

## Original Research Article

# Vasodilating effect of *Hypericum revolutum* (Vahl) (Clusiaceae) methanol extract in rats

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Sent for review: 15 December 2020

Revised accepted: 22 April 2021

### Abstract

**Purpose:** To investigate the vasodilating effect of *Hypericum revolutum* Vahl (Clusiaceae) in rats.

**Methods:** *H. revolutum* aerial parts were extracted with methanol. The total methanol extract was fractionated with chloroform to yield fraction I. The remaining aqueous solution was chromatographed on Diaion HP-20 using water, 50 % methanol, and methanol to yield three fractions (II- IV). Total methanol extract and fractions I-IV were applied to phenylephrine-pre-contracted (10  $\mu$ M) rat aortic rings at doses of 1, 3, and 10  $\mu$ g/mL. Subsequent decreases in aortic tension were recorded by an isometric force transducer to evaluate the vasodilation. Column chromatography was utilized to separate the active components of the bioactive fraction.

**Results:** Remarkable decreases in aortic tension ( $p < 0.05$ ) revealed that fraction I (3 and 10  $\mu$ g/mL) produced a vasodilating effect, whereas fractions III and IV did not possess any substantial effect. Vasodilation induced by fraction I was endothelial-dependent because it was significantly ( $p < 0.05$ ) blocked by endothelial denudation. Phytochemical inspection of fraction I led to the isolation of  $\beta$ -sitosterol (1), 1,7-dihydroxyxanthone (euxanthone) (2), and 2,3,4-trimethoxy xanthone (3).

**Conclusion:** Fraction I of the *H. revolutum* extract was responsible for its vasodilating effect. This fraction may be used as a possible anti-hypertensive preparation after *in vivo* testing and successful clinical trials.

**Keywords:** Vasodilation, *Hypericum revolutum*, Clusiaceae, Nitric oxide

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## INTRODUCTION

Medicinal plants use represents the basis of global healthcare. Medicinal plants are advantageous for treating various diseases. Most developing countries, particularly in Asia and

Africa, have reported a considerable increase in the use of such medications. Previous studies have shown that >75% of the global population relies on herbal medicine [1]. The Saudi flora consists of approximately 2,250 plants that are equally disseminated all over the country [2]. A

substantial number of these plants have been used by the locals for the management of hypertension [3]. Hypertension is a major worldwide problem that is frequently accompanied with metabolic syndrome, obesity, and diabetes. In fact, many people are at risk of cardiovascular diseases when they are affected by metabolic syndrome [4]. This is based on the fact that vascular destruction occurs as a result of oxidative stress and hyperglycaemia [5,6]. Vasodilators are an important class of anti-hypertensives to treat coronary artery disease and vascular insufficiency [7].

The *Hypericum* genus belongs to the Clusiaceae family, which was formerly known as Guttiferae [8]. Diversified *Hypericum* species possess anti-viral, anti-inflammatory antioxidant, and anti-bacterial properties [9]. Naphthodianthrones, chlorogenic acid, phloroglucinols, flavonoids, xanthenes, proanthocyanidins, phenylpropanes, benzofuran, and benzopyrans are widely reported from this genus [10-13].

General screening of the methanol extracts from some of the Saudi plants has revealed a promising vasodilating effect of *H. revolutum*. Therefore, this study targeted to determine the possible vasodilating potential of *H. revolutum* through a bio-guided approach using the isolated artery technique [14]. The methanol extract was fractionated to determine the bioactive fraction, which was then further fractionated to separate the bioactive compounds.

## EXPERIMENTAL

### Extraction

Plant material was collected from Al-Baha governorate (KSA), and was certified by Dr Emad Al- Sharif (taxonomist at Faculty of Science, Jeddah University). A specimen (# HR-0438) was preserved at the Department of Natural Products and Alternative Medicine's herbarium (KAU, Faculty of Pharmacy). Powdered *H. revolutum* aerial parts (1 kg) were extracted with cold methanol utilizing an Ultraturrex homogenizer. The pooled methanol extract was concentrated to yield a brown viscous extract. The total methanol extract was fractionated with chloroform to yield fraction I. The remaining aqueous solution was chromatographed in Diaion HP-20 using water, 50 % methanol, and methanol to yield fractions (II-IV). Thin-layer chromatography revealed that fraction II was free from any phytochemical components. The chloroform fraction was subjected to SiO<sub>2</sub> CC (column chromatography)

using an *n*-hexane:EtOAc gradient to yield compounds **1**, **2**, and **3**.

### Drugs and chemicals

Acetyl choline (ACh), DMSO (dimethyl sulfoxide), and phenyl ephedrine (PE) were procured from Sigma-Aldrich (Dorset, UK).

