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Research Article

Characterization And Antihypertensive Effect of Ginger Extract in Combination with Vincamine Drug

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ABSTRACT

Vincamine is a monoterpenoid indole alkaloid found in the leaves of vincamine minor which acts as a vasodilator in cerebral vascular conditions. Ginger is known to lower blood pressure through ACE inhibition, reduction of inflammation, and vasodilation via binding interaction between the bioactive compounds in red ginger as ligands and ACE protein as receptors. The current research is envisaged to explore and scientifically validate the synergistic antihypertensive potential of *Zingiber officinale* (ginger) extract in combination with vincamine, a well-known peripheral vasodilator alkaloid derived from *Vinca minor*. The in vitro antihypertensive activity was evaluated using the Angiotensin-Converting Enzyme (ACE) inhibition assay, which demonstrated significant enzyme inhibition by both ginger extract and vincamine, with enhanced activity observed in the combination, suggesting a potential synergistic effect. This herbal-drug combination approach may provide an effective, safe, and integrative option for hypertensive therapy, warranting further in vivo and clinical studies.

INTRODUCTION

Hypertension is commonly referred to as the "silent killer" because it usually has no symptoms in its early stages but can lead to life-threatening complications if left untreated. The World Health Organization (WHO) estimates that 1.28 billion persons worldwide suffer with hypertension, a condition that is quite common in the world [1].

The use of medicinal herbs continues to be an alternative treatment approach for several diseases including cardiovascular diseases (CVDs). CVDs are a variety of diseases including peripheral vascular diseases, coronary heart disease (CHD), heart failure, heart attack (myocardial infarction), stroke, cardiomyopathies, dyslipidemias, and hypertension, among others [2]. Currently, there is

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an unprecedented drive for the use of herbal preparations in modern medicinal systems [3]. For ages, herbal remedies have been used extensively in traditional medical systems including Ayurveda, Traditional Chinese Medicine, and Unani to prevent and treat cardiovascular illnesses (CVDs). Since cardiovascular diseases (CVDs) are responsible for over 17.9 million deaths worldwide each year, there is growing interest in using plant-based medicines that provide cardiovascular protection with fewer side effects than traditional medications. Many herbs have been clinically tested for their cardiovascular effects, such as *Camellia sinensis* (green tea), *Terminalia arjuna*, *Zingiber officinale* (ginger), and *Allium sativum* (garlic). For example, ginger has demonstrated ACE-inhibitory and vasodilatory properties, which lower blood pressure and enhance endothelial function [4-6]. Similarly, *vinca minor*'s monoterpenoid indole alkaloid vincamine dilates cerebral and peripheral blood arteries and may be used in conjunction with herbal remedies like ginger to treat hypertension and vascular insufficiency [7]. Ginger contains bioactive compounds such as gingerol, shogaol, and zingerone [8,9]. These compounds have potent anti-inflammatory, antioxidant, and antihypertensive properties [9-12]. Ginger is known to lower blood pressure through ACE inhibition, reduction of inflammation, and vasodilation via binding interaction between the bioactive compounds in red ginger as ligands and ACE protein as receptors [13,14]. Vincamine is a naturally occurring alkaloid primarily derived from the plant *Vinca minor* (commonly known as lesser periwinkle), as well as other species within the Apocynaceae family. Vincamine has gained attention for its pharmacological properties, particularly its use as a vasodilator in cerebral vascular conditions [15]. Vincamine is a monoterpenoid indole alkaloid found in the leaves of *vincamine minor* (lesser periwinkle),

comprising about 25–65% of its indole alkaloids by weight. Together, vincamine and ginger may activate distinct metabolic pathways that result in a more comprehensive and potent vasodilatory response. Ginger may improve systemic circulation vasodilation, but vincamine concentrates on boosting blood flow to the brain. Due to this combined impact, blood pressure may be lowered more effectively without significantly lowering blood pressure in non-cerebral arteries [16]. With both ginger and vincamine increasing nitric oxide availability and reducing oxidative damage to the endothelium, there could be a more pronounced improvement in the regulation of blood vessel tone, leading to enhanced vasodilation and reduced blood pressure. The potential for a synergistic antihypertensive effect between vincamine and ginger extract is not well-established in scientific literature. While both vincamine and ginger have shown antihypertensive properties individually, research specifically focusing on their combined effect is limited. The current research is envisaged to explore and scientifically validate the synergistic antihypertensive potential of *Zingiber officinale* (ginger) extract in combination with vincamine.

