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Original Research Article

Phytochemical Composition and Inhibitory Activities of *Hibiscus sabdariffa* and *Cucumis sativus* Infusions Against Angiotensin-Converting Enzyme

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ABSTRACT

Hibiscus sabdariffa and *Cucumis sativus* are often used in ethnomedicinal practice for treating several health conditions, including hypertension. The present study aimed to investigate the *in vitro* inhibitory activities of *H. sabdariffa* and *C. sativus* against angiotensin-converting enzyme (ACE) and their phytochemical properties directly from their infusions. Each infusion was prepared with a concentration of 10, 20, and 30 grams of plant material in 300 ml of hot distilled water. Combinations of both plants were also prepared in 1:1, 2:1, and 1:2 ratios. The inhibitory activities were determined by the colorimetric method. The results showed that the highest inhibition rates of *H. sabdariffa* and *C. sativus* infusions were 88.741±0.001% and 92.180±0.001%, respectively. Meanwhile, the highest inhibition rate of the combination infusion was obtained from the ratio of *H. sabdariffa*: *C. sativus* (1:2), which was 96.062±0.001%, although this result was still below the inhibitory activity of Captopril (97.393±0.001%). The phytochemical screening results indicated that *H. sabdariffa* infusions contain saponins and tannins, and *C. sativus* infusions contain alkaloids, saponins, and terpenoids. This study concluded that the infusion with the ratio of *H. sabdariffa: C. sativus* (1:2) demonstrated the strongest inhibitory activity against ACE and, therefore, could potentially be utilized as a functional drink for managing hypertension.

Keywords: antihypertensive, cucumber, roselle, ACE, infusion

Introduction

Patients with hypertension require drug therapy to control their blood pressure and maintain normal conditions.[1] Several drugs used for treating hypertension belong to the angiotensin-converting enzyme (ACE) inhibitor class, which work by inhibiting the conversion of angiotensin-I to angiotensin-II, a vasopressor that triggers an increase in blood pressure.[2,3] However, antihypertensive drugs have limitations because they must be adjusted to the patient's health condition, especially those with contraindications.[1] In addition, the long-term use of synthetic drugs can cause undesired side effects, such as hypersensitivity reactions, itching, hypotension, and upper respiratory tract infections.[4]

Ethnomedicinal alternatives widely used for treating hypertension are *Hibiscus sabdariffa* and *Cucumis sativus*.[5-7] Previous studies showed that *H. sabdariffa* contains anthocyanins, flavonoids, phenolic compounds, glycosides, tannins, alkaloids, terpenoids, vitamin C, and organic acids,[8-10] which can be useful as antioxidants,[11,12] antibacterials,[9] antidiabetics,[13] anti-inflammatory agents and immunomodulators,[14] and anticancer agents.[15] Previous research also showed that *H. sabdariffa* helps lower cholesterol levels,[16,17] reduce obesity,[18] lower blood pressure,[19-22] and improve kidney function in people with hypertension.[23]

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Meanwhile, *C. sativus* contains phenolic compounds, flavonoids, and vitamin C,[24,25] and is rich in cucurbitacins (terpenoids),[26,27] which can be useful as antibacterial and anticancer,[28] anti-inflammatory,[29,30] and antihypertensive agents,[7] as well as helping wound healing,[31] and reducing pain in osteoarthritis.[32]

One of the physiological properties of the phytochemicals contained in *H. sabdariffa* and *C. sativus* is their ability to inhibit ACE.[2,7,33] In several studies, observations of ACE-inhibitory activity were carried out by initially extracting the plants in the form of decoction/infusion,[22,23] but the doses used were still varied, so the therapeutic effects observed were still inconsistent.[34] Furthermore, no previous study has directly measured the ACE inhibitory activities of *H. sabdariffa* and *C. sativus* from their infusions. Thus, this study aimed to determine the inhibitory activities of *H. sabdariffa* and *C. sativus* infusions against ACE and their phytochemical properties.

Materials and Methods

Assay kits, chemicals, and instrumentation

The ACE inhibition kit (ACE kit-WST) used was produced by Dojindo Laboratories, Japan. Captopril as a positive control was manufactured by Kimia Farma, Indonesia. All chemical reagents used were of analytical grade. Multiskan FC Microplate Reader (Thermo Scientific) was used to measure the inhibitory activities of the infusions.

Collection and identification of plant materials

Dried *H. sabdariffa* calyces were purchased from Indo Herbal, Tangerang, Indonesia, between March and June 2022. Fruits of *C. sativus* were collected in July 2022 from a traditional market in Tangerang, Indonesia. Both plant materials were then identified and authenticated by Dr. Lamijan of the Balai Materia Medica, Batu, Indonesia, with voucher specimen numbers 074/713/102.20-A/2022 (*H. sabdariffa*) and 074/712/102.20-A/2022 (*C. sativus*).

