bacterial enzymatic activities, and inhibiting bacterial energy production and nucleotide synthesis.[23] Baicalin increased the permeability of *E. coli* cell membrane by causing damage, leading to the infiltration of bacterial, and achieving the bacteriostatic effect.[24]

The results of HPLC showed that WHQ has the most Baicalin than the other two extracts, but it has the least Baicalein, which is less than 1% w/w. EtOHHQ has the most Baicalein than the other two extractions (13.8% w/w) but with the least Baicalin compound. EtOAcHQ has almost the same amount of Baicalin and Baicalein, with 2.6% w/w and 3.1% w/w. EtOH and EtOAc can extract more Baicalein from *S. baicalensis*, which makes the extraction more effective in inhibiting *E. coli* while compared with WHQ. However, EtOHHQ and EtOAcHQ did not showed significantly different which suggests that the inhibition zone might be the at the functional saturation point.

EtOH and EtOAc are common solvents in plant compound extraction and product making. EtOH is considered a universal solvent due to its molecular structure, allowing it to dissolve polar, hydrophilic, and nonpolar, hydrophobic compounds. The chemical formula of ethanol is CH3CH2OH. EtOAc is the ester of EtOH and acetic acid, manufactured on a large scale as a solvent with low cost, low toxicity, and agreeable odor.[25] The chemical formula of EtOAc is C4H8O2. These solvents showed a difference in the extraction of *S. baicalensis*; EtOAc might be more suitable for baicalein extraction. *S. baicalensis* was widely regarded as a safe and nontoxic herb in China. Jinsu Lim's[26] study showed that water extract *S. baicalensis* had a lower value in total phenolic and total flavonoid content, which caused lower antioxidant activities. The result is similar to the result of this study; this could be the reason for the lack of anti-microbial in WHQ.

Research tested the acute toxicity of the *S. baicalensis* extraction.[27] The results showed that the maximal tolerated dose of the aqueous extracts of *S. baicalensis* in mice was 72.0 g/kg, and the median lethal concentration value of 80.0% ethanol

extracts of *S. baicalensis* was 39.6g/kg. There are no obvious adverse events on in-vivo *S. baicalensis*. The study showed that the aqueous extracts of *S. baicalensis* had no significant changes in body weight, clinical symptoms, and mortality in rabbits and guinea pigs during dermal stimulation/ corrosion and skin sensitization tests.[28] Another study showed 300 mg/kg, 1250 mg/kg, and 2500 mg/kg of ethanol extracts of *S. baicalensis*; only 2500 mg/kg per day, the liver tissue of the rats showed some reversible inflammatory changes.[29]

*In previous studies*, *S. baicalensis* did not show many toxicity activities or serious adverse events but showed the effects on antimicrobial activities and treatments in clinical applications. It has been a practical choice for the application in hospitals to antimicrobial and treat patients. Baicalein protected Vero cells from cytotoxicity of Stx1 and Stx2 by binding to the cytoplasmic membrane of the cell and altering its function.[30] *S. baicalensis* has the effect of *E. coli* inhibition with high concentration, making it a potential option for *E. coli* infection.

## Conclusion

*S. baicalensis* extracted by EtOH and EtOAc has the potential to inhibit the growth of *E. coli*. The results showed the difference between water extracts and organic solvent extracts of *S. baicalensis* and the effect of antimicrobials against *E. coli*. This has given a key to the importance of extraction methods in maximizing the pharmacological potential of herbal medicines. Future research should prioritize optimizing extraction, such as ethanol or ethyl acetate extraction, to enhance the yield of hydrophobic compounds. Future studies should also explore synergistic combinations of *S. baicalensis* flavones with conventional antibiotics to lower the effective dose and overcome resistance in Gram-negative pathogens. The findings point toward the potential use of *S. baicalensis* compounds as complementary agents in antimicrobial therapy.

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## Conflict of interest

Authors declare that no conflict of interest in the research.

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Case Report

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# Severe Limbs Ischemia with Retiform Purpura: Serious Manifestations in Acute Meningococcemia

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**Abstract:**

Acute meningococcemia often presents with severe skin manifestations, such as limb ischemia and purpura fulminans. Prompt diagnosis and timely treatment are critical to avoid serious complications, including permanent limb loss. We report the case of a 22-year-old man living in a military camp, referred from a community hospital, who developed acute high-grade fever, altered consciousness, and retiform purpura that evolved into purpura fulminans alongside septic shock. His condition rapidly worsened, complicated by bilateral limb ischemia and necrotizing fasciitis, eventually requiring bilateral below knee amputations. This case highlights the importance of early recognition and aggressive management of meningococcemia to improve patient outcomes and prevent irreversible damage.

**Keywords:** Acute meningococcemia, Retiform purpura, Purpura fulminans, Limbs ischemia

## Introduction

*Neisseria meningitidis* is an encapsulated, aerobic gram-negative diplococcus that colonizes in human nasopharynx.[1] Transmission occurs via respiratory droplets or direct close contact. Of the 13 identified serogroups, A, B, C, W-135, X, and Y are most commonly associated with invasive disease.[2] In Thailand, meningococcal infections remain sporadic, with serogroup B being the predominant strain.[3] Acute meningococcemia commonly presents with fever and neurological symptoms such as headache, seizures, and altered consciousness. A hallmark skin sign is petechial rashes with a "smudged" appearance resembling splattered mud, often including retiform purpura.[4] In severe cases, ischemic necrosis and purpura fulminans may develop, characterized by rapid onset of widespread hemorrhagic skin lesions and systemic complications.[5] Possible complications include limb amputation, abnormal bone growth in children, and sensorineural hearing loss or deafness.[6]

