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MONITORING OF THE HYPOTENSIVE EFFECT OF *HIBISCUS SABDARIFFA* EXTRACT USING DIFFERENT EXTRACTION CONDITIONS

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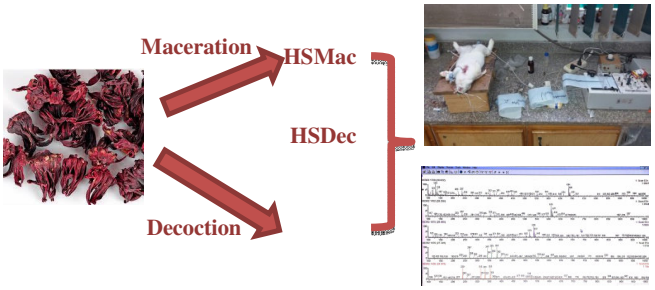
Hypertension management poses a challenge in developing countries, where poverty, illiteracy and poor nutrition combine to exacerbate the condition. The study was carried out to examine the active constituents of aqueous extract of *Hibiscus sabbdariffa* calyces' using LC/MS, together with evaluation of its *in-vivo* hypotensive properties upon using two different techniques in extraction. The obtained LC/MS chromatograms revealed the presence of difference in the constitution of both extracts HSMac, extracted with maceration, and HSDec extracted with decoction. The oscillographic technique was employed on a live rabbit to evaluate the *in-vivo* antihypertensive properties of the extract. In the *in-vivo* studies, the HSMac extract moderately reduced the mean arterial blood pressure (mmHg) of an anaesthesized normotensive rabbit from a control value of 110 (when normal saline was administered) to 90 at does 1g/kg, while the HSDec extract didn't shown any significance change on the mean arterial blood pressure. These findings corroborate the use of macerated *H. sabdariffa* calyces' extract as an antihypertensive agent in ethno-medicine.



INTRODUCTION

Cardiovascular diseases are the major cause of death worldwide. Hypertension is considered as one of the major health problems in the developing countries[1]. Hence, the detection of hypertension and blood pressure control are critically important for reducing the risk of heart attacks and strokes[2].

Blood pressure can be controlled by several mechanisms, using different lines of treatments. However, antihypertensive drugs have many side-effects as reduced renal functions, dry cough and angioedema. Therefore, researchers were motivated to find new medicines in metabolites or extracts from medicinal plants to control hypertension. Recently, several ethnobotanical studies showed that hundreds of plants are used worldwide for empirical hypertension treatment with larger safety margin and less side effects[3].

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Hibiscus sabdariffa, in the folk medicine, considered as one of the non-pharmacological treatments, in which the calyces' aqueous infusion used in treatment of several conditions including hypertension[4]. The reported possible mechanism underlying the hypotensive effect of *H. sabdariffa* relies mainly on its vasodilatation effect. It was reported that anthocyanins and proanthocyanidin compounds detected in abundance in aqueous infusion of the hibiscus calyces, could be the bioactive compounds responsible for the lowering of the blood pressure through the inhibition of the angiotensin converting enzymes II and hence a vasodilatation effect, in addition to the diuretic effect and the increased concentration of urinary sodium while maintaining normal potassium levels[4]. Also, Ajay demonstrated in his study the vasodilatation effect in the isolated aortas of the spontaneously hypertensive rats via endothelium dependent and independent vasodilator pathway[5]. Our study aimed to spot the best way for the extraction of *H. sabdariffa* calyces'active constitutes which demonstrate the most efficient hypotensive action on human being.

MATERIAL AND METHODS

Preparation plant extract

Calyces of *Hibiscus sabdariffa* were obtained from herbarium of Pharmcognosy department, Faculty of Pharmacy, Assiut University, Assiut, Egypt. The samples were identified and authenticated by late Prof. Dr. Ahmed El-Moghazy, Professor of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt. About 400 g of the obtained samples were properly cleaned from any external contaminants and then crushed into fine powder. The powder was divided into two equal portions (200 g each) in which both portions were extracted using distilled water as a solvent. However, the first portion was extracted by maceration and the obtained extract was denoted HSMac, while the second portion was extracted by decoction and its extract was denoted as HSDec.