Preparation of plant materials
The dried calyces of *H. sabdariffa* were rinsed under running tap water to remove contaminants and then dried in an oven. Dried calyces were ground into a coarse powder using an electric blender. Calyces powder was stored in a clean container for infusion. Fresh *C. sativus* fruits were washed with tap water and cut into cuboid shapes of about 5x5 mm to obtain more active substances that diffused during the infusion.

Preparation of infusions
Powdered *H. sabdariffa* (HS) was weighed at 10, 20, and 30 grams each and put into conical Beakers, coded with HS1, HS2, and HS3. Freshly cut *C. sativus* (CS) was weighed in a similar procedure, and the samples were coded with CS1, CS2, and CS3. The combination of *H. sabdariffa* and *C. sativus* with a ratio of 1:1, 2:1, and 1:2 (with a total of 30 grams each) were also prepared and coded with HC1, HC2, and HC3. Every sample was steeped with 300 ml of hot distilled water (90^0C) and shaken gently until all plant materials were soaked. All mixtures were left at room temperature and were kept in contact for 1 hour to obtain infusions. The infusions were then filtered with Whatman no. 1 filter paper (125 mm). The filtrates were stored in clean plastic containers and refrigerated at 4^0C before analysis.

Phytochemical screening
The samples were screened for phytochemical compounds: flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids. The screening followed modified methods adapted from Harborne.[35]

Determination of ACE inhibitory activities
The ACE-inhibitory activities of all infusions were determined by the colorimetric method using the ACE kit-WST,[36] following the manufacturer's technical manual.[37] Captopril (25 ppm) was prepared as a positive control. The absorbance was read at 450 nm using a Thermo Multiskan FC Microplate Reader, and each sample was measured in 5 replicates. The inhibitory activity (%) of each sample was calculated using the formula written in the manual.

Statistical analysis
All data obtained from the experiment were presented as a mean±standard deviation (SD). The statistical analysis was performed with Microsoft Excel (2019 edition).

Results and Discussion

Phytochemical composition of the infusions
Qualitative analyses of secondary metabolites were conducted to detect the presence of flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids in each infusion.
The phytochemical composition detected in all aqueous infusions varied (Table 1). Flavonoids and steroids were not detectable in all infusions. In contrast, saponins were found in all infusions, which indicated that saponins were successfully extracted from *H. sabdariffa* and *C. sativus*, respectively. Alkaloids were absent in all HS infusions but were present in all CS and HC infusions, which suggested that these phytochemicals were effectively extracted from *C. sativus* in an aqueous condition. In comparison, tannins were detectable in all HS and HC infusions but were absent in all CS infusions, which implied that *H. sabdariffa* was the source of these phytochemicals. Meanwhile, terpenoids were only present in CS3 and HC3, which contained higher amounts of *C. sativus*. The presence of secondary metabolites in each infusion depends on the effectiveness of the infusion process, which is influenced by the extraction time and the polarity of the solvent.[38-40] All samples were soaked in hot distilled water for 1 hour, and this process was probably not long enough to extract metabolites from the plant materials. In addition, the solvent used, which was polar, had limited ability to extract nonpolar or semipolar metabolites due to the difference in polarity. The amount of plant material weighed may also affect the metabolites yielded from the extraction.[38-40]

ACE inhibitory activities of the HS, CS, and HC infusions
The antihypertensive potency of a plant can be evaluated through its inhibitory activity against ACE. Naturally, ACE converts angiotensin-I to angiotensin-II, a potent vasopressor, causing an increase in blood pressure. The conversion must be prevented to maintain normal blood pressure.[3] The ACE inhibitory activities of the HS, CS, and HC infusions were determined using the ACE kit-WST (Dojindo Laboratories, Japan). The assay is based on the detection of 3-hydroxybutyric acid (3HB) generated from the cleavage of the substrate 3-hydroxybutyrylglycylglycylglycine (3HBGGG) in the presence of ACE and aminoacylase.[36,37] Samples containing phytochemicals with inhibitory activity will prevent the generation of 3HB. Therefore, the lower the readable absorbance, the higher the measured inhibitory activity.
As shown in Figure 1, the ACE inhibitory activities of HS and CS infusions showed that the inhibition rate increased with increasing concentration. The highest ACE inhibition rate of *H. sabdariffa* infusion was obtained at a concentration of 30 g/300 ml, which was 88.741±0.001%. In comparison, the highest inhibition rate of *C. sativus* infusion at the same concentration was 92.180±0.001%. In general, CS infusions showed higher inhibitory activities than HS infusions at the same concentration. The inhibitory activities of the combined *H. sabdariffa* and *C. sativus* infusions were also observed. Figure 2 shows that the infusion HC3 with a ratio of HS:CS (1:2) gave the highest inhibition rate among all combination infusions, which was 96.062±0.001%, even though it was still below the inhibition rate shown by Captopril (97.393±0.001%). According to the findings, when the HS and CS were combined, the inhibitory activities against ACE increased. The potential biological activities demonstrated by all infusions were due to the presence of phytochemical constituents. The high inhibitory activities of *C. sativus* infusions were likely associated with the presence of alkaloids, saponins, and terpenoids, and the high inhibitory activities of *H. sabdariffa* were possibly due to their metabolites such as saponins and tannins. Previous studies reported that these phytochemical compounds showed inhibitory activities against ACE and could lower blood pressure.[7,27,41-44]