2. MATERIALS AND METHODS

Vincamine, was purchased from Sigma-Aldrich with 99.97% purity. Fresh edible rhizomes intact with whole plant of ginger were purchased from a local market in Baddi. All chemicals and solvents were of high-performance liquid chromatography grade. Other required chemicals were acquired from standard commercial chemical suppliers.

2.1. Extraction of Ginger

The collected rhizomes were first cleaned three to four times with tap water to get rid of the dirt, and then twice with deionized water. It was then cut into pieces, allowed to dry at room temperature in



the dark, and then crushed using an electric grinder before being stored for later use. In the Soxhlet apparatus, 80% methanol was used to percolate the powdered ginger rhizomes at 60–65°C. For aqueous extraction distilled water was used to percolate the ginger rhizomes. After being filtered, this percolate was dried off by evaporating it in a water bath that was not over 40°C. The obtained extract was kept at a temperature below 5°C in airtight bottles.

2.2. Phytochemical screening

The study of the phytochemicals for the presence of alkaloids, saponins, flavonoids, tannins, reducing sugars, and cardiac glycosides were carried out according to similar studies with some modifications.

2.2.1. Test for alkaloids: Few drops of Mayer's reagent and few drops of dil. HCl were added to 500 µL of extract. Creamy precipitates show the presence of alkaloids

2.2.2. Test for reducing sugar: 500 µL of Fehling's solution A & B were added to 500 µL of extract and heated for 10 min. Red precipitates show the presence of reducing sugar.

2.2.3. Test for glycosides: 300 µL glacial acetic acid, 1 drop of FeCl₃ (5%) and 100 µL H₂SO₄ (conc.) were added to 500 µL extract. Blue color indicates the presence of glycosides.

2.2.4. Test for tannins: 500 µL of ferric chloride solution was added to 500 µL extract. Blue, green or violet color indicates the presence of tannins.

2.2.5. Test for saponin: 2 mL water was added to 2mL extract and heated. Froth formation confirms the presence of saponin.

2.2.6. Test for flavonoids: Extract was treated with sodium hydroxide solution then dilute acid

was added. Intense yellow color which becomes colorless after some time indicates the presence of flavonoids.

2.3. Characterization techniques

2.3.1. UV-Visible spectroscopy:

UV Spectrophotometer (SHIMADZU 1800) was utilized for this study. Solution of rhizome extract and vincamine were prepared in methanol and scanned between 200 to 400 nm.

2.3.2. FTIR spectroscopy:

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. FTIR Spectrophotometer (Perkin Elmer) was utilized for the study. A small quantity (5 mg) of the extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The sample was scanned from 4000 – 400 cm⁻¹. For the study, HPLC (Waters 1650) was used. IPA was obtained from Alkem Labs. Ltd., Baddi and all chemicals and solvents utilized were of A.R. grade. In a water-bath, 3 g of the coarsely ground material being examined was refluxed with 100 mL of methanol for 15 minutes, then cooled and filtered. The residue was further cooled and filtered after further refluxing with methanol until the final extract was colorless. The concentrate and all of the filtrates were combined. A 0.1 percent w/v solution of 6-gingerol RS in methanol was prepared as a reference. Methanol:water with volume ratios of 65:35, 70:30, 80:20 and 90:10 were prepared to check most suitable ratio. A 25 cm x 4.6 mm stainless steel column filled with octadecylsilane bonded to a porous silica (5 µm) mobile phase—55 volumes of acetonitrile and 45 volumes of water made up the chromatographic

apparatus. The spectrophotometer was set at 278 nm with a 20 μ L loop injector, and the flow rate was set at 1.2 mL per minute.

2.3.3.2. For vincamine:

Preparation of standard and sample solution: 5 mg of vincamine were precisely weighed and then transferred to a 50 mL volumetric flask. 30-35 mL of methanol were added, sonicated to dissolve it entirely, and then added to bring the volume up to the desired level. After passing the solution through an appropriate 0.45 μ filter, three to five

milliliters of the filtrate was discarded. The mobile phase was added to further dilute 1 mL to 10 mL. Selection of analytical wavelength from the spectrophotometric method: The wavelength of maximal absorption from the spectrophotometric study was chosen as the analytical wavelength for the investigation. Between 200 and 400 nm, the standard solution was scanned. For the medication vincamine, the wavelength of maximal absorption was found to be 228 nm.