LC/MS

LC/MS analysis was carried out using HPLC Agilent 1200 series instrument (Santa Clara, CA) consisting of a degasser, binary pump, auto sampler, and column heater. The chromatographic separation was held on Gemini 3 µm C18 110 Å from Phenomenex with dimensions 100 × 1 mm i.d., protected with RP C18 100 Å guard column with dimensions (5 mm × 300 lm i.d., 5 µm). The elution was done isocratically using system 90% MeOH, 2% acetic acid at a flow rate of 50 µL/min. The sample was dissolved in 5% MeOH and 2% acetic acid while the sample injection volume was 10 µL. A Fourier transform ion cyclotron resonance mass analyzer was equipped with an electrospray ionization (ESI) system and controlled by XcaliburVR software (Thermo Electron, San Jose, CA). Detection was performed in the positive ion mode with scan range from *m/z* 100 to 650, applying a capillary voltage of 36 V and a temperature of 275°C. The API source voltage was adjusted to 5 kV, and the desolvation temperature to 275°C.

Antihypertensive activity

Animals

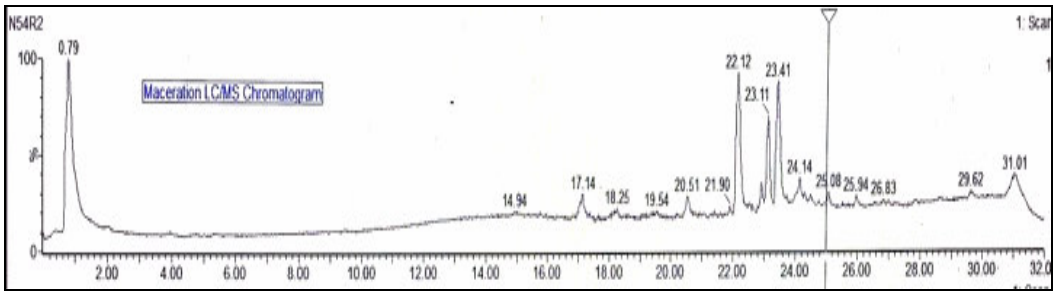
The present study was conducted on about 12 rabbits (~ 1Kg) for the monitoring of the hypotensive activity of *H. sabdariffa*. They were obtained from animal house of Pharmacology Department, Faculty of Medicine, Assiut University. They were housed in conventional cages with free access to water and food at 20-22°C, with a 12-h light/dark cycle. Animals were divided into four groups, 3 rabbits each. Group I includes negative control animals treated with saline, group II includes animals treated with HSMac, group III includes animals treated with HSDec and group IV treated with positive control Capotril® (EIPICO, Cairo, Egypt).

In-vivo studies of *H. sabdariffa* extract

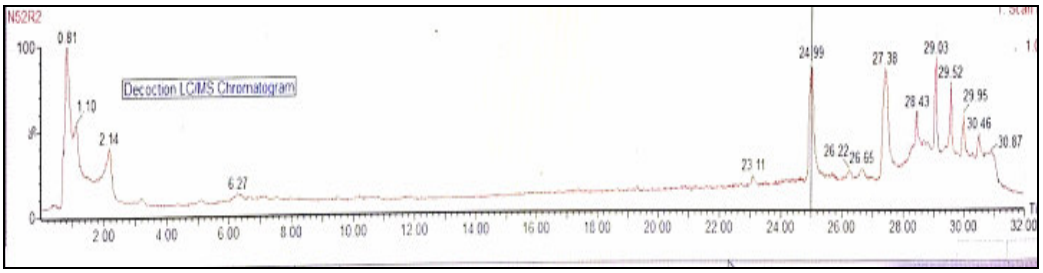
Rabbits were anaesthetized with a 5 mL/kg using urethane (10%) given intraperitoneally and the carotid artery was exposed and intubated with a cannula and used to record the changes in blood pressure. The arterial blood pressure was monitored from the carotid via an arterial cannula connected to a pressure transducer coupled to an oscillograph (Harvard Apparatus Ltd, Kent, England) as described by Niazmand, *et al.*[6].

RESULTS AND DISCUSSION

The LC/MS chromatograms of HSMac and HSDec revealed the presence of difference between both extracts as shown in figure 1a & 1b, which emphasize the difference of the chemical constitution of both extracts. In addition, both extracts showed different activity in the *in-vivo* hypotensive study, in which the i.p. injection of HSMac extract with a dose 1g/kg led to moderate decrease in mean arterial blood pressure of an anaesthesized normotensive rabbit from a control value of 110 mmHg (when normal saline was administered) to 90 mmHg at dose 1g/kg after 15 min. from the injection and continue to decrease for over an hour after injection (Fig. 2a & 2c), while the HSDec extract didn't shown any significance change on the mean arterial blood pressure (Fig. 2b), in comparison to the Capotril®, the positive control used, which reduced the mean arterial blood pressure of normotensive rabbit to 60 mmHg after 15 mins. from injection as shown in figure 2c.



