Research article

Antihypertensive activity and vascular reactivity mechanisms of *Vitex pubescens* leaf extracts in spontaneously hypertensive rats

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ABSTRACT

Background: *Vitex pubescens* has been used traditionally in hypertension treatment but not yet scientifically assessed. The objective of the study is to investigate the antihypertensive and vasorelaxant activities of *V. pubescens*, study its underlying pharmacological mechanisms, and identify the relevant vasoactive compounds.

Methods: Successive extractions of *V. pubescens* leaf were carried out to produce petroleum ether (VPPE), chloroform (VPCE), methanol (VPME), and water (VPWE) extracts. Spontaneously hypertensive rats (SHRs) received a daily oral administration of the extracts (500 mg/kg/day; n = 6) or verapamil (15 mg/kg/day; n = 6) for 2 weeks, while the systolic and diastolic blood pressures were measured using non-invasive tail-cuff method. Vasorelaxation assays of the extracts were later conducted using phenylephrine (PE, 1 μM) pre-contracted aortic ring preparation. Mechanisms of vasorelaxation by the most potent fraction were studied using vasorelaxation assays with selected blockers/inhibitors. GC-MS was conducted to determine the active compounds.

Results: VPPE elicited the most significant diminution in systolic and diastolic blood pressure of treated SHRs and produced the most significant vasorelaxation in the aortic rings. Vasorelaxant effects of F2-VPPE were significantly reduced in endothelium-denuded aortic rings by glibenclamide (3 μM), whereas calcium chloride and PE-induced contractions were significantly suppressed. Endothelium removal of the aortic rings or incubation with indomethacin (10 μM), atropine (1 μM), methylene blue (10 μM), propranolol (1 μM) and L-NAME (10 μM) did not significantly alter F2-VPPE-induced vasorelaxation. Seven compounds were identified using GC-MS, including spathulenol.

Conclusion: F2-VPPE exerted its endothelium-independent vasorelaxation by inhibition of vascular smooth muscle contraction induced by extracellular Ca²⁺ influx through trans-membrane Ca²⁺ channels and/or Ca²⁺ release from intracellular stores, and by activation of KATP channels. The vasorelaxation effects of *V. pubescens* could be mediated by the compound, spathulenol.

1. Introduction

Hypertension is one of the most common causes of premature morbidity and death worldwide. More than 5.8% of the total deaths and more than 45% of deaths result from heart diseases are caused by hypertension [1]. The slight increase in blood pressure if untreated raises the risk of cardiovascular diseases and organ damage [2]. Hypertension can be strongly controlled by vasorelaxation that decreases peripheral resistance and blood flow [3]. Vasorelaxation is achieved by activating endogenous or exogenous vasoactive compounds that act on the receptors, channels, or enzymes located on vascular endothelium or vascular smooth muscle [4, 5]. Therefore, finding new drugs that are capable of employing multiple vasorelaxant mechanisms is highly desired.

All over the world, medicinal plants have been used as part of society due to many factors, including affordability, accessibility, and less toxicity [6]. *Vitex pubescens* Vahl. (Verbenaceae) locally called Halban in the Peninsular of Malaysia has been used traditionally to treat hypertension and gastrointestinal disorders [7, 8]. In Brunei folk medicine, young leaves of *V. pubescens* are eaten raw for treating hypertension and fever [9]. Bhakuni et al. [10] have reported hypotensive activity of *V. pubescens* from *in vitro* study of cardiovascular effects of ethanol (50 %).
extract on dogs. Vitexin is an important flavonoid with a potent hypotensive effect [11] and induces vasorelaxation in rat aorta [12]. Thenmozhi et al. [13] have reported the isolation of vitexin, an apigenin flavone glycoside, from hydroalcoholic leaves extract of V. pubescens.

Although the hypotensive activity of V. pubescens has been reported, there is no study on the antihypertensive and vasorelaxant activities of this plant so far. Therefore, this study investigated the antihypertensive activity of V. pubescens using conscious SHRs and the mechanism of its vasorelaxant activity using isolated SHR aortic rings.

