Acta Biologica Marisiensis

TANNINS IN *HEINSIA CRINITA* EXTRACT INHIBIT CARDIAC ARGINASE *IN SILICO* AND *IN VITRO*: POSSIBLE ROLE IN HYPERTENSION

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Received: 10 November 2025; **Accepted:** 12 January 2026; **Published:** 30 June 2026

Abstract: Hypertension is afflicting millions worldwide. The present study investigated *in vitro* anti-hypertension of *Heinsia crinita* targeting cardiac arginase in experimental and *in silico* analysis. The methanolic extract of *H. crinita* was subjected to gas chromatography flame ionization detection (GC-FID); Total antioxidant capacity (TAC), DPPH antiradical and inhibition of lipid peroxidation were analyzed. Cardiac arginase inhibitory activity was experimented *in vitro*, also selected tannins from GC-FID chromatography were docked into the active pocket of cardiac arginase PDB: 4HWW. The experimental data reveal tannins, steroids, phenols, flavonoids and saponins. Quantitative phytochemical detected were alkaloids, flavanol, flavonoid, phenols and tannins. Also, the total antioxidant capacity in *H. crinita* was 25.33±0.57 µg ascorbic acid equivalent per gram (AAE/g) the extract of *H. crinita* revealed the following tannins: geranin, epigallocatechin gallate, agrimoniin, punicalagin, praecoxin, amongst others. *H. crinita* displayed antioxidant and antiradical properties. It also inhibited cardiac arginase in dose dependent fashion. The molecular docking results revealed that punicacortein, grenatin a and b, castalin, praecoxin a and punicalagin show better binding affinity of -6.48, -6.29, -4.94, -5.16, -5.53 and -5.16 kcal/mol respectively. In conclusion *H. crinita* extract can be used for the management of cardiac arginase related hypertension.

Keywords: tannins, chromatography, molecular docking, hypertension, cardiac arginase

1. Introduction

It is statistically proven that by the year 2030 deaths from hypertension, heart failure and atherosclerosis will be more than 23 million (Dumor et al., 2018). Hypertension is a major risk factor for sudden death globally (Agunloye et al., 2023). A lifestyle devoid of light exercise, very low K^+