(a)



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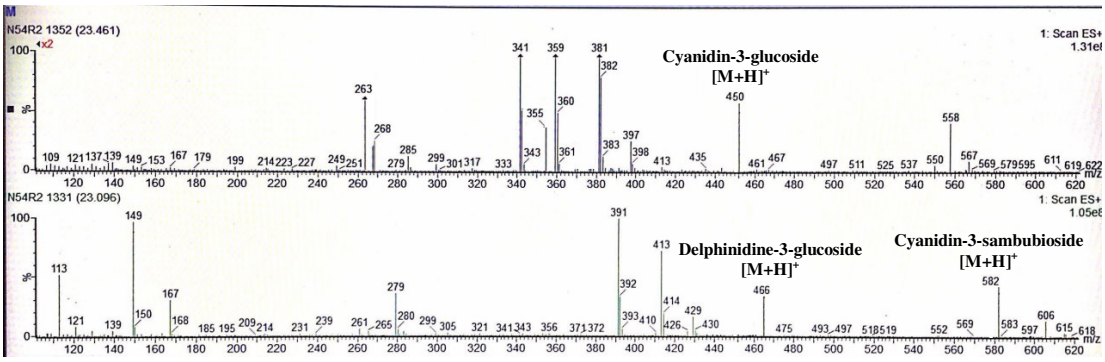
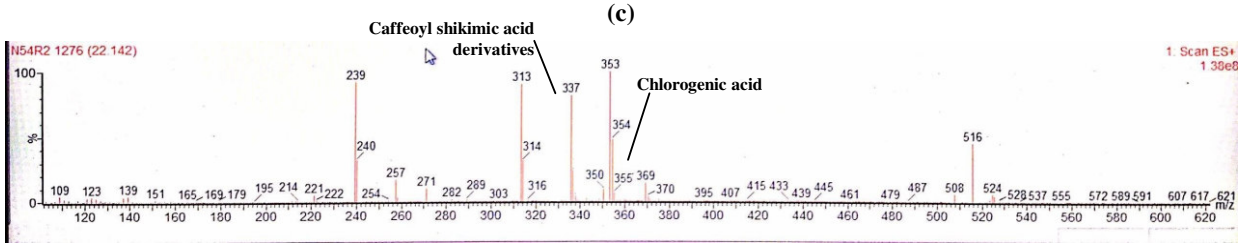


(c)



(d)

Fig. 1: a) LC/MS chromatogram of HSMac extract b) LC/MS chromatogram of HSDec extract
c) Mass spectra of the major peaks in HSMac extract d) Mass spectra of the major peaks in HSDes extract