Chromatographic Conditions: Chromatographic conditions for vincamine determination are given in Table 1.

Table 1. Chromatographic conditions

Parameter/condition	Description
Column name	Kromasil C18 (250 mm X 4.6 mm i.d.) 5 μ m
Detector UV-3000-M	UV-3000-M
Injection Volume	20 μ L
Wavelength	228 nm
Mobile Phase Acetonitrile: Ammonium acetate solution	(90:10)
Diluent	Methanol
Programme	isocratic
Flow rate	1.0 mL/min
Column Oven temp	35° C
Run time	15 min

Acceptance Criteria:

RSD should not be more than 2.0 % for six replicate injections of standard. USP Tailing Factor/ Asymmetry Factor is not more than 2.0. The column efficiency as determined for Plate Count should be more than 2000.

2.3.4. Thin layer chromatography (TLC) profiling

TLC analytical plates covered with 0.2 mm thick silica gel-G were used to evaluate the extracts. The solvent system employed a 4:1:1:1 v/v mixture of butanol, acetic acid, and water. The capillary

action caused this combination to migrate on the silica-coated plates. After the coated plate was fully produced, it was allowed to air dry before being heated for 20 to 25 minutes. To find the bands, a 0.2% freshly made ninhydrin solution was sprayed onto the plate. Its retention factor (Rf) was expressed by the following equation:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

2.4. Antihypertensive assay

2.4.1. In vitro angiotensin-converting enzyme (ACE) inhibition assay [17]:



ACE inhibitory activity was assessed using the ACE kit-WST. The ACE enzyme catalyzes the conversion of angiotensin I (an inactive decapeptide) to angiotensin II (a potent vasoconstrictor). ACE also degrades bradykinin, a vasodilator. Inhibiting ACE helps lower blood pressure, and this mechanism is the basis for many antihypertensive drugs. A commonly used in vitro method is based on the hydrolysis of the synthetic substrate hippuryl-histidyl-leucine (HHL) by ACE, which releases hippuric acid. The reaction can be monitored using spectrophotometry or HPLC.

2.4.2. Procedure (spectrophotometric method):

Preparation of reaction mixture: 50 μ L of ACE enzyme solution was mixed with 150 μ L of test sample (or buffer for control). The solution was incubated at 37°C for 10 minutes.

Substrate addition: 100 μ L of 5 mM HHL substrate solution was added and the solution was incubated again at 37°C for 30–60 minutes.

2.4.2.3. Stop reaction: 250 μ L of 1N HCl (or extract hippuric acid with ethyl acetate) was added.

2.4.2.4. Detection: The absorbance of hippuric acid was measured at 228 nm (UV-visible spectrophotometer).

2.4.3. Determination of IC₅₀ value [17]

Serial dilutions of the test compound were prepared and % ACE inhibition vs. concentration graph were plotted to calculate the IC₅₀ (the concentration at which 50% ACE activity is inhibited).

3. RESULTS & DISCUSSION

3.1. Phytochemical screening

Various tests were carried out to check the presence of various phytochemical constituents in both the aqueous as well as methanolic extract of ginger and the results are shown in Table 2.

Table 2. Phytochemical Screening of Zingiber officinale Rhizome Extract

Chemical constituent	Aqueous extract	Methanolic extract
Cardiac Glycoside	Present	Present
Alkaloids	Present	Present
Saponins	Present	Present
Tannins	Absent	Present
Flavonoids	Present	Present
Reducing Sugar	Present	Present

3.2. Characterization techniques

3.2.1. UV-Visible spectroscopy

UV Spectrophotometer (SHIMADZU 1800) was utilized for this study. Solutions of ginger extract and vincamine were prepared in methanol and scanned in the range 200 to 400 nm. Maximum absorbance of ginger extract was observed at wavelength of 281.40

nm as shown in Figure 1. On the other hand, maximum absorbance of vincamine was shown at the wavelength of 268.5 nm as shown in Figure 2. The absorption falls within the typical range for conjugated systems such as gingerols and shogaols. The observed UV absorption supports the presence of bioactive compounds known for cardiovascular and antioxidant properties. The data is in agreement with prior reports on ginger extract characterization [18-20].