2. Materials and methods

2.1. Chemicals

Petroleum ether, chloroform, methanol, n-hexane, and acetone were purchased from Fischer Scientific (Selangor, Malaysia). Phenylephrine (PE), acetylcholine (ACh), N-nitro-L-arginine methyl ester (L-NAME), methylene blue (MB), atropine, indomethacin, glibenclamide, propranolol hydrochloride, and verapamil hydrochloride were purchased from Sigma-Aldrich Company (St Louis, Mo, USA). All chemical substances were of analytical quality.

2.2. Animals

Male spontaneously hypertensive rats (SHRs, 250–320 g) were obtained from and housed in the Animal Research and Service Centre Universiti Sains Malaysia (ARASC-USM). The SHRs were kept at 27°C with a 12-h light/12-h dark cycle with standard rat diet (Gold Coin) and oven-dried at 45°C for 24 h. All animals were handled according to the Guide for the Use and Care of Laboratory Animals and approved by the Animal Ethics Committee, Universiti Sains Malaysia. [No. of Animal Ethics Approval: USM/IACUC/2017/ (110) (897)].

2.3. Plant material and preparation of V. pubescens extracts

Fresh green leaf samples of V. pubescens were gathered from the main campus of Universiti Sains Malaysia, Penang, Malaysia. The plant was authenticated by Rahmad Zakaria, Ph.D. at the Herbarium, School of Biological Sciences, Universiti Sains Malaysia (Voucher specimen registration no. 11750). 2.5 kg of V. pubescens crushed dried leaves were subjected to successive extraction by maceration at 45°C in a water bath sequentially, with petroleum ether, chloroform, methanol, and water. These extracts were filtered with Whatman filter paper (No. 1). The filtrates were concentrated by a rotary evaporator (Buchi, Switzerland), and oven-dried at 45°C for the petroleum ether, chloroform, and methanol extracts, and freeze-dried for the water extract.

2.4. Fractionation of V. pubescens petroleum ether extract

Based on the vasorelaxation study, V. pubescens petroleum ether extract had been established as the most potent extract and was selected for fractionation. 10 g of the extract was further fractionated by column chromatography using silica gel (230–400 mesh) as the stationary phase. The column was eluted with n-hexane containing increasing concentrations of acetone (10, 20, 30, 40, and 50% acetone) as the mobile phase [1-4]. Fractions were collected and then analyzed on TLC plates using hexane/acetone (7:3).

2.5. Antihypertensive study

36 SHRs were divided randomly into 6 groups of six; positive control (verapamil, 15 mg/kg/day), negative control (10% Tween 80 in distilled water, 10 mL/kg/day), four groups of V. pubescens, namely petroleum ether (VPPE), chloroform (VPCE), methanol (VPME), and water (VPWE) (500 mg/kg/day). Each SHR received a daily oral feeding of respective treatment for two weeks. Systolic and diastolic blood pressures of the SHRs were measured at day 0 (before oral feeding), 3, 7, and 14 for the two weeks’ duration using a non-invasive tail-cuff method (CODA system, Kent Scientific, USA). The SHR was placed in an animal restrainer on a heating pad at 38°C, and the tail was inserted in the occlusion and volume pressure recording (VPR) cuffs for the blood pressure reading. At the end of the day 14 blood pressure measurements, the SHRs were euthanized in ARASC-USM using carbon dioxide (CO2) gas inhalation (10%–30% per minute) in a chamber.