Although retiform purpura in critically ill patients often raises concern for infectious etiologies particularly meningococcemia. It is important to maintain a broad differential diagnosis. Retiform purpura can be classified based on the underlying pathology into two major categories: vessel wall damage and vessel lumen occlusion. When the disease

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process centers on the vessel wall, potential causes include depositional disorders (e.g., calciphylaxis), infections (e.g., meningococcemia, angioinvasive fungal infections), and vasculitides, such as antineutrophil cytoplasmic antibody (ANCA) associated vasculitis and mixed cryoglobulinemia (types II and III). In contrast, occlusion of cutaneous blood vessels can result from a variety of mechanisms, including hypercoagulable states such as disseminated intravascular coagulation (DIC) and embolic processes, such as cholesterol embolization.[4] Microbiological cultures remain a key diagnostic tool in distinguishing between various infectious causes, particularly in critically ill patients, by enabling the identification of specific pathogens and supporting appropriate antimicrobial management. Specifically, in suspected cases of meningococcemia, the gold standard for diagnosis is culture of clinical specimens.[4]

Once the diagnosis is established, prompt management is crucial. The management of acute meningococcemia is time sensitive, and early initiation of antibiotic therapy is essential to improve patient outcomes. The first line empirical treatment consists of third generation cephalosporins, such as cefotaxime or ceftriaxone.[7]

**Case presentation**

A 22-year-old cadet residing in a military barrack presented with acute onset of high-grade fever, altered mental status, and non-blanchable purpuric macules and patches. The cutaneous lesions initially appeared as smudged appearance (Figure 1A) and progressed to retiform purpura (Figure 1B). He rapidly deteriorated into purpura fulminans, peripheral limbs ischemia (Figure 2). Given the acute fever with neurological involvement and characteristic skin findings, an infectious etiology was suspected, with *Neisseria meningitidis* considered the most likely pathogen based on his risk factors, including residence in a crowded setting.[8]

The patient was diagnosed with acute meningococcemia, and early empiric antibiotic therapy with intravenous ceftriaxone (2 grams) was promptly initiated. Subsequent laboratory investigations revealed sepsis with multiorgan dysfunction, including disseminated intravascular coagulation (DIC), ischemic tubular necrosis, and ischemic hepatitis. In this case, DIC was a key driver in the development of purpura fulminans. Due to the presence of DIC, a known contraindication to lumbar puncture (LP), cerebrospinal fluid (CSF) analysis was deferred. In addition, the patient initially presented with altered consciousness, prompting an urgent non-contrast computed tomography (CT) scan of the brain, which revealed no evidence of infarction or intracranial hemorrhage.

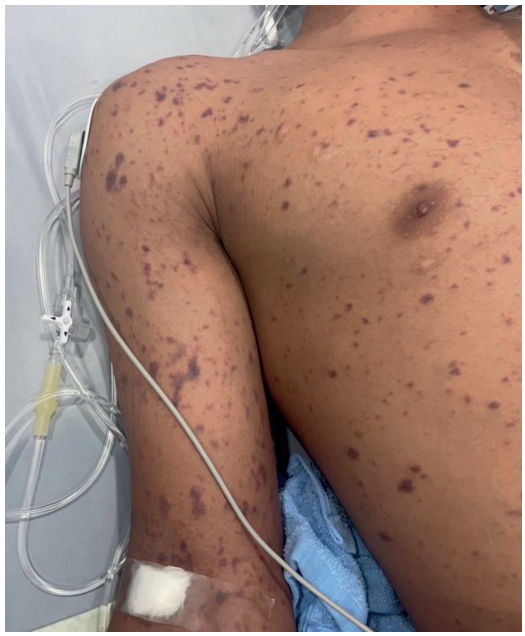

**Figure 1A** Multiple well-defined border non-blanchable purpuric macules on trunk and all extremities (smudged appearance)

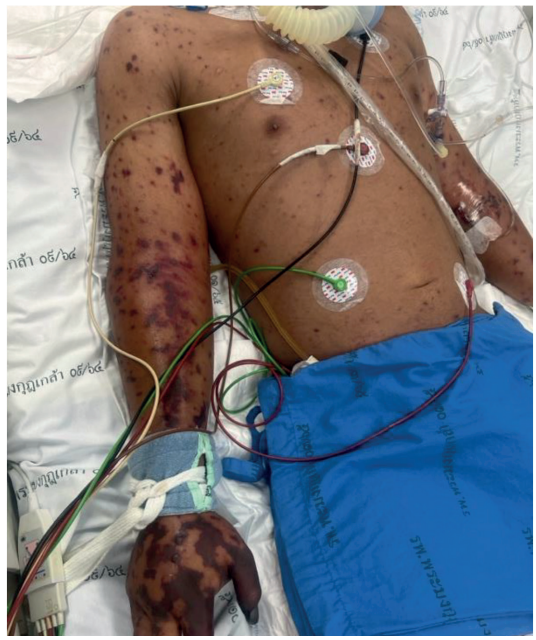

**Figure 1B** Multiple well-defined border non-blanchable purpuric patches on all extremities (retiform purpura)

Definitive diagnosis was established by blood cultures, both of which yielded *Neisseria meningitidis* serogroup B, confirming the clinical suspicion of meningococcemia.