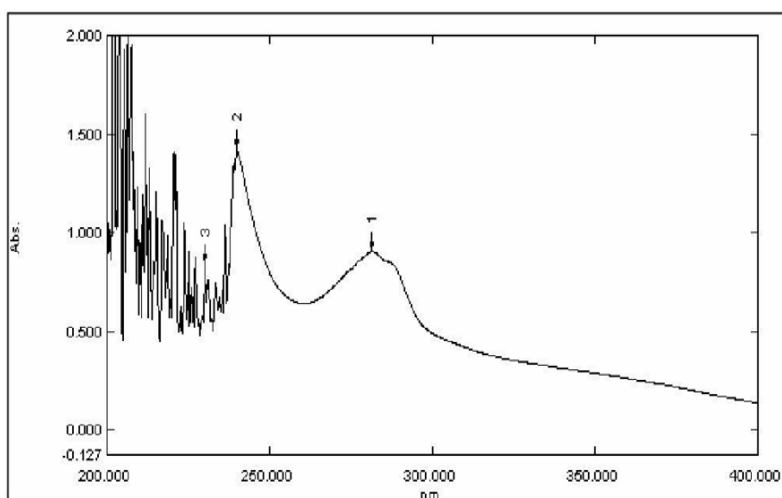


Figure 1. UV spectra of ginger (*Zingiber officinale*) extract

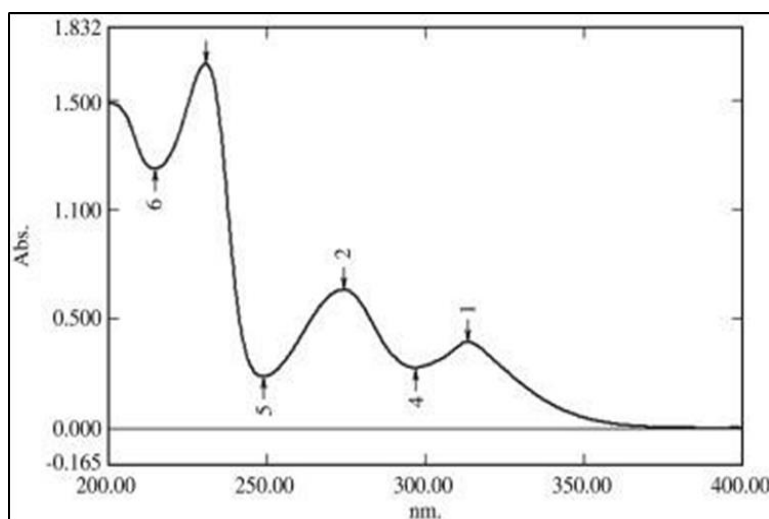


Figure 2. UV spectra of vincamine

3.2.2. FTIR

The ginger extract was scanned from 4000 – 400 cm^{-1} by using FTIR Spectrophotometer. Presence of various peaks in spectrum of ginger extract gives a hint toward the presence of different phytochemicals in ginger extract as shown in Figure 3. The FTIR profile confirms the presence

of hydroxyl, carbonyl, and aromatic functional groups, supporting the presence of key phytoconstituents like gingerols, shogaols, and flavonoids [21-25]. The spectra of vincamine as shown in Figure 4 gave strong and broad band beyond 3000 cm^{-1} due to presence of -OH group and another strong band at 1750 cm^{-1} due to presence of ester group which confirms the presence of the compound vincamine.

