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In Vitro ACE Inhibitory Activity and Phytochemical Characterization of Hibiscus sabdariffa and Cucumis sativus Infusions

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Abstract

Hibiscus sabdariffa and Cucumis sativus are commonly employed in traditional medicine to manage various ailments, including high blood pressure. This research sought to evaluate the in vitro ACE-inhibitory effects of infusions derived from H. sabdariffa and C. sativus, along with their phytochemical profiles. Infusions were made using 10, 20, and 30 grams of each plant material in 300 ml of hot distilled water. Mixtures of the two plants were additionally prepared in 1:1, 2:1, and 1:2 ratios. ACE inhibition was assessed via a colorimetric assay. Findings revealed that the maximum inhibition achieved by H. sabdariffa and C. sativus infusions reached 88.741±0.001% and 92.180±0.001%, respectively. The combination yielding the greatest inhibition was the H. sabdariffa: C. sativus ratio of 1:2, at 96.062±0.001%, though this remained lower than that of Captopril (97.393±0.001%). Phytochemical analysis showed that H. sabdariffa infusions contained saponins and tannins, whereas C. sativus infusions possessed alkaloids, saponins, and terpenoids. The study suggests that the 1:2 ratio infusion of H. sabdariffa and C. sativus exhibited the most potent ACE inhibition and may serve as a potential functional beverage for hypertension control.

Keywords: Antihypertensive, Cucumber, Roselle, ACE, Infusion

Introduction

Individuals diagnosed with hypertension typically need pharmacological intervention to regulate blood pressure and sustain healthy levels [1]. A major category of antihypertensive medications comprises angiotensin-converting enzyme (ACE) inhibitors, which function by blocking the transformation of angiotensin-I into angiotensin-II, a potent vasoconstrictor responsible for elevating blood pressure [2, 3]. Nevertheless, these medications present challenges, as dosages must be tailored to individual patient profiles, particularly in cases involving contraindications [1]. Moreover, prolonged administration of synthetic agents may lead to adverse reactions, including allergic responses, pruritus, low blood pressure, and infections of the upper respiratory tract [4].

Traditional herbal options frequently utilized for hypertension management include Hibiscus sabdariffa and Cucumis sativus [5-7]. Earlier investigations have identified that H. sabdariffa is rich in anthocyanins, flavonoids, phenolics, glycosides, tannins, alkaloids, terpenoids, vitamin C, and organic acids [8-10], conferring properties such as antioxidant [11, 12], antibacterial [9], antidiabetic [13], anti-inflammatory and immunomodulatory [14], and anticancer effects [15]. Additional studies indicate that H. sabdariffa aids in reducing cholesterol [16, 17], alleviating obesity [18], decreasing blood pressure [19-22], and enhancing renal function in hypertensive patients [23].

In parallel, C. sativus is abundant in phenolic compounds, flavonoids, and vitamin C [24, 25], as well as cucurbitacins (a type of terpenoid) [26, 27], providing benefits including antibacterial and anticancer activity [28], anti-inflammatory action [29, 30], antihypertensive

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potential [7], promotion of wound healing [31], and pain relief in osteoarthritis [32].

A key biological attribute of the phytochemicals in both H. sabdariffa and C. sativus is their capacity to suppress ACE activity [2, 7, 33]. Prior research has evaluated ACE inhibition primarily through decoctions or infusions [22, 23], yet the concentrations applied varied considerably, resulting in inconsistent therapeutic outcomes [34]. Notably, direct assessment of ACE-inhibitory potential from infusions of these plants has not been previously reported. Consequently, the objective of this investigation was to examine the ACE-inhibitory effects and phytochemical composition of infusions prepared from H. sabdariffa and C. sativus.

Materials and Methods

Assay kits, chemicals, and instrumentation

The ACE inhibition assay employed the ACE kit-WST from Dojindo Laboratories, Japan. Captopril, serving as the positive control, was sourced from Kimia Farma, Indonesia. All reagents were of analytical grade. Absorbance measurements for inhibition activity were conducted using a Multiskan FC Microplate Reader (Thermo Scientific).

Collection and identification of plant materials

Dried calyces of H. sabdariffa were acquired from Indo Herbal in Tangerang, Indonesia, during March to June 2022. Fresh C. sativus fruits were obtained in July 2022 from a local market in Tangerang, Indonesia. Authentication of both materials was performed by Dr. Lamijan at Balai Materia Medica, Batu, Indonesia, assigning voucher numbers 074/713/102.20-A/2022 for H. sabdariffa and 074/712/102.20-A/2022 for C. sativus.