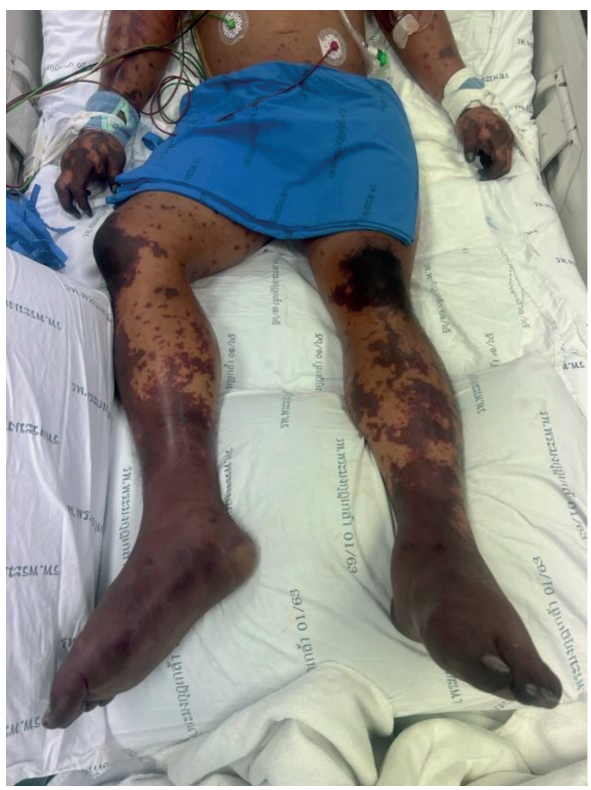

**Figure 2** Purpura fulminans and peripheral limbs ischemia

In light of the clinical deterioration, computed tomography angiography (CTA) of the lower extremities was performed. The imaging confirmed extensive bilateral limb ischemia and necrotizing fasciitis, both recognized as severe and limb threatening complications of acute meningococcemia.

The patient was diagnosed with acute meningococcemia complicated by bilateral limb ischemia and necrotizing fasciitis. Management included prompt hemodynamic resuscitation and antibiotic therapy with intravenous ceftriaxone at 2 grams every 12 hours. Given the extent of ischemic damage and soft tissue necrosis, bilateral below knee amputations were performed to control local disease progression and mitigate systemic deterioration. Following surgery, the patient showed significant clinical improvement. To prevent secondary transmission, chemoprophylaxis with a single oral dose of ciprofloxacin 500 mg was administered to all close contacts within the military camp.

Given the severity of meningococcal infection in this patient and the extent of its complications, the possibility of an underlying immunodeficiency was considered. Secondary causes of immunosuppression, including HIV infection and diabetes mellitus, were first excluded, with all results found to be within normal limits. A targeted immunologic evaluation was subsequently performed, and complement testing, including both the classical (C3) and terminal (C5-C9) pathways, yielded results within normal limits.

## Discussion

According to a report from the Centers for Disease Control and Prevention (CDC), *Neisseria meningitidis* serogroup B has the highest incidence in children under one year of age, with a second peak observed among adolescents aged 16 to 23 years.[7] In Thailand, although the overall incidence of meningococcemia remains relatively low and has not reached pandemic levels, serogroup B is reported to be the most commonly identified strain.[3] Commonly recognized risk factors for meningococcal infection include immunocompromised states such as HIV infection, recent upper respiratory tract infections, and young adults living in crowded environments, particularly military barracks, as well as infants and young children attending daycare. However, certain key risk factors are often underappreciated for example, individuals with complement component deficiencies (e.g., C3, C5-C9, properdin, or factor D) are at increased risk.[6] Patients with meningococcal disease who warrant an evaluation for underlying primary immunodeficiency include those with unusually severe disease, recurrent infections, frequent sinopulmonary infections, previous episodes of meningitis, or a family history of meningococcal disease.[3]

Clinically, *Neisseria meningitidis* infection can manifest with varying severity, ranging from isolated meningitis to acute or chronic meningococcemia. Disseminated meningococcal infection may present as meningitis alone, acute meningococcemia with or without meningitis, or chronic meningococcemia.[6] The clinical features of acute meningococcemia typically include high grade fever and neurologic symptoms such as headache, seizures, muscle rigidity, and altered mental status. Cutaneous findings are often among the earliest clues, particularly petechial rashes with a characteristic "smudged" appearance. Retiform purpura may subsequently develop, and in severe cases complicated by disseminated intravascular coagulation, purpura fulminans can occur. These lesions may progress to hemorrhagic bullae and areas of ischemic necrosis, predominantly involving the trunk

and extremities.[4] In the present case, the patient was a 22-year-old Thai male residing in a military camp, an environment associated with increased risk of meningococcal transmission. On initial physical examination, characteristic smudged petechiae and retiform purpura were observed on the extremities. Neurological examination demonstrated a cooperative patient without signs of meningeal irritation; notably, neck stiffness was absent. Despite the lack of overt meningeal signs, the combination of clinical presentation and cutaneous findings strongly pointed toward invasive meningococcal disease. Empirical intravenous antibiotic therapy with a third - generation cephalosporin (ceftriaxone) was initiated without delay. Within hours, the patient's condition rapidly deteriorated. He developed septic shock and DIC, followed by the progression of purpuric lesions into widespread purpura fulminans and bilateral limb ischemia hallmarks of life-threatening disease. This cascade highlights the importance of early clinical suspicion, prompt antimicrobial administration, and aggressive supportive care in suspected meningococcemia to prevent irreversible complications or death.

The gold standard for confirming the diagnosis of meningococcal disease is microbiological culture.[4] These cultures are essential for isolating the causative organism and establishing a definitive diagnosis. In systemic infections such as sepsis or meningitis, blood and CSF play a central role.[9] Lumbar puncture is a key diagnostic procedure in suspected meningococcal meningitis, allowing for CSF analysis and identification of gram negative diplococci.[10] However, lumbar puncture must be deferred in the presence of contraindications, such as cardiorespiratory instability, elevated intracranial pressure, or coagulopathy, due to the risk of serious complications. Although skin biopsy may be performed in patients with purpuric lesions, histopathological findings are often non-specific and do not reliably establish diagnosis.[9] In this case, blood cultures were obtained before initiating antibiotic therapy. Although the patient presented with neurological symptoms suggestive of possible meningitis, a lumbar puncture was not performed due to the presence of coagulopathy, which significantly increased the risk of bleeding. The blood cultures later yielded *Neisseria meningitidis* serogroup B, confirming the clinical suspicion of meningococcemia.