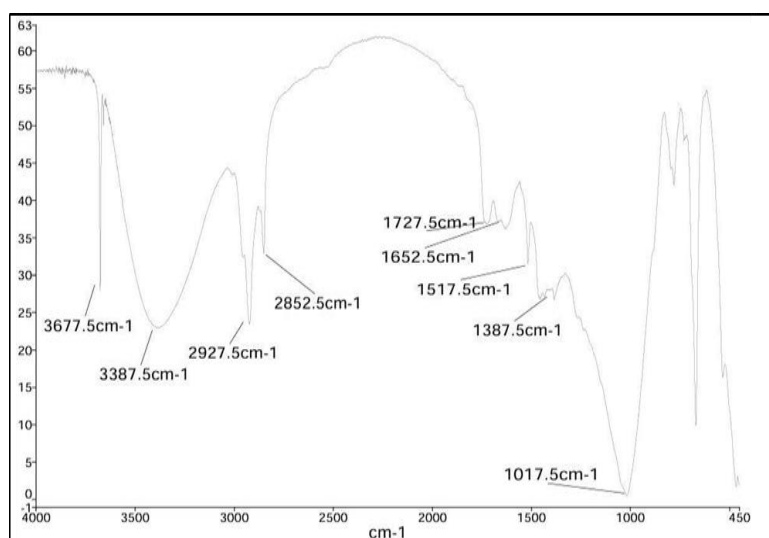


Figure 3. FTIR spectra of ginger (*Zingiber officinale*) extract

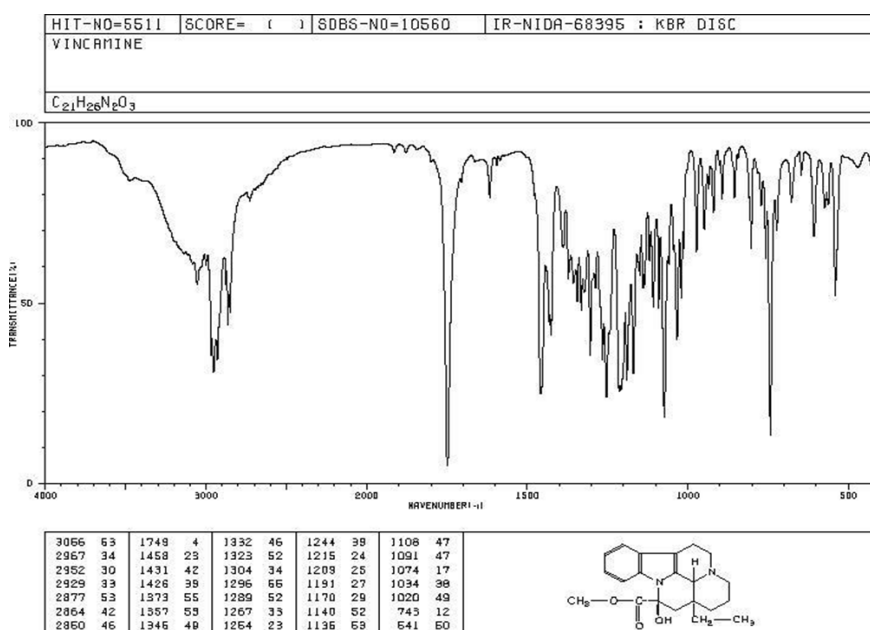


Figure 4. FTIR spectra of vincamine

3.2.3. HPLC

For the study, HPLC Waters 1650 was used. The HPLC chromatogram of the ginger extract is presented in Figure 5. According to earlier studies on HPLC assessments of 6-gingerol in ginger, an octadecylsilane (ODS) column is preferred. Methanol is a frequently utilized mobile phase in HPLC analysis of numerous natural compounds that use ODS columns. Methanol was selected as the mobile phase for the HPLC of 6-gingerol in

this investigation. First, optimization was done to get the ideal methanol content. The ability to elute 6-gingerol in a clear, narrow peak and the ability to elute 6-gingerol with the shortest retention time were the two parameters used to choose the optimal concentration. Methanol: water (v/v) with ratios of 65:35, 70:30, 80:20 and 90:10 were used. Based on the chromatographic data as shown in Figure 5, the 90:10 methanol: water showed the shortest retention time for 6-gingerol observed at 29.052 minutes. It also showed the highest detection for the compound by having the largest

peak area as clearly shown in Figure 5. Hence, the optimum methanol: water ratio was chosen to be 90:10 (v/v), and was used for all the subsequent

analyses in this study. Retention time for 6-gingerol and 6-shogaol were found to be 29.052 and 41.181 minutes, respectively.

Table 2. Retention time for 6-Gingerol standard at different volume ratios of methanol: water

Sr. No.	Methanol & Water Ratio	Retention Time
1	65:35 (v/v)	46.43 minutes
2	70:30 (v/v)	39.84 minutes
3	80:20 (v/v)	35.42 minutes
4	90:10 (v/v)	29.052 minutes