Table 1: Phytochemical contents of all infusions

Secondary metabolites	HS Infusions			CS Infusions			HC Infusions		
	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

Apart from the phytochemical screening results, infusions containing *H. sabdariffa* demonstrated a specific color of anthocyanins. Anthocyanins presented in *H. sabdariffa* infusions were marked by the reddish-purple color of the aqueous solution. Phytochemicals such as anthocyanins and flavonoids could inhibit ACE activity due to the formation of chelate complexes with the zinc atom within the enzyme's active center.[33,45] The complexes caused the enzyme to lose its affinity to bind with the substrate, resulting in no conversion of angiotensin-I to angiotensin-II.[2] In addition, phenolic acids and anthocyanins in *H. sabdariffa* have a vasorelaxant effect, which helps lower blood pressure.[46]

This study showed that the infusions of *H. sabdariffa*, *C. sativus*, and their combinations demonstrated powerful inhibitory effects against ACE. Among all formulations, the infusion HC3 showed the highest inhibitory activity against ACE, even though the effect shown by Captopril remained the strongest compared to all infusions. Nevertheless, the infusion HC3 may be an alternative for hypertensive patients undergoing nonpharmacological treatment. Furthermore, the formulations used in this study were safe since all infusion doses prepared were below the median lethal dose of *H. sabdariffa*, which was >5000 mg/kg body weight,[5,22,23] and *C. sativus* itself has no dose-dependent side effects in its application.[30]

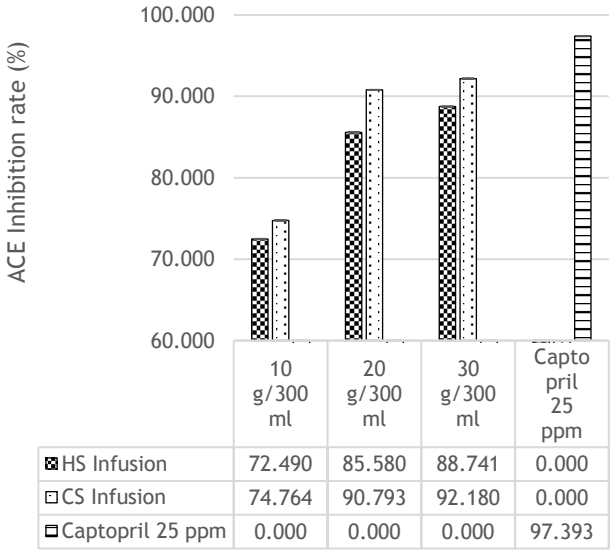

Figure 1: ACE inhibitory activities of HS and CS infusions

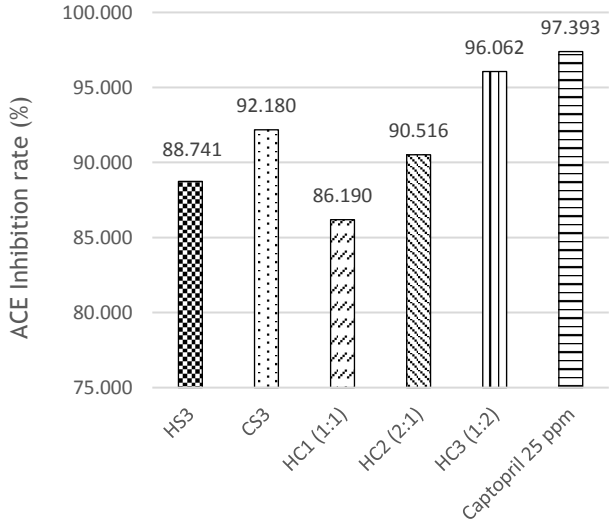

Figure 2: ACE inhibitory activities of combination infusions compared to HS3 and CS3

Conclusion

The findings of this study revealed that the infusions of *H. sabdariffa*, *C. sativus*, and their combination showed potent inhibitory activities against ACE. The strongest inhibitory activity is demonstrated by the infusion HC3, which was close to that of Captopril. The phytochemical evaluation confirmed that *H. sabdariffa* infusions contained saponins and tannins, *C. sativus* infusions contained alkaloids, saponins, and terpenoids, and the combination infusions contained all the phytochemicals that existed in both plants' infusions. Since the doses of *H. sabdariffa* used in this study were far below its median lethal dose and *C. sativus* also has no dose-dependent side effects, the infusion HC3 could potentially be utilized as a functional drink for managing hypertension.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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