The management of acute meningococcemia is time sensitive and potentially lifesaving. Early initiation of antibiotic therapy is crucial for improving clinical outcomes. First line empirical treatment involves third generation cephalosporins, such as cefotaxime or ceftriaxone. Alternative regimens may include penicillin G or ampicillin. For patients with documented allergies to both penicillin and cephalosporins, chloramphenicol serves as an alternative option.[7] Antibiotic administration should be initiated alongside aggressive hemodynamic resuscitation. Of note, corticosteroid therapy with dexamethasone has not demonstrated benefit in meningococcal meningitis and is not recommended for routine use in this context.[3] Supportive measures should also include proper wound care and implementation of droplet precautions to limit disease transmission. In the present case, although lumbar puncture was not performed due to coagulopathy, neurological signs raised concern for possible meningitis. As a result, the patient was started on intravenous ceftriaxone 2 grams intravenous every 12 hours, in conjunction with hemodynamic stabilization and strict droplet precautions. Given the development of bilateral limb ischemia and necrotizing fasciitis, both of

which are life threatening complications of severe meningococcemia. The patient underwent bilateral below knee amputations. Following the surgery, in combination with appropriate antimicrobial therapy and intensive supportive care, the patient's clinical condition improved significantly. He was subsequently discharged in a stable condition.

Preventive strategies play a critical role in the control of meningococcal disease. The two main components are chemoprophylaxis and vaccination. Administering chemoprophylaxis to close contacts and providing vaccination to high risk groups can significantly reduce disease transmission and help prevent future outbreaks. The most commonly used agents for post exposure chemoprophylaxis include rifampin, ciprofloxacin, and ceftriaxone, each demonstrating efficacy rates of 90-95% (Table 1).[7] Vaccination remains the most effective long term strategy for preventing meningococcal disease. The quadrivalent MenACWY vaccine is recommended for high risk groups, including individuals with complement deficiencies, asplenia, HIV infection, or those living in close quarter environments such as military barracks. However, the MenB vaccine is currently not included in the national immunization program. In this case, to prevent secondary transmission, chemoprophylaxis with a single oral dose of ciprofloxacin 500 mg was promptly administered to all close contacts within the military camp. No additional symptomatic cases were observed among the exposed individuals during the follow up period.

## Conclusion

This case describes a 22-year-old Thai male residing in a military camp who presented with acute meningococcemia due to *Neisseria meningitidis* serogroup B. He initially developed high grade fever, altered mental status, and rapidly evolving purpuric skin lesions. Although there were no overt meningeal signs, and lumbar puncture was contraindicated due to coagulopathy, the clinical presentation raised strong suspicion for invasive meningococcal infection. Empirical therapy with high dose intravenous ceftriaxone was initiated promptly alongside hemodynamic resuscitation. Blood cultures subsequently confirmed the diagnosis. Despite aggressive medical management, the patient developed bilateral limb ischemia and necrotizing fasciitis, necessitating bilateral below knee amputations. Following surgical intervention and continued supportive care, his condition improved, and he was discharged in stable condition.

**Table 1** Recommended chemoprophylaxis regimens for close contacts of persons with invasive meningococcal disease[7]

| Drug | Age | Dose | Duration | Efficacy (%) | Cautions |
|---|---|---|---|---|---|
| **Rifampin** | < 1 month | 5 mg/kg, orally, every 12 hours | 2 days | NA | Discussion with an expert for infants < 1 month. |
| | ≥ 1 month | 10 mg/kg (maximum 600 mg), orally, every 12 hours | 2 days | 90-95 | Can interfere with efficacy of oral contraceptives and some seizure prevention and anticoagulant medications; may stain soft contact lenses. Not recommended for pregnant women. |
| **Ceftriaxone** | < 15 years | 125 mg, intramuscularly | Single dose | 90-95 | To decrease pain at injection site, dilute with 1% lidocaine. |
| | ≥ 15 years | 250 mg, intramuscularly | Single dose | 90-95 | |
| **Ciprofloxacin** | ≥ 1 month | 20 mg/kg (maximum 500 mg), orally | Single dose | 90-95 | Not recommended for pregnant women. |
| **Azithromycin** | | 10 mg/kg (maximum 500 mg) | Single dose | 90 | Not recommended routinely. Equivalent to rifampin for eradication of *N. meningitidis* from nasopharynx in one study. |

This case highlights the critical importance of early identification and treatment of meningococcal disease to prevent severe complications such as limb loss. Rapid initiation of appropriate antimicrobial therapy, combined with chemoprophylaxis for close contacts and targeted vaccination of high-risk populations, remains essential to reducing morbidity, mortality, and the risk of secondary transmission.

## Conflict of interest

The authors have no relevant conflicts of interest to disclose.

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Review Article

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# Lipoprotein(a) and Cardiovascular Disease: A Review of Current Evidence and Future Directions

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**Abstract:**

Lipoprotein(a), Lp(a), is a type of low-density lipoprotein (LDL) that is now widely understood to be an independent and direct risk factor for atherosclerotic cardiovascular disease (ASCVD) and calcific aortic valve stenosis (CAVS). Plasma Lp(a) levels are predominantly (over 90%) genetically determined, making them relatively stable throughout life and unresponsive to lifestyle modifications or most currently available lipid-lowering therapies. The pathophysiology of Lp(a) is complex, involving pro-atherogenic, pro-inflammatory, and pro-thrombotic mechanisms, primarily driven by its unique protein component, apolipoprotein (a) (apo(a)), and its role as the primary carrier of oxidized phospholipids (OxPL). Despite challenges in measurement standardization, a global clinical consensus is emerging, recommending at least a one-time screening for Lp(a) in all adults. The field is on the cusp of a major therapeutic breakthrough with the development of specific Lp(a)-lowering RNA-based therapies, such as pelacarsen and olpasiran, as well as a novel oral agent, muvalaplin, which are in late-stage trials and promise to address this long-recognized risk factor for the first time.