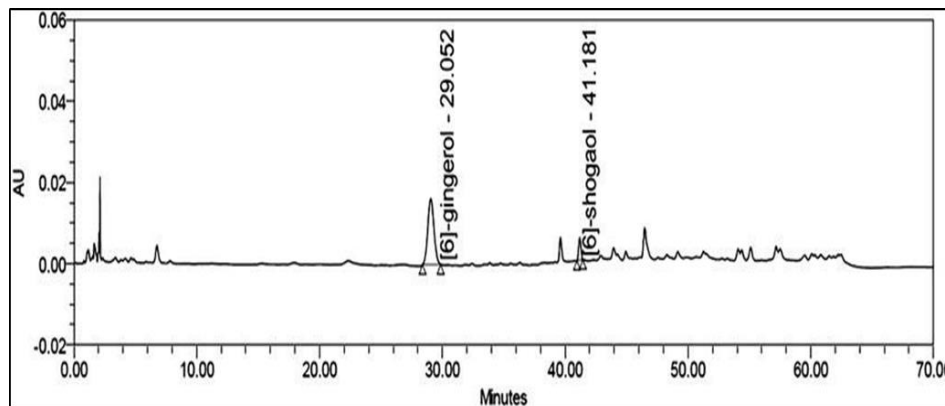


Figure 5 HPLC Analysis of Ginger (*Zingiber officinale*) Extract

3.2.4. Thin Layer Chromatography (TLC) Profiling

Results for the methanolic extract are displayed by the sample's thin layer chromatography. As seen in Figure

6 and Table 3, there were three sites with Rf values of 0.62, 0.70, and 0.78, respectively. TLC confirmed the presence of gingerols in the extract. Sharp and distinct spots indicate high purity and stability, validating the suitability of the formulation for further pharmacological evaluation.

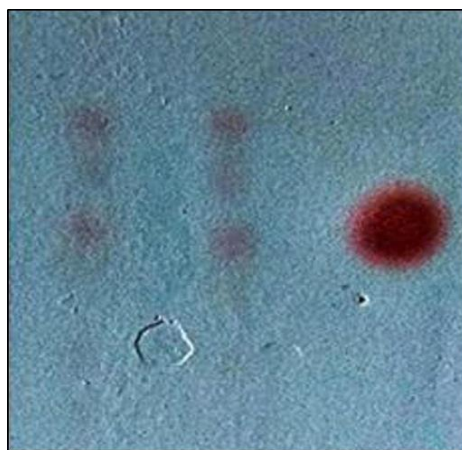


Figure 6. TLC plate of ginger (*Zingiber officinale*) extract

Table 3. Rf value of ginger (*Zingiber officinale*) extract

Sr. No.	Extract	Spot No.	Rf Value
1	Standard	1	0.69
2	Ginger (<i>Zingiber officinale</i>)	3	0.62, 0.70, 0.78

3.3. Antihypertensive Assay

ACE inhibitory activity was assessed using the ACE kit-WST (Redcells Diagnostics Pvt. Ltd.). The assay was carried out according to the standard operating procedure. Ginger extract was tested in various concentration and mean % ACE inhibition value was calculated as shown in Table 4. A graph was plotted illustrating concentration vs % ACE inhibition of ginger extract shown in figure 7. Results reveals that ginger extract's ACE inhibition IC₅₀ was found to be 85.21 µg/mL. Vincamine was also tested for same concentrations and mean % ACE inhibition value was calculated as shown in Table 5. A graph was plotted illustrating concentration vs % ACE inhibition of vincamine as shown in Figure 8. In case of vincamine ACE inhibition IC₅₀ was found to be 80.28 µg/mL. Standard drug captopril was tested for same concentrations and mean % ACE inhibition value was calculated as shown in Table 6. A graph was plotted illustrating concentration vs % ACE inhibition of captopril shown in Figure 9. Vincamine and ginger

extract combination was tested in same concentrations and mean % ACE inhibition value was calculated as shown in Table 7. A graph was plotted illustrating concentration vs % ACE inhibition of vincamine and ginger extract combination shown in Figure 10. Results reveal that in vincamine and ginger extract combination, ACE inhibition IC₅₀ was found to be 71.97 µg/mL. In case of the standard drug captopril, ACE inhibition IC₅₀ was found to be 54.88 µg/mL as shown in Table 8. Standard drug captopril was found to be most effective in inhibiting ACE activity as IC₅₀ value was found to be lowest in this case. In comparison to ginger extract, vincamine showed slightly less value of IC₅₀ which makes it more potent than ginger for inhibition of ACE activity. IC₅₀ value for the combination of vincamine and ginger extract was slightly higher as compared to the standard drug captopril but it was on the lower side when we compared it with vincamine and ginger extract alone. Hence, vincamine in combination with ginger extract produced synergistic effect.