Preparation of plant materials

Dried H. sabdariffa calyces were cleaned under running water to eliminate impurities and oven-dried. The material was then pulverized into coarse powder with an electric blender and stored in a sealed container. Fresh C. sativus fruits were rinsed with tap water and diced into approximately 5x5 mm cubes to facilitate greater release of bioactive compounds during infusion.

Preparation of infusions

H. sabdariffa powder (HS) was measured at 10, 20, and 30 grams and placed in conical beakers, labeled HS1, HS2, and HS3. Cut C. sativus (CS) portions were prepared similarly and designated CS1, CS2, and CS3. Combined samples totaling 30 grams were formulated in 1:1, 2:1, and 1:2 ratios (H. sabdariffa to C. sativus) and coded HC1, HC2, and HC3. Each preparation was infused with 300 ml of distilled water heated to 90°C, gently agitated to ensure complete submersion, and allowed to steep for 1 hour at room temperature. Resulting infusions were filtered through Whatman no. 1 paper (125 mm), collected in plastic containers, and stored at 4°C prior to testing.

Phytochemical screening

Infusions were tested for the presence of flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids using adapted procedures based on Harborne's methods [35].

Determination of ACE inhibitory activities

ACE inhibition was quantified colorimetrically with the ACE kit-WST [36], adhering to the manufacturer's protocol [37]. A 25 ppm solution of Captopril was used as the positive control. Absorbance was recorded at 450 nm on a Thermo Multiskan FC Microplate Reader, with five replicates per sample. Percentage inhibition was computed according to the kit's specified formula.

Statistical analysis

Experimental results were expressed as mean ± standard deviation (SD). Data analysis was carried out using Microsoft Excel (2019 edition).

Results and Discussion

Phytochemical composition of the infusions

Qualitative tests for secondary metabolites were performed to identify the occurrence of flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids across the various infusions.

The secondary metabolite profiles varied among the aqueous infusions **(Table 1)**. Neither flavonoids nor steroids were identified in any of the preparations. Conversely, saponins appeared in every infusion, confirming their effective extraction from both H. sabdariffa and C. sativus. Alkaloids were undetectable in all HS infusions yet were found in every CS and HC infusion, indicating successful aqueous extraction primarily from C. sativus. Tannins, on the other hand, were present in all HS and HC infusions but missing from CS infusions, pointing to H. sabdariffa as their origin.

Terpenoids were detected solely in CS3 and HC3, the preparations with greater proportions of C. sativus. The detection of these compounds in the infusions relies on the efficiency of the extraction process, affected by duration and solvent polarity [38-40]. In this study, materials were steeped in hot distilled water for 1 hour, a period that may have been insufficient for complete extraction of certain metabolites. Furthermore, the polar nature of water limits its capacity to dissolve nonpolar or semi-polar compounds due to polarity mismatches. The quantity of plant material used can also influence the yield of extracted metabolites [38-40].

ACE inhibitory activities of the HS, CS, and HC infusions

The potential of plants to combat hypertension can be assessed by measuring their ability to inhibit angiotensin-converting enzyme (ACE). Under normal conditions, ACE catalyzes the formation of angiotensin-II from angiotensin-I, leading to vasoconstriction and elevated blood pressure. Blocking this process is essential for blood pressure regulation [3]. Inhibitory effects of the HS, CS, and HC infusions on ACE were measured using the ACE kit-WST (Dojindo Laboratories, Japan). The method relies on quantifying 3-hydroxybutyric acid (3HB) released from the substrate 3-hydroxybutyrylglycylglycylglycine (3HB-GGG) through enzymatic action of ACE followed by aminoacylase [36, 37]. Compounds with inhibitory properties reduce 3HB production, resulting in lower absorbance and correspondingly higher calculated inhibition percentages.

As illustrated in **Figure 1**, ACE inhibition by both HS and CS infusions rose with increasing plant material concentration. The maximum inhibition for H. sabdariffa infusion, achieved at 30 g/300 ml, was 88.741±0.001%. For C. sativus infusion at the identical concentration, the peak value reached 92.180±0.001%. Overall, CS infusions exhibited stronger inhibition than HS infusions at equivalent concentrations. Combined infusions of H. sabdariffa and C. sativus were also evaluated. **Figure 2** indicates that HC3, prepared in an HS:CS ratio of 1:2, displayed the greatest inhibition among combinations at 96.062±0.001%, although this remained slightly less than the reference standard Captopril (97.393±0.001%). Combining the two plants evidently enhanced ACE inhibition compared to individual infusions. The observed effects can be attributed to the detected phytochemicals. The superior performance of C. sativus infusions may stem from its alkaloids, saponins, and terpenoids, while that of H. sabdariffa infusions likely relates to saponins and tannins. Earlier research has established that such compounds possess ACE-inhibitory capabilities and contribute to blood pressure reduction [7, 27, 41-44].