**Keywords:** Lipoprotein(a), low-density lipoprotein (LDL), Oxidized phospholipids (OxPL), Atherosclerotic cardiovascular disease (ASCVD), Calcific aortic valve stenosis (CAV)

## Introduction: The Reemergence of a Causal Risk Factor

### Search Strategy

The authors conducted a comprehensive search of PubMed, Scopus, and Google Scholar using keywords: "Lipoprotein(a)", "Low-density lipoprotein (LDL)", "Oxidized phospholipids (OxPL)", "ASCVD", and "CAVS". The search focused on articles published up to July 2025, including meta-analyses, Genome-Wide Association Studies (GWAS), Mendelian randomization, and results from Phase 2 and 3 clinical trials.

### Historical Context and Clinical Inertia

Lipoprotein(a) was first discovered in 1963 by Kåre Berg as an LDL antigen.[1] For decades, its role in cardiovascular disease was widely debated, partly due to inconsistent results from early studies that

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used isoform-sensitive assays for apo(a), which failed to measure Lp(a) concentration accurately.[2] This long history led to clinical inertia and a widespread lack of Lp(a) testing, a situation that is only now beginning to change.

Understanding the history of Lp(a) is a critical case study in identifying cardio vascular risk factors. It demonstrates how technical and methodological limitations (i.e., measurement technology) can delay the clinical acceptance of a genuine causal risk factor for decades. Early studies yielded conflicting results regarding the link between Lp(a) and CVD. It was later discovered that early immunoassays were biased by the variable size of apo(a) isoforms, leading to inaccurate quantification.[3] Specifically, smaller, more pathogenic Lp(a) isoforms were often underestimated. The development of isoform-insensitive assays, combined with the power of large-scale genetic studies such as Mendelian randomization, has overcome these limitations.[4] This combination of improved measurement and enhanced causal methodology provided definitive, robust evidence that finally cemented Lp(a)'s role. This is a key lesson for cardiologists: we must critically evaluate not only the clinical data but also the measurement technology and study design behind it when assessing new biomarkers. The "noise" from poor assays obscured the clear "signal" of this risk factor for nearly 50 years.[2]

## The Paradigm Shift: Establishing Causality

The turning point in the Lp(a) story occurred around 2009, driven by high-quality epidemiological data, large-scale meta-analyses, Genome-Wide Association Studies (GWAS), and, crucially, Mendelian randomization studies.[5,6] These genetic studies provided strong evidence that elevated Lp(a) is a causal risk factor for ASCVD and CAVS, not merely a biomarker, as they are less susceptible to confounding and reverse causation than observational studies.[2]

## The Problem of Residual Risk

Residual cardiovascular risk refers to the risk of cardiovascular events that persists even after patients have achieved guideline-recommended targets for LDL-C, blood pressure, and other modifiable risk factors. Elevated Lp(a) is a significant factor that contributes to this ongoing risk, as it still poses a substantial threat even for patients taking strong statin medications and who have their LDL-C levels well controlled. Approximately 20-25% of the global population, or over 1.4 billion people, have elevated Lp(a) levels (e.g., >50 mg/dL or >125 nmol/L).[2]

## The Lp(a) Particle: Structure, Genetics, and Metabolism

### A Unique Molecular Architecture

The Lp(a) particle has a core like LDL, which includes lipids and one molecule of apolipoprotein B-100 (apoB), and it has a large protein called apolipoprotein A (apo(a)) attached to it by a single disulfide bond. The Lp(a) particle has a core like LDL, which includes lipids and one molecule of apolipoprotein B-100 (apoB), and it has a large protein called apolipoprotein A (apoA) attached to it by a single disulfide bond.

The structure of apo(a) is remarkable for its high homology to plasminogen, comprising multiple copies of a kringle IV (KIV) domain (specifically KIV-2 repeats), one kringle V (KV) domain, and a proteolytically inactive protease domain.[7] This structural mimicry is the basis for Lp(a)'s antifibrinolytic properties.[7,8]

### The Genetic Basis of Lp(a) Levels

Plasma Lp(a) levels are overwhelmingly (70% to ≥ 90%) genetically determined, making it one of the most heritable cardiovas-

cular risk factors. The primary genetic locus is the LPA gene on chromosome 6q2.6-2.7, which evolved from the plasminogen (PLG) gene approximately 40 million years ago in Old World primates.[9]

The most important genetic determinant is the KIV-2 copy number variation (CNV), which is strongly and inversely correlated with plasma Lp(a) concentration.[10] A lower number of KIV-2 repeats results in a smaller apo(a) isoform, which is more efficiently synthesized and secreted from hepatocytes, leading to higher plasma Lp(a) levels. Conversely, larger isoforms are more prone to intracellular degradation.[10] This inverse relationship between KIV-2 CNV and Lp(a) concentration is a central tenet linking genetics, molecular biology, and clinical risk. An LPA gene with many KIV-2 repeats produces a large, complex apo(a) protein that is more difficult to fold and secrete, leading to increased intracellular retention and degradation.