2.6. Isolated rat thoracic aortic rings preparation and vasorelaxation study of V. pubescens extracts

Vasorelaxation assay was conducted as described by Bello et al. [15]. The SHR was immobilized in the carbon dioxide (CO2) gas chamber and then exsanguinated. After opening the SHR’s chest, the aorta was carefully taken out and placed in Krebs’s Physiological Solution (KPS) consisting of 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 4.2 mM NaHCO3, 1.2 mM MgSO4, 10 mM glucose, and 2 mM CaCl2. The aorta was cleaned of the connective tissues and cut into 3–5 mm long aortic rings. These rings were hunged up horizontally in a tissue bath containing 10 mL KPS being continuously ventilated with carbogen gas (95% oxygen and 5% carbon dioxide) at 37°C. Equilibration of the aortic rings was set at a basal tension of 1 g for at least 30 min. During the experiment, the KPS in the aortic ring chamber was replaced every 15 min. For endothelium intact aortic ring preparation, the endothelium integrity was assessed by more than 90% relaxation of acetylcholine (1 μM) to phenylephrine (PE, 1 μM) precontracted rings. For the endothelium-denuded aortic ring preparation, endothelium denudation was attained by rubbing the intima with forceps and was later affirmed by the lack of relaxation to ACh (1 μM) of PE-precontracted rings [16]. The vasorelaxant effects of the extracts/fractions that were added cumulatively (0.25, 0.5, 1, 2, and 4 mg/mL) to PE-precontracted aortic rings were determined. Percentage vasorelaxation was measured using the formula:

\[
\% \text{ Relaxation} = \left( \frac{T_c - T_t}{T_c} \right) \times 100
\]

Where Tc = gm contraction of aortic rings with PE (1 μM) and Tt = gm relaxation of aortic rings with extract/fraction. The tension was measured with a force-displacement transducer (GRASS FT03, U.K.). Signals were amplified by PowerLab 26T (AD Instrument, Australia) and read by LabChart 7 software [17, 18].

2.7. Effects of endothelium-dependent pathways in vasorelaxation mechanisms of the most active fraction of V. pubescens

Involvements of muscarinic cholinergic receptors, endothelium-derived nitric oxide, prostacyclin (PGI2) and cyclic guanosine monophosphate (cGMP) in the vasorelaxation mechanism were examined using endothelium-intact aortic rings incubated with atropine (1 μM; a competitive non-selective muscarinic receptor antagonist), L-NAME (10 μM; a nonspecific nitric oxide synthase inhibitor), indomethacin (10 μM; a non-selective cyclooxygenase inhibitor), and methylene blue (MB, 10 μM; a cGMP inhibitor), respectively. The aortic rings were precontracted with PE (1 μM), and then cumulative concentrations of the most active fraction of V. pubescens (0.25–4 mg/mL) were added. This was followed by the aortic rings incubation with a blocker, and the vasorelaxation procedures were repeated [19].

2.8. Effects of the most active fraction of V. pubescens on β-adrenergic receptors and K⁺ channels using endothelium-denuded aortic rings

K⁺ channels and β-adrenergic receptors involvements in the vasoactive mechanisms of V. pubescens most active fraction were examined using glibenclamide (1 mM; a selective ATP-sensitive K⁺ channel blocker) and propranolol (1 mM; a non-selective β-adrenergic receptor blocker),
The aortic rings were precontracted with PE (1 μM), and then cumulative concentrations of the most active fraction (0.25–4 mg/mL) were added. This was followed by the aortic rings incubation with a blocker, and the vasorelaxation procedures were repeated [20, 21].

2.9. Effects of the most active fraction of V. pubescens on extracellular Ca$^{2+}$-induced contraction using endothelium-denuded aortic rings

Inhibitions of Ca$^{2+}$ influx through voltage-dependent calcium channels (VDCC) by the most active fraction of V. pubescens were assessed as described by Oliveira et al. [22]. Denuded aortic rings were equilibrated for 30 min in normal KPS. The solution was then replaced with Ca$^{2+}$ free KPS containing EDTA (0.1 mM), and the aortic rings were continually being immersed for 30 min to get rid of Ca$^{2+}$ from the tissue. The solution was further replaced with Ca$^{2+}$ free, K$^+$ rich KPS (92.6 mM NaCl, 16.09 mM KCl, 1.2 mM KH$_2$PO$_4$, 16.67 mM NaHCO$_3$, 9.98 mM MgSO$_4$, 11 mM glucose) for 30 min. The contraction effects of cumulative additions of Ca$^{2+}$ (0.1–10 mM) were compared before and after incubation with different concentrations of the V. pubescens most active fraction (0.5, 1, and 2 mg/mL) or verapamil (0.01 and 0.1 μM), respectively.