### Animals

Seven-week-old Wister male rats (200g) were supplied from the King Abdulaziz University's animal facilities and were used in this study. The animal studies were legitimated by the Faculty of Pharmacy's Research Ethical Committee, King Abdulaziz University (approval no. 126-1439). The thoracic aortas were carefully excised after the rats were sacrificed by decapitation.

### Analysis of the direct vasodilating effect of the extract and its fractions

Vasodilating effects of the total extract as well as its different fractions and pure isolates were assessed by the isolated artery technique, as formerly stated [15,16]. Briefly, the isolated aortic rings were suspended in a thermostatically controlled organ bath containing Krebs-Hens light buffer at 37 °C. The aortic rings were subjected to 150 mg of isometric tension, similar to physiological conditions in living animals, for 1 h before testing the vasodilating activities. Then, the aortic rings were pre-contracted with 10 µM phenylephrine, followed by the cumulative addition of the total extract and fractions I, III, and IV. Only the vehicle was added in the time control group. The decreases in tension following each addition were noted by a sensitive isometric force transducer linked to a computer running Labchart v.8 software (ADInstruments, Australia). Endothelial tissues were mechanically denuded for additional analyses to examine the effect on vasodilation.

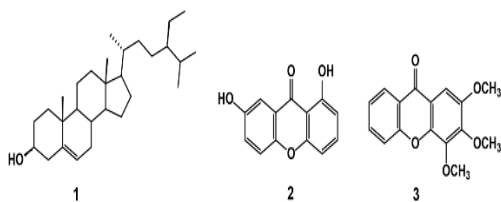
### Statistical analysis

The obtained data were recorded as the mean ± standard error of the mean (SEM) by two-way ANOVA (analysis of variance) followed by Dunnett's post hoc test. The analysis was carried out using GraphPad Prism software with a defined  $\alpha$  of 0.05.

## RESULTS

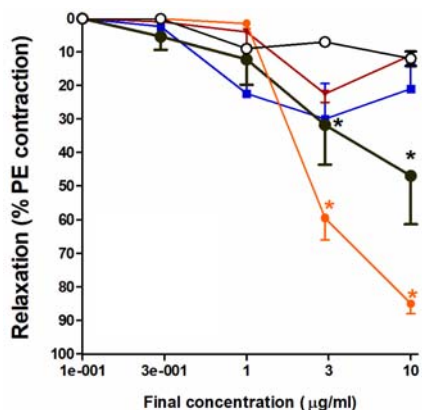
Chemical examination of the *H. revolutum* chloroform fraction resulted the separation of three known compounds (**1-3**) (Figure 1). Their structures were confirmed by spectroscopic data

( $^{13}\text{C}$  and  $^1\text{H}$  NMR) and comparison with the available literature. The compounds were determined to be  $\beta$ -sitosterol (**1**) [17], 1,7-dihydroxyxanthone (euxanthone) (**2**) [18], and 2,3,4-trimethoxy xanthone (**3**) [19].



**Figure 1:** Isolated compounds (1-3) from *H. revolutum*

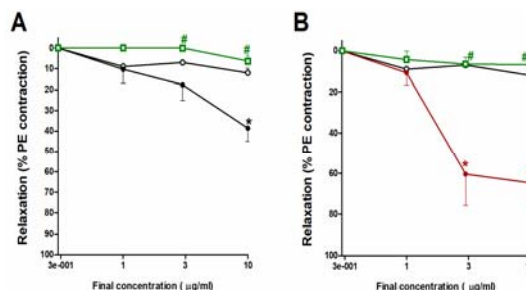
The vasodilating effect of methanol extract and fractions were examined using the isolated artery technique, as previously described [15,16]. Mechanical denudation of endothelial tissues was used for further investigation of the role of the endothelium in the observed vasodilating effect. Total methanol extract (3 and 10  $\mu\text{g}/\text{mL}$ ) caused a significant decrease in tension ( $p < 0.05$ ) and the subsequent vasodilation of phenylephrine (PE,  $10^{-6.5}$  M) pre-contracted aorta in a dose-reliant manner (Figure 2).