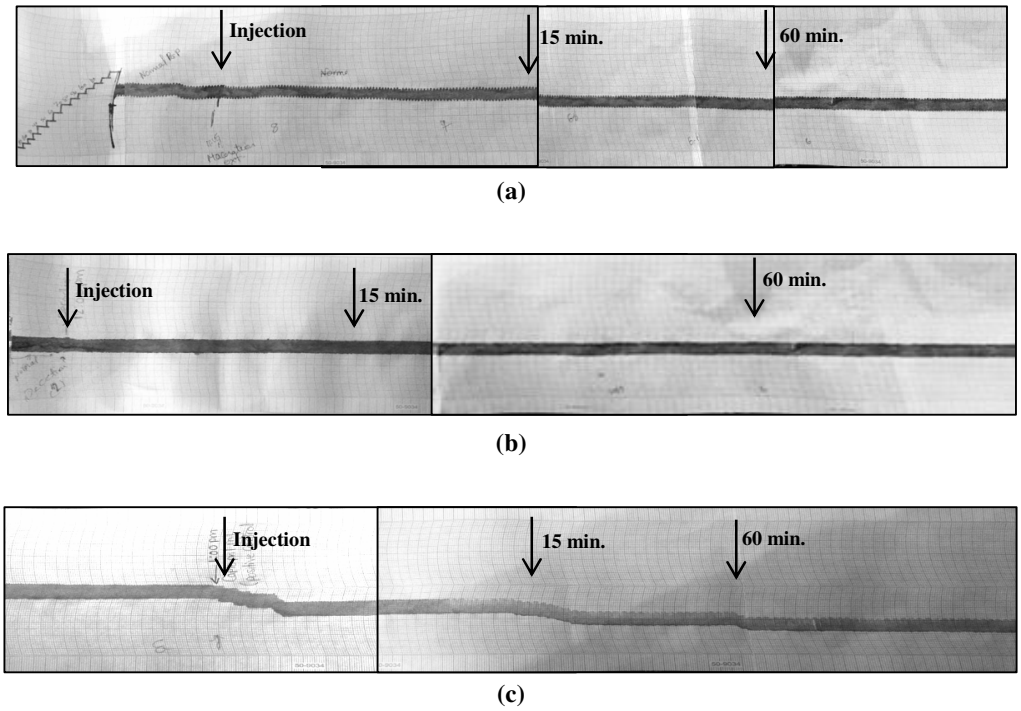


Fig. 2: One centimeter (1cm) of the standard ruler of the recorder corresponds to 10 mmHg pressure change in glass sphygmomanometer. On the tracing the values from the baseline to the lowest border of the tracing represent the diastolic pressure while from the baseline to upper border represent the systolic pressure, (a) Hypotensive effect of HSMac extract, (b) Hypotensive effect of HSDes extract, and (c) Hyptotensive effect of captopril (positive control).

The obtained results recommends the possibility of change in the anthocyanins content in HSDec due to its degradation upon the prolonged application of heat leading to less hypotensive action comparable to HSMac which was obtained without heating. This assumption was confirmed by inspection of LC/MS chromatograms of both extracts which show difference in their chemical constitution. The MS spectra of HSMac extract (Fig. 1c) show the presence of major peaks characteristic for some anthocyanins which were previously reported from *H. sabddariffa* extract[7]. At 23.09 min, $[M+H]^+$ ion peak found at *m/z* 450 corresponds to cyanidin-3-*O*-glucoside, while at 23.46, the $[M+H]^+$ ion peaks at *m/z* 466 and 582 correspond to delphinidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside. On the other hand, MS spectra of HSDec revealed the absence or the less abundance of these compounds (Fig. 1d). Formerly, Maciel *et al.*[8] concluded from his study that the temperature of 60°C and time of 20 min were the best conditions for the extraction of the highest content of anthocyanins, while raising the temperature between 80°C and 100°C lead to a marked increase in the rate of degradation of anthocyanins, which support our results.

Conclusion and prospectus work

Our study shed the light on the better method of extraction that should be used while extracting calyces of *H. sabdariffa.* It revealed that applying of high temperatures during extraction may result in alteration of the extract constitution and consequently will affect the hypotensive effect of the extract. Our prospectus work will include more deep characterization for the extract constituents, quantitative analysis of the anthocyanin content, studying the hypotensive action of the extract on humans through the incorporation of the extract in an appropriate dosage form.

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مراقبة التأثير الخافض للضغط لمستخلص الكركديه باستخدام طرق الإستخلاص المختلفة

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يعتبر مرض إرتفاع ضغط الدم من أكثر الأمراض إنتشاراً وخاصتاً في الدول النامية حيث للفقر وسوء التغذية. ومن ثم ، فإن الكشف عن أدوية لعلاج والتحكم في ارتفاع ضغط الدم يعتبر تحدياً وذلك للتقليل من مخاطر الإصابة بالنوبات القلبية والسكتات الدماغية. ولذلك أجريت هذه الدراسة لفحص تأثير الحرارة على المستخلص المائي لكؤوس زهرة الكركديه الذي تم تحضيره بطريقتي النقع والإغلاء. حيث تم فحص المواد الفعاله الموجودة بالمستخلصان باستخدام تقنية ال LC/MS ، إلى جانب تقييم قدرة المستخلصان على خفض الضغط. كشفت الدراسة عن وجود اختلاف في تكوين كل من المستخلصين; HSMac المستخلصين بالنقع ، و HSDec المستخلص بالغلاء وأرجأ ذلك لاختلاف الحرارة المستخدمة لتحضير كلا من المستخلصين. كما اوضحت ايضا الدراسه فعالية مستخلص HSMac على خفض متوسط ضغط الدم الشرياني باستخدام تقنية رسم الذبذبات.