Table 4. ACE inhibitory activity of ginger (*Zingiber officinale*) extract

Sr. No.	Concentration (µg/mL)	% ACE inhibition			
		T1	T2	T3	Mean
1.	10	9.46	9.59	9.7	9.58
2.	25	21.12	21.35	21.79	21.42
3.	50	39.99	39.72	39.82	39.84
4.	100	61.34	61.47	61.23	61.35
5.	150	75.43	75.33	75.78	75.51

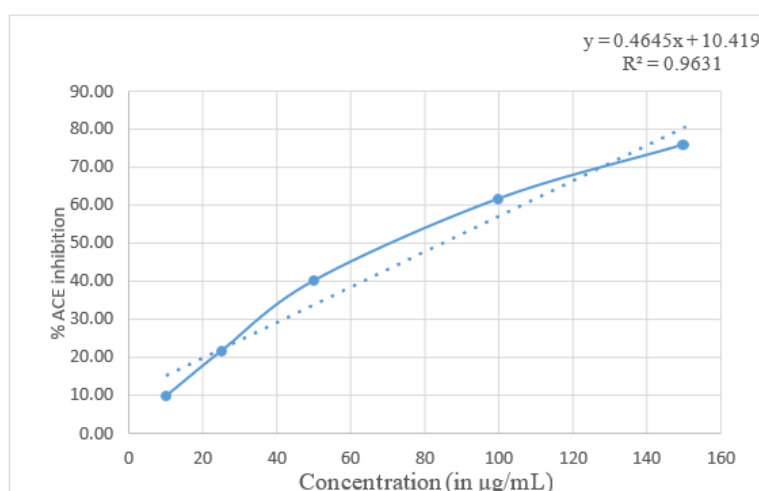


Figure 7. Concentration vs % ACE inhibition of ginger extract

Table 5. ACE inhibitory activity of vincamine

Sr. No.	Concentration (µg/mL)	% ACE inhibition			
		T1	T2	T3	Mean
1.	10	12.14	12.27	12.30	12.24
2.	25	25.22	24.98	25.46	25.22
3.	50	42.24	42.56	42.13	42.31
4.	100	61.34	61.47	61.23	61.35
5.	150	78.32	78.44	78.90	78.55

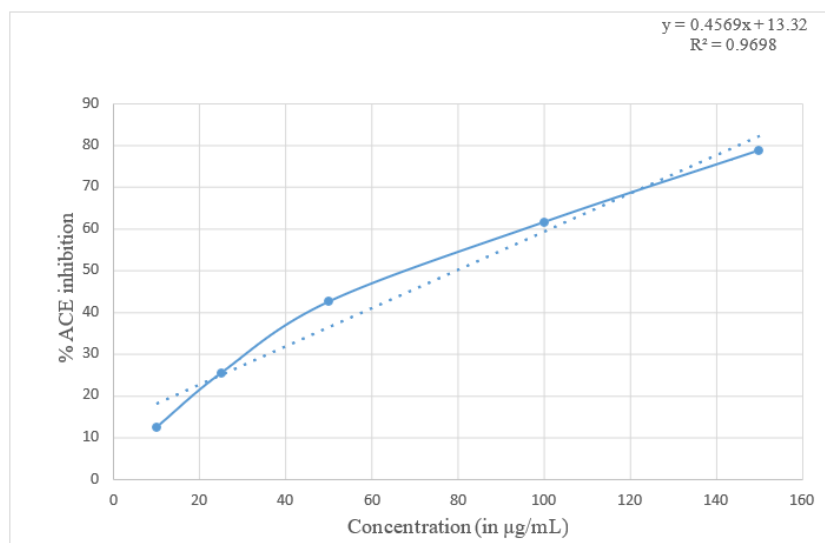


Figure 8. Concentration vs % ACE inhibition of vincamine drug

Table 6 ACE inhibitory activity of standard drug captopril

Sr. No.	Concentration (µg/mL)	% ACE inhibition			
		T1	T2	T3	Mean
6.	10	14.32	13.99	14.21	14.17
7.	25	35.76	35.23	35.77	35.59
8.	50	58.72	58.22	58.43	58.46
9.	100	81.23	81.42	81.92	81.52
10.	150	92.44	92.77	92.32	92.51