**Figure 2:** Effects of the total extract  $\bullet$  and fractions I  $\blacktriangle$ , III  $\blacksquare$  and VI  $\blacktriangledown$  of *H. revolutum* on isolated aorta pre-contracted with phenylephrine (PE) compared with the time control  $\circ$ . Results are expressed as the mean  $\pm$  standard error of the mean (SEM,  $n = 8$ ). \*Significantly varies from the corresponding time control values ( $p < 0.05$ ) by two-way repeated measures ANOVA and the Newman-Keuls post-hoc test

Bio-guided fractionation signified that chloroform fraction (fraction I) caused the vasodilating effect of the total extract because fraction I addition (3 and 10  $\mu\text{g}/\text{mL}$ ) led to significant ( $p < 0.05$ ) vasodilation, similar to that of the total methanol extract. Neither fraction III nor VI produced a significant effect (Figure 2). Removal of the

endothelial layer by aorta denudation remarkably decreased the vasodilating effect of *H. revolutum* total extract (3 and 10  $\mu\text{g}/\text{mL}$ ) ( $p < 0.05$ ) (Figure 3 A). Likewise, the vasodilating impact of fraction I (3 and 10  $\mu\text{g}/\text{mL}$ ) was also blocked by endothelial denudation ( $p < 0.05$ , Figure 3 B).



**Figure 3:** Effects of the total extract of *H. revolutum*  $\bullet$  (A) and fraction I  $\blacktriangle$  (B) on isolated endothelium-denuded and normal aorta  $\square$  compared with the time control  $\circ$ . Results were expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n=8$ ). \*Significantly varies from the corresponding time control values ( $p < 0.05$ ). #Significantly varies from the corresponding fraction I or total extract values ( $p < 0.05$ ) by two-way ANOVA and the Newman-Keuls post-hoc test

## DISCUSSION

Hypertension is a worldwide problem that is correlated with diabetes, obesity, and metabolic syndrome. Moreover, hypertension is the main cause of death due to the development of various cardiovascular diseases [4]. This is because vascular destruction occurs as a result of oxidative stress and hyperglycaemia [5,6]. In this regard, vasodilators are an important class of anti-hypertensives to treat coronary artery disease and vascular insufficiency [7]. Conventional vasodilators often show different side effects. Therefore, there is an urgent need for new bioactive anti-hypertensives with few or negligible side effects. Many medicinal plants have been examined for their vasodilating effects; however, only a few of them have shown pronounced activity [20].

The current study is the first to show that the *H. revolutum* total methanol extract induces a concentration-dependent vasodilation in phenylephrine pre-contracted isolated aortas. Bio-guided fractionation has demonstrated that the chloroform fraction is accountable for the vasodilation activity of the total extract. Similar vasodilation activity has been cited for other plant extracts, including the methanol extracts of *Garcinia mangostana* and *Mentha longifolia*, which have been reported to produce direct vasorelaxation effects in response to phenylephrine-induced vasoconstriction and in

an experimental model of angina, respectively [21,22]. The observed vasodilating effect of the plant extract may be due to its major bioactive components, and a review of the data has revealed that this effect may be attributed to the presence of xanthenes and  $\beta$ -sitosterol.

Xanthenes are reported to have potent anti-hypertensive activity through a calcium channel blocking mechanism [23]. It has been reported that 1,7-dihydroxyxanthone (euxanthone) (2) produces vasodilating effects through the repression of a calcium-sensitive mechanism accelerated by protein kinase C [24]. Also,  $\beta$ -sitosterol (1) has been shown to ameliorate hypertension in rats through the restoration of basal liver and kidney functions [25]. Further studies are required to isolate other major compounds that may contribute to this vasodilating effect. In addition, a detailed mechanism of action for each of the isolated compounds will be considered for further publications.

## CONCLUSION

The methanol extract from the aerial parts of *H. revolutum* exerts a significant vasodilating effect in a concentration dependent manner in rats. The vasodilating activity is endothelial-dependent and is attributed to the presence of xanthenes and  $\beta$ -sitosterol, which have previously been investigated as vasorelaxants. These findings suggest that *H. revolutum* has potentials for treating hypertension; however, additional toxicological and clinical studies are warranted.

## DECLARATIONS

### Acknowledgement

This project was funded by King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia (no. P-S 1928-37).

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by all the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The contribution of authors is given below for each role using their initials: conceptualization, Hossam M Abdallah and Ali M El-Halawany;

methodology, Noha Z Timraz, and Hany M El-Bassossy; validation, Noha Z Timraz, and Hany M El-Bassossy; formal analysis, Noha Z Timraz, and Sabrin RM Ibrahim; investigation, Noha Z Timraz, and Sabrin RM Ibrahim; resources, Hossam M Abdallah and Omar S Aljohani; data curation, Noha Z Timraz and Hossam M Abdallah; writing—original draft preparation, NT and HB; writing—review and editing, Hossam M Abdallah, Ali M El-Halawany, Sabrin RM Ibrahim and Ibrahim A Shehata; visualization, Noha Z Timraz, and Hany M El-Bassossy; supervision, Hossam M Abdallah, Ali M El-Halawany and Ibrahim A Shehata; project administration, Hossam M Abdallah.

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