Figure 1. The effects of 14 days daily oral administration of V. pubescens petroleum ether (VPPE), chloroform (VPCE), methanol (VPME), water (VPWE) extracts (500 mg/kg), negative control and verapamil (15 mg/kg) on SBP of SHRs. Means comparison between treatment days were analyzed by one-way ANOVA, followed by Tukey’s multiple comparison test. Values are expressed as mean ± SEM (n = 6 SHRs). **P < 0.01, ***P < 0.001 as compared to day 0.

Figure 2. The effects of 14 days daily oral administration of V. pubescens petroleum ether (VPPE), chloroform (VPCE), methanol (VPME), water (VPWE) extracts (500 mg/kg), negative control and verapamil (15 mg/kg) on DBP of SHRs. Means comparison between treatment days were analyzed by one-way ANOVA, followed by Tukey’s multiple comparison test. Values are expressed as mean ± SEM (n = 6 SHRs). *P < 0.05, **P < 0.01, ***P < 0.001 as compared to day 0.
containing EDTA (0.1 mM) before inducing the constrictor response. Denuded aortic rings were equilibrated in a Ca²⁺-free solution. After that, the aortic rings were equilibrated and then incubated with the active fraction of V. pubescens extract in phenylephrine pre-contracted intact aortic ring preparations. Means comparison between treatment groups were analyzed by two-way ANOVA, followed by Tukey's multiple comparison test. Values are expressed as mean ± SEM (n = 6 SHRds). **P < 0.01, ***P < 0.001 as compared to VPCE, VPME and VPWE.

2.10. Effects of the most active fraction of V. pubescens on intracellular Ca²⁺-induced contractions using endothelium-denuded aortic rings

The inhibitions of PE-induced intracellular Ca²⁺ release by the most active fraction of V. pubescens were examined as described by Senejoux et al. [23]. Denuded aortic rings were equilibrated in a Ca²⁺-free KPS containing EDTA (0.1 mM) before inducing the first transient contraction (T₁) by PE (1 μM). The solution was then washed twice with the same solution. After that, the aortic rings were equilibrated and then incubated with the V. pubescens most active fraction (1 and 2 mg/mL, respectively) for 15 min before inducing the second transient contraction (T₂) by PE (1 μM). The ratio of the second transient contraction to the first (T₂/T₁) was calculated.

2.11. GC-MS analysis of the most active fraction

As described by Zuo et al. [24], gas chromatography-mass spectrometry (GC-MS) analysis of the most active fraction (1 mg/mL) of V. pubescens was carried out using Agilent Technologies 6890N Network GC System coupled with an Agilent Technologies 5973 Mass Selective Detector and equipped with an Agilent 19091S-433 HP-5MS (5% Phenyl Methyl Siloxane) capillary column (30 m length × 250 μm diameter × 0.25 μm film thickness). The mass spectrum was acquired by an electron ionization at 69.9 eV and scanned from m/z 35 to 650 at a rate of 2 scans/sec. Helium gas was used as a carrier gas with a steady flow rate of 1.2 mL/min, and 2 μl of injection volume was used. The oven initial and maximum temperatures were 70 °C and 325 °C, respectively. The front inlet temperature was 280 °C with pressure at 10.97 psi in the splitless mode. The total running time was 32.50 min. The relative percentages of the determined compounds were calculated by the total ion chromatogram using ChemStation software. The mass-spectrum was interpreted by the database of the National Institute Standard and Technology (NIST02). The phytochemicals with their retention time (RT), chemical formula, chemical structure, and ethnopharmacological uses were listed.