In contrast, a gene with fewer repeats produces a smaller, simpler apo(a) protein that is synthesized and secreted much more efficiently. Thus, a "smaller gene" (fewer repeats) leads to a "bigger clinical problem" (higher plasma Lp(a)). This theory explains why Lp(a) is a lifelong trait, not regulated by feedback mechanisms like LDL-C, but rather a consequence of the inherent efficiency of a genetically determined production line, accounting for the 1,000-fold variation in levels across the population.[2]

Other genetic factors, such as single-nucleotide polymorphisms (SNPs) in and around the LPA locus (e.g., rs10455872, rs3798220), also independently influence Lp(a) levels and are associated with ASCVD risk. Lp(a) levels and LPA gene architecture also vary significantly between ethnicities. For example, individuals of African ancestry have, on average, 2- to 3-fold higher Lp(a) concentrations than those of European or Asian descent.[2]

## Synthesis and Catabolism

ApoA is synthesized primarily in the liver. The assembly of the mature Lp(a) particle (covalent linkage of apo(a) to apoB on an LDL particle) is thought to occur extracellularly, possibly on the hepatocyte surface.[11] The catabolic pathway for Lp(a) is not fully understood. Still, it appears to be largely independent of the LDL receptor, which is why statins are not effective at lowering Lp(a) levels. The kidney is known to play a role in the excretion of apo(a) fragments.[12]

## Pathophysiology: The Triple Threat of Atherogenesis, Inflammation, and Thrombosis

The pathophysiology of Lp(a) can be understood through three synergistic mechanisms, rendering it a "triple threat." It delivers cholesterol to the plaque (atherogenesis), incites potent inflammation via OxPL (inflammation), and impairs the body's ability to dissolve clots (thrombosis), creating a perfect storm for atherothrombotic events.

## Pro-Atherogenic Effects

Like LDL, the Lp(a) particle can penetrate the endothelium and accumulate in the arterial intima. The LDL-cholesterol component of the Lp(a) particle directly contributes to the lipid content of the plaque and promotes foam cell formation. On an equimolar basis, Lp(a) is considered more atherogenic than LDL.[13]

## Pro-Inflammatory Cascade

This is a key mechanism that distinguishes Lp(a) from LDL. Lp(a) is the primary carrier of pro-inflammatory oxidized phospholipids (OxPL) in human plasma.[14,15] Lp(a)'s role as the primary carrier of OxPL is perhaps its most critical pathological feature, making it a "Trojan horse" that delivers a potent inflammatory payload

directly to the vessel wall. This characteristic explains why its cardiovascular risk is greater than what would be predicted by its cholesterol content alone.[14] Measuring Lp(a) is not just measuring another cholesterol particle; it is assessing the body's burden of a highly inflammatory and prothrombotic molecule. This is why simply lowering LDL-C is insufficient in patients with high Lp(a), as the inflammatory and thrombotic risk persists.

These OxPL promote endothelial dysfunction, induce the expression of adhesion molecules like VCAM-1, and stimulate monocyte recruitment into the vessel wall. Furthermore, OxPL stimulates macrophages to adopt a pro-inflammatory phenotype, secreting cytokines such as IL-1β, IL-6, and TNF-α, perpetuating a local inflammatory cycle within the plaque.[15,16]

### Pro-Thrombotic and Antifibrinolytic Actions

This mechanism is a direct consequence of the structural homology between apo(a) and plasminogen.[7] Apo(a) competes with plasminogen for binding to fibrin and cell surfaces, thereby inhibiting the conversion of plasminogen to plasmin, the primary enzyme for clot dissolution. This antifibrinolytic effect promotes clot persistence and stabilization, which is particularly dangerous in the context of plaque rupture, directly linking atherosclerosis to thrombosis.[8]

## The Clinical Spectrum of Lp(a)-Associated Disease

### Atherosclerotic Cardiovascular Disease (ASCVD)

* Coronary Artery Disease (CAD): There is robust evidence from meta-analyses and Mendelian randomization studies showing a continuous, independent, and causal relationship between Lp(a) levels and the risk of Myocardial Infarction (MI) and CAD. The association is influential in the development of premature ASCVD. For instance, one meta-analysis found that elevated Lp(a) increased the odds ratio for premature CAD by 2.44.[17]

* Ischemic Stroke and Peripheral Artery Disease (PAD): The association extends to ischemic stroke and PAD, although the relationship may not be as strong as for CAD. A meta-analysis found an odds ratio of 2.56 for premature PAD.[18]

### Calcific Aortic Valve Stenosis (CAVS)

CAVS is a significant health issue that is strongly linked to high levels of Lp(a), as demonstrated by various studies and genetic research.[19-21] The proposed mechanism is that Lp(a) particles and their OxPL cargo infiltrate the aortic valve leaflets, promoting inflammation, osteogenic differentiation of valvular interstitial cells, and microcalcification, which drives the progression from aortic sclerosis to clinically significant stenosis.[20]

The causal link between Lp(a) and CAVS has challenged the traditional view of aortic stenosis as a purely "degenerative" or "wear-and-tear" disease of aging, reframing it as a lipid-driven, inflammatory disease process akin to atherosclerosis. This is a significant paradigm shift. Suppose CAVS is a modifiable, lipid-driven disease. In that scenario, it presents a novel opportunity for the development of pharmacological therapies aimed at slowing or preventing the progression of CAVS, which are currently unavailable. The cardiovascular outcome trials of Lp(a)-lowering drugs (e.g., Lp(a) HORIZON, OCEAN(a)-Outcomes) are therefore not just testing the hypothesis for ASCVD, but also for CAVS. A positive result would revolutionize the management of valvular heart disease.

Evidence also suggests that high Lp(a) is associated not only with the incidence of CAVS but also with faster hemodynamic progression and a higher risk of adverse outcomes, including the need for aortic valve replacement (AVR).[4,22,23] One study found that patients with Lp(a) ≥125 nmol/L had a 58% higher risk of AVR.