Table 1. Phytochemical contents of all infusions

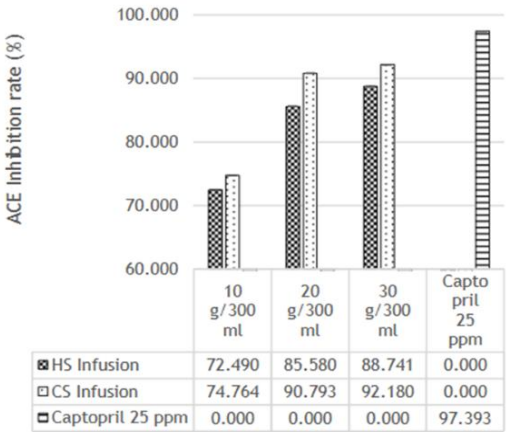
	HS Infusions			CS Infusions			HC Infusions		
Secondary metabolites									
	HS1	**HS2**	**HS3**	**CS1**	**CS2**	**CS3**	**HC1**	**HC2**	**HC3**
Flavonoids	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	-	-	-	+	+	+
Steroids	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	+	-	-	+

Note: 1) HS: H. sabdariffa, CS: C. sativus, HC: combination of HS and CS; 2) +: present; -: absent

Beyond the outcomes of phytochemical testing, preparations incorporating H. sabdariffa exhibited a distinctive hue attributable to anthocyanins. The presence of anthocyanins in these infusions was evidenced by the deep red-purple tint of the resulting aqueous extracts. Compounds like anthocyanins and flavonoids can suppress ACE function by creating chelate bonds with the zinc ion located at the enzyme's active site [33, 45]. These bonds diminish the enzyme's ability to interact with its substrate, thereby preventing the transformation of angiotensin-I into angiotensin-II [2]. Moreover, phenolic acids and anthocyanins found in H. sabdariffa promote vascular relaxation, contributing to reduced blood pressure [46].

The current investigation demonstrated that infusions derived from H. sabdariffa, C. sativus, and their mixtures

exhibited substantial ACE-inhibitory potential. Of all the tested preparations, HC3 displayed the most pronounced inhibition, although Captopril continued to outperform every infusion in terms of efficacy. Nonetheless, HC3 represents a promising option for individuals managing hypertension through non-drug approaches. Additionally, the preparations employed here were deemed safe, as all concentrations remained well under the median lethal dose for H. sabdariffa, reported as >5000 mg/kg body weight [5, 22, 23], while C. sativus shows no concentration-related adverse effects [30].



	10 g/300 ml	20 g/300 ml	30 g/300 ml	Captopril 25 ppm
HS Infusion	72.490	85.580	88.741	0.000
CS Infusion	74.764	90.793	92.180	0.000
Captopril 25 ppm	0.000	0.000	0.000	97.393

Figure 1. ACE inhibitory activities of HS and CS infusions

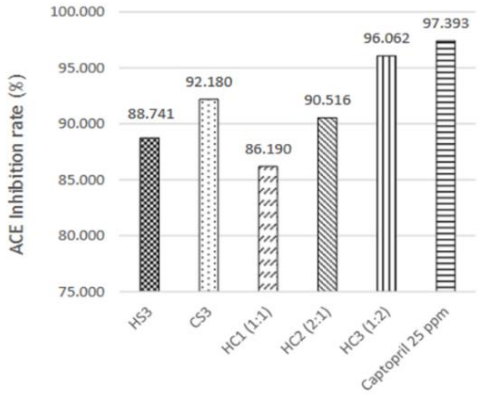


Figure 2. ACE inhibitory activities of combination infusions compared to HS3 and CS3

Conclusion

This research established that aqueous infusions from H. sabdariffa, C. sativus, and their blended forms possess significant capacity to inhibit ACE. The most effective inhibition was observed in the HC3 preparation, approaching the level achieved by Captopril. Phytochemical assessment verified the presence of saponins and tannins in H. sabdariffa infusions, alkaloids, saponins, and terpenoids in C. sativus infusions, and a combination of all these compounds in the mixed infusions. Given that the amounts of H. sabdariffa applied were considerably lower than its median lethal dose and C. sativus exhibits no dose-related toxicity, the HC3 infusion holds potential as a functional beverage for hypertension management.

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Conflict of Interest: None

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