2.12. Statistical analysis and data presentation

All data were presented as mean ± SEM and n = 6. The effect of 14 days daily oral administration of extracts and control groups on SBP and DBP of SHRds were analyzed by one-way ANOVA, followed by Tukey's multiple comparison test. The other vasorelaxation experiments were analyzed by two-way ANOVA, followed by Tukey's multiple comparison test. All analyses were carried out using Graph Pad Prism statistical software (version 7). A significant difference was set at P < 0.05, **P < 0.01, and ***P < 0.001.

3. Results

3.1. Extraction and fractionation of V. pubescens

The yields of the V. pubescens extracts petroleum ether (VPPE; 36.2 g), chloroform (VPCE; 27.8 g), methanol (VPME; 301.5 g), and water (VPWE; 183.0 g). The yields of the fractions of VPPE: F1-VPPE; 0.163 g, F2-VPPE; 1.620 g, F3-VPPE; 0.323 g, and F4-VPPE; 1.112 g.

3.2. Antihypertensive effects of V. pubescens extracts

For the evaluation of antihypertensive effects of V. pubescens extracts, VPPE significantly reduced (P < 0.001) SBP and DBP as early as day 3 during the 14 treatment days in a comparable manner as verapamil. VPME produced a significant reduction (P < 0.01) only on day 14 (Figure 1 and Figure 2).

3.3. Vasorelaxation effects of V. pubescens extracts/fractions on the endothelium-intact aortic rings

The cumulative additions of V. pubescens extracts to the tissue chamber elicited a concentration-dependent relaxation of PE-precontracted endothelium-intact aortic rings (Figure 3). VPPE significantly produced greater vasorelaxation (108.3 ± 4.3%, P < 0.001) at 4 mg/mL concentration than VPCE (61.8 ± 4.2%), VPME (68.2 ± 4.7%), and VPWE (62.7 ± 3.7%), respectively. Whereas between the VPPE fractions, F2-VPPE significantly relaxed (P < 0.001) the PE (1 μM)-precontracted intact aortic rings greater than F4-VPPE, F3-VPPE, and F1-VPPE, respectively (Figure 4). Therefore, F2-VPPE was selected for the vasorelaxation mechanism study using aortic rings.
3.4. Effects of endothelium-dependent pathways in vasorelaxation mechanisms of F2-VPPE

F2-VPPE induced a concentration-dependent vasorelaxation in both endothelium-intact and endothelium-denuded aortic ring preparations pre-contracted with PE (1 μM) without any significant difference between the two aortic preparations (Figure 5). F2-VPPE also did not produce any significant decrease in concentration-dependent vasorelaxation in endothelium intact aortic rings following treatments with L-NAME, methylene blue (Figure 6), atropine, and indomethacin (Figure 7).

3.5. Effects of F2-VPPE on β-adrenergic receptors and K⁺ channels using endothelium-denuded aortic rings

Glibenclamide significantly decreased (P < 0.001) the vasorelaxation effects of F2-VPPE (from a concentration of 0.25–4 mg/mL) on PE pre-contracted endothelium-denuded aortic ring preparations but not propranolol (Figure 8).

3.6. Effects of F2-VPPE on extracellular Ca²⁺-induced contraction using endothelium-denuded aortic rings

Cumulative additions of CaCl₂ (0.1–10 mM) to endothelium-denuded aortic rings in high K⁺ and Ca²⁺ free KPS induced concentration-dependent contractions. Both, F2-VPPE (0.5, 1 and 2 mg/mL) and verapamil (0.01 and 0.1 μM) significantly (P < 0.001) attenuated the CaCl₂-induced vasoconstriction of endothelium-denuded aortic ring preparation (Figure 9 A and B) in a comparable manner.
3.7. Effects of F2-VPPE on intracellular Ca\(^{2+}\)-induced contractions using endothelium-denuded aortic rings

F2-VPPE (1 and 2 mg/mL) significantly attenuated (\(P < 0.001\)) PE-induced vasoconstrictions of denuded aortic rings (Figure 10).