## Measurement and Clinical Application

### The Standardization Challenge

A major challenge in measuring Lp(a) is the vast size heterogeneity of the apo(a) protein among individuals, due to the KIV-2 CNV. Many early immunoassays used antibodies that bound to the repetitive KIV-2 domains, making them "isoform-sensitive." This phenomenon led to an underestimation of Lp(a) in individuals with minor, high-risk isoforms and an overestimation in those with large, lower-risk isoforms. Modern, standardized assays are therefore designed to be "isoform-insensitive", a critical requirement for accurate risk assessment.[24,25]

### Units of Measurement: Mass (mg/dL) vs Molar (nmol/L)

Lp(a) is reported in two central units: mass (mg/dL), which measures the total weight of the Lp(a) particle (protein, lipid, carbohydrate), and molar concentration (nmol/L), which measures the number of Lp(a) particles. There is a clear consensus from expert bodies (e.g., IFCC, EAS) that nmol/L is the preferred unit because it reflects the particle number, which is the actual driver of risk, and is not confounded by the variable molecular weight of different apo(a) isoforms.[3,26]

Crucially, there is no reliable universal conversion factor between mg/dL and nmol/L, as the conversion depends on the patient's specific apo(a) isoform size. While a rough approximation of nmol/L ~2.0-2.5 x mg/dL is sometimes used, it is imprecise and should be avoided for clinical decision-making purposes.[3]

### Screening and Risk Assessment Guidelines

Recommendations from major professional societies are becoming increasingly aligned, with a growing consensus in favor of universal screening (see Table 1).

* 2022 EAS Consensus & 2019 ESC/EAS Guidelines: Recommend measuring Lp(a) at least once in every adult's lifetime. They state that having a very high level of Lp(a) over 180 mg/dL (or over 430 nmol/L) indicates a lifetime risk like that of someone with heterozygous familial hypercholesterolemia (HeFH).

* 2018 AHA/ACC Guideline: Classifies Lp(a) ≥50 mg/dL (or ≥125 nmol/L) as a "risk-enhancing factor" that can be used to guide the decision to initiate statin therapy in patients with borderline or intermediate 10-year ASCVD risk.

* National Lipid Association (NLA) & Canadian Cardiovascular Society (CCS): Also recommend screening in adults, particularly those with a personal or family history of premature ASCVD, using risk thresholds around 50 mg/dL or 100-125 nmol/L.

**Table 1** Summary of International Professional Society Guideline Recommendations for Lp(a)

| Society/Guideline | Screening Recommendation | Key Risk Thresholds |
|---|---|---|
| 2022 EAS Consensus[2] | Recommends measuring Lp(a) at least once in all adults to assess lifetime ASCVD risk. | ≥50 mg/dL (≥125 nmol/L) considered a risk factor. |
| 2019 ESC/EAS Guidelines[27] | Lp(a) measurement should be considered at least once in each adult's lifetime to identify those with very high inherited levels. | >180 mg/dL (>430 nmol/L) confers a lifetime risk equivalent to HeFH. |
| 2018 AHA/ACC Guideline[28] | Measurement may be considered to aid in clinical decision-making for statins in adults with borderline (5% to <7.5%) and intermediate (≥7.5% to <20%) 10-year risk. | ≥50 mg/dL (≥125 nmol/L) considered a "risk-enhancing factor." |
| 2021 Canadian Cardiovascular Society (CCS)[29] | Recommends a one-time measurement of Lp(a) in all adults to refine risk assessment. | >50 mg/dL (>100 nmol/L) considered high risk. |
| 2019 HEART UK[30] | Lp(a) should be measured in those with a personal/family history of premature ASCVD, first-degree relatives with high Lp(a), FH, or borderline 10-year risk. | Graded risk: Moderate (90-200 nmol/L), High (200-400 nmol/L), Very High (>400 nmol/L). |
| 2019 National Lipid Association (NLA)[31] | Measurement is reasonable for risk assessment in adults with a family history of premature ASCVD, a personal history of premature ASCVD, or severe hypercholesterolemia. | ≥50 mg/dL (≥100 nmol/L). |

## Pharmacological Management: From Current Limitations to Emerging Hope

### Effects of Current Lipid-Lowering Therapies

* Lifestyle Modification: Diet and exercise have little to no effect on Lp(a) levels.[2]

* Statins: The effect is controversial and variable. Some meta-analyses suggest statins may modestly increase Lp(a) levels (8-24%), while others show no significant change. However, statins remain critical for reducing overall ASCVD risk via LDL-C lowering.[32]

* Ezetimibe: Reported to have a modest ~7% lowering effect on Lp(a), which is likely not clinically significant, and some studies show no effect.[33]

* Niacin: While niacin can lower Lp(a) by ~20-25%, significant side effects and a lack of evidence for cardiovascular event reduction in the statin era limit its use.[34,35]

* PCSK9 Inhibitors (Evolocumab, Alirocumab): These agents moderately lower Lp(a) by ~20-30%. Post-hoc analyses of the cardiovascular outcome trials (e.g., FOURIER, ODYSSEY OUTCOMES) suggest that patients with higher baseline Lp(a) derive greater absolute benefit from treatment, likely due to the combination of profound LDL-C reduction and moderate Lp(a) lowering.[36,37]

* Lipoprotein Apheresis: This is the only currently approved and highly effective treatment, achieving a mean interval reduction of 25-40%.[38] However, it is invasive, expensive, and accessible to only a minimal number of high-risk patients.

## The New Frontier: Specific Lp(a)-Lowering Drugs

This is the most exciting area of current research, with drugs specifically designed to inhibit the synthesis of apo(a) in the liver.