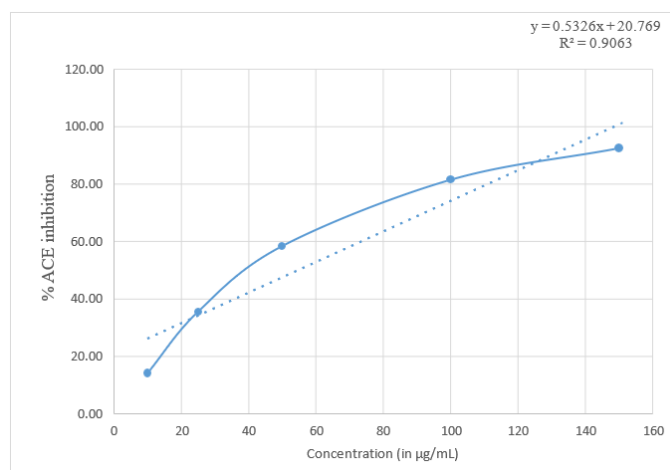
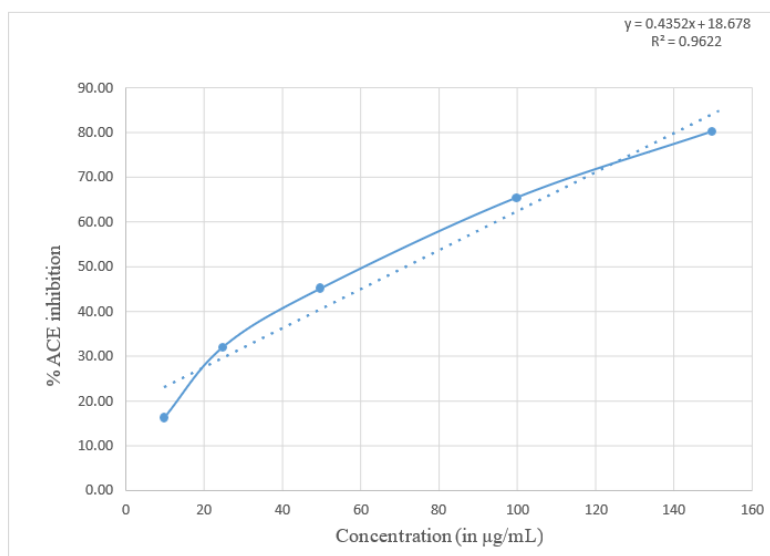


Figure 9. Concentration vs % ACE inhibition of standard drug captopril

Table 7. ACE inhibitory activity of vincamine and ginger extract combination

Sr. No.	Concentration (µg/mL)	% ACE inhibition			
		T1	T2	T3	Mean
11.	10	16.23	16.32	16.22	16.26
12.	25	32.12	31.98	32.07	32.06
13.	50	45.12	45.27	45.14	45.18
14.	100	65.44	65.33	65.54	65.44
15.	150	80.43	80.11	80.21	80.25

**Figure 10. Concentration vs % ACE inhibition of vincamine and ginger extract combination****Table 8. ACE inhibition IC₅₀ values of ginger extract, vincamine and standard drug captopril**

Source	ACE inhibition IC ₅₀ (µg/mL)
Ginger	85.21
Vincamine	80.28
Vincamine + Ginger Extract	71.97
Captopril	54.88

4. CONCLUSION

The present study was undertaken to investigate the antihypertensive potential of ginger (*Zingiber officinale*) extract in combination with vincamine, a known vasodilator alkaloid, through in vitro evaluation and characterization techniques. Initially, the ginger rhizomes were subjected to extraction. The extract was then characterized using UV spectroscopy, FTIR, and TLC to identify and confirm the presence of key phytoconstituents such as gingerols and shogaols, which are known for their cardiovascular benefits. The in vitro

antihypertensive activity was evaluated using the Angiotensin-Converting Enzyme (ACE) inhibition assay, which demonstrated significant enzyme inhibition by both ginger extract and vincamine, with enhanced activity observed in the combination, suggesting a potential synergistic effect. The combination of ginger extract and vincamine showed superior antihypertensive activity in comparison to either agent alone, supporting the hypothesis that herbal-bioactive and synthetic drug combinations can work in synergy to offer better therapeutic outcomes. The findings of this study suggest that ginger extract

possesses significant in vitro antihypertensive activity. Vincamine complements this effect through its vasodilatory properties. The combination exhibits enhanced ACE inhibition, indicating a promising synergistic potential in the management of hypertension. This herbal-drug combination approach may provide an effective, safe, and integrative option for hypertensive therapy, warranting further in vivo and clinical studies. Thus, the research provides a scientific basis for the combined use of *Zingiber officinale* and vincamine in antihypertensive therapy and contributes to the development of plant-based cardiovascular remedies with modern pharmacological validation.

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