3.8. GC-MS analysis

The GC-MS analysis of F2-VPPE indicated the presence of a total of 76 volatile components. Seven compounds with 95% similarity and above based on NIST 02 library had been identified (Table 1). These compounds are sesquiterpenes alcohol, phytosterols, and terpenes that have been reported with main pharmacological activities. The quantitative analysis showed the majority presence of \(\alpha\)-amyrin, spathulenol, \(\beta\)-amyrin, and phytol. However, only spathulenol has been reported to be a potent vasorelaxant of smooth muscles.

4. Discussion

The present study is the first bioactivity-guided approach for antihypertensive activities of \(V.\) pubescens leaf using conscious SHRs and the mechanism of its vasorelaxant activity using isolated SHR aortic rings. Based on the antihypertensive evaluation, VPPE produced the most significant blood pressure-lowering activity in SHRs, which was comparable to verapamil, a calcium channel blocker. Vasorelaxation is an important mechanism in lowering blood pressure as it decreases systemic vascular resistance. As in the whole animal measurement, VPPE exhibited the most significant concentration-dependent relaxation on isolated PE-precontracted rat thoracic aorta rings. Both findings suggested that the antihypertensive effects of VPPE might be largely due to its vasorelaxation activities which lead to a reduction in the peripheral resistance, and consequently lower diastolic and systolic blood pressure. We also established that F2-VPPE was the most potent fraction for vasorelaxation effects and was selected for the vasoactive mechanism study.

The endothelium regulates vascular activities by modulating the action of the contractile agents existed on the vascular smooth muscle layer of blood vessels through the production of endothelium-derived relaxing factors (EDRFs), including nitric oxide (NO), prostacyclin and various hyperpolarizing factors derived from endothelium [25, 26]. Our results showed that F2-VPPE-induced vasorelaxation effects were not influenced by the presence or absence of an intact endothelium. Further support of endothelium independence in F2-VPPE-induced vasorelaxation was confirmed by examining the effect of the selective blockers/inhibitors of EDRFs. This assumption has been reported in many therapeutic plants such as \(Ligusticum\) chuanxiong [27, 28, 29]. Studies with L-NAME, indomethacin, methylene blue, and atropine further supported the irrelevancy of the nitric oxide and prostacyclin pathways, cGMP, and muscarinic receptors roles in the vasorelaxation properties of F2-VPPE.

Having established the role of endothelium and EDRFs, we investigated the participation of vascular smooth muscles in the vasorelaxation of \(V.\) pubescens. Roles of \(\beta\)-adrenergic receptors, potassium channels, and calcium channels in the vascular smooth muscles on F2-VPPE induced relaxation in endothelium-denuded aortic ring preparations were studied. Glibenclamide significantly reduced the F2-VPPE-induced relaxation but not propranolol, implying that \(K_{ATP}\) channel activation is largely involved in the vasorelaxation mechanism of F2-VPPE and not are \(\beta\)-adrenoceptors. The opening of the \(K_{ATP}\) channel leads to hyperpolarization, causing inhibition of \(Ca^{2+}\) influx through voltage-dependent \(Ca^{2+}\) channels and relaxation of vascular smooth muscle [30]. Extracellular \(Ca^{2+}\) influx induces vascular smooth muscle contraction by opening.
voltage-dependent Ca\textsuperscript{2+} channels (VDCC) \[31\]. The present results showed that F2-VPPE and verapamil (Ca\textsuperscript{2+} channel blocker) almost completely abolished the contraction prompted by the cumulative addition of CaCl\textsubscript{2}. This may imply that F2-VPPE inhibited the extracellular Ca\textsuperscript{2+} inflow through VDCC in a similar way as verapamil \[32\].