### Antisense Oligonucleotides (ASOs): Pelacarsen (TQJ230)

* Mechanism of Action: A GalNAc-conjugated ASO that specifically targets hepatocytes. It binds to the LPA mRNA, leading to its degradation by RNase H and preventing the translation of the apo(a) protein.[39]

* Clinical Data: Phase 2 results showed a potent, dose-dependent reduction in Lp(a) of up to 80%.[40,41]

* Pivotal Trial: Lp(a) HORIZON (NCT04023552): An ongoing Phase 3 Cardiovascular Outcome Trial (CVOT) of 8,325 participants with established CVD and Lp(a) ≥70 mg/dL, testing pelacarsen 80 mg subcutaneously monthly vs. placebo, with MACE as the primary endpoint. Topline results are expected in 2025.

### Small Interfering RNA (siRNA): Olpasiran (AMG 890), Zerlasiran, and others

* Mechanism of Action: Also, GalNAc-conjugated, these siRNAs use the RNA interference (RNAi) mechanism to cleave and degrade LPA mRNA, thereby inhibiting apo(a) synthesis.[42]

* Clinical Data (Olpasiran): The Phase 2 OCEAN (a)-DOSE study demonstrated profound Lp(a) reductions of >95% with doses of 75 mg or 225 mg every 12 weeks. The effect is durable, with a ~40-50% reduction maintained nearly a year after the last dose.[42,43]

* Pivotal Trial: OCEAN (a)-Outcomes (NCT05581303): An ongoing and fully enrolled Phase 3 CVOT of ~7,000 patients with ASCVD and Lp(a) ≥ 200 nmol/L, testing olpasiran vs. placebo every 12 weeks. The primary endpoint is CHD death, MI, or urgent coronary revascularization. Results are anticipated around 2026.

### Novel Oral Agent: Muvalaplin

* Mechanism of Action: A first-in-class oral small molecule that acts via a different mechanism, disrupting the non-covalent interaction between apo(a) and apoB, thereby inhibiting the final step of Lp(a) particle assembly.[44]

* Clinical Data: Phase 1 results showed a 63-65% reduction in Lp(a) versus placebo. The Phase 2 study (ALPACA) is now fully enrolled. This represents a desirable option for patients who prefer an oral therapy.[44]

**Table 2** Efficacy of Current and Emerging Therapies in Lowering Lp(a)

| Therapy/Class | Mechanism of Action | Average Percent Lp(a) Reduction | Key Evidence/Trials |
|---|---|---|---|
| Statins | HMG-CoA reductase inhibitor | 8% to 24% or no effect | Meta-analyses[32,45] |
| Ezetimibe | NPC1L1 inhibitor | ~7% reduction or no effect | Meta-analyses33 |
| Niacin | Unclear | ~20-25% reduction | AIM-HIGH, HPS2-THRIVE[34,35] |
| PCSK9 Inhibitors | Inhibit PCSK9, upregulate LDLR | ~20-30% reduction | FOURIER, ODYSSEY OUTCOMES[36,37] |
| Lipoprotein Apheresis | Removes apoB-containing lipoproteins | ~25-40% reduction (mean interval) | Observational studies[38] |
| Pelacarsen (ASO) | Degrades LPA mRNA (RNase H) | ~80% reduction | Phase 2 trial[39,40,41] |
| Olpasiran (siRNA) | Degrades LPA mRNA (RNAi) | >95% reduction | OCEAN(a)-DOSE (Phase 2)[42,46,47] |
| Muvalaplin (Oral) | Inhibits Lp(a) assembly | ~63-65% reduction | Phase 1 trial[14,48] |

**Table 3** Design and Key Features of Pivotal Phase 3 Lp(a)-Lowering Trials

| Trial Name | Investigational Drug | Mechanism of Action | Patient Population | Lp(a) Inclusion Criteria | Primary End-point | Expected Completion |
|---|---|---|---|---|---|---|
| Lp(a) HORIZON[49] | Pelacarsen | Antisense Oligonucleotide (ASO) | Patients with established ASCVD | ≥70 mg/dL | MACE-4 (CV death, non-fatal MI, non-fatal stroke, urgent coronary revascularization) | 2025 |
| OCEAN(a)-Outcomes[50,51] | Olpasiran | Small Interfering RNA (siRNA) | Patients with established ASCVD | ≥200 nmol/L | MACE-3 (CHD death, MI, urgent coronary revascularization) | ~2026 |

## Conclusion and Future Directions

### Synthesizing the Evidence

Lp(a) is no longer an enigmatic biomarker but a validated, causal therapeutic target. The congruent evidence from genetics, epidemiology, and pathophysiology is undeniable. The immediate clinical imperative is to identify patients with high Lp(a) through screening and to aggressively manage all other modifiable risk factors (especially LDL-C and blood pressure) to mitigate their heightened global risk.

### Testing the Lp(a) Hypothesis

The key unanswered question is the "Lp(a) hypothesis": will specific and substantial lowering of Lp(a) translate into a reduction in cardiovascular events? The ongoing Phase 3 CVOTs (Lp(a)HORIZON, OCEAN(a)-Outcomes) are designed to answer this question definitively.[52] Their results will be practice-changing, either by establishing Lp(a) as a new pillar of cardiovascular prevention or by questioning its role as a therapeutic target despite its causal association.

### Unanswered Questions and the Path Forward

The field will still face important future questions: What is the optimal degree of Lp(a) lowering for clinical benefit? Are there any long-term, off-target effects of near-total Lp(a) elimination? What will be the role of these agents in primary prevention, especially in those with very high genetic risk but no overt disease? And finally, how will cost-effectiveness and access shape their role in clinical practice?

In conclusion, the field of preventive cardiology is poised for a new era. The clinical validation of Lp(a) lowering would represent one of the most significant advances since the introduction of statins.

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