Phenylephrine increases extracellular Ca\textsuperscript{2+} inflow and induces vascular smooth muscle contraction by opening receptor-operated Ca\textsuperscript{2+} channels (ROCC). Furthermore, phenylephrine induces contraction in the absence of extracellular Ca\textsuperscript{2+} by the release of intracellular Ca\textsuperscript{2+} from the sarcoplasmic reticulum Ca\textsuperscript{2+} store via 1,4,5-triphosphate inositol receptors (IP3Rs) which bind to IP3 \[33\]. Our results also showed that F2-VPPE inhibited PE-induced contraction that could be due to an inhibition of intracellular Ca\textsuperscript{2+} release from Ca\textsuperscript{2+} store and/or inhibition of extracellular Ca\textsuperscript{2+} inflow through ROCC. From these results, it is most likely that F2-VPPE-induced vasorelaxation involves K\textsubscript{ATP} channels activation, inhibition of the extracellular Ca\textsuperscript{2+} inflow through VDCC, and inhibition of intracellular Ca\textsuperscript{2+} release from Ca\textsuperscript{2+} store and/or inhibition of extracellular Ca\textsuperscript{2+} inflow through ROCC in the vascular smooth muscles.

Based on the GC-MS analysis of F2-VPPE, spathulenol was one of the main compounds identified. It has been reported by Dib et al. \[34\] that spathulenol is a predominant compound in therapeutic plants that possess vasorelaxant activity. Perez et al. \[35\] stated that spathulenol, isolated from Lepechinia caulescens, induces relaxation of smooth muscles by inhibiting the contraction prompted by the cumulative addition of CaCl\textsubscript{2} and thus inhibiting the Ca\textsuperscript{2+} inflow through VDCC.

5. Conclusion

These findings showed that V. pubescens possesses antihypertensive effects. The most potent fraction, F2-VPPE, exerted its endothelium-independent vasorelaxation by inhibition of vascular smooth muscle contraction induced by extracellular Ca\textsuperscript{2+} influx through transmembrane Ca\textsuperscript{2+} channels and/or Ca\textsuperscript{2+} release from intracellular stores, and by activation of K\textsubscript{ATP} channels. The vasorelaxation effects of V. pubescens could be due to the compound, spathulenol.

Declarations

Author contribution statement

A.A. Al-Akwaa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

M.Z. Asmawi: Conceived and designed the experiments; Wrote the paper.

A. Dewa: Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. Mahmud: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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| RT (min) | Peak Area | Compound name | Chemical formula/structure | Pharmacological actions | References |
|---------|-----------|---------------|-----------------------------|-------------------------|------------|
| 9.04    | 5.49      | Spathulenol   | C\textsubscript{15}H\textsubscript{24}O | Vasorelaxation of smooth muscle, antiinflammatory, antimicrobial and antioxidant | \[35, 36\] |
| 9.47    | 0.70      | \(\alpha\)-cadinol | C\textsubscript{15}H\textsubscript{26}O | Antifungal, anticancer and pesticide | \[37, 38\] |
| 11.71   | 3.90      | Phytol       | C\textsubscript{20}H\textsubscript{40}O | Antibacterial, antiinflammatory, and antiallergic | \[39, 40\] |
| 22.29   | 0.96      | Stigmasterol | C\textsubscript{29}H\textsubscript{48}O | Antiinflammatory, antihypercholesterolemia, antitumor, antioxidant and antibacterial | \[41, 42\] |
| 23.59   | 2.98      | \(\beta\)-sitosterol | C\textsubscript{29}H\textsubscript{50}O | Antiinflammatory antihypercholesterolemia, anticancer and antidiabetic | \[43\] |
| 24.49   | 5.15      | \(\beta\)-amyrin | C\textsubscript{30}H\textsubscript{50}O | Gastroprotective and antiplatelet | \[44, 45\] |
| 25.68   | 13.7      | \(\alpha\)-amyrin | C\textsubscript{30}H\textsubscript{50}O | Gastroprotective and antiplatelet | \[44, 45\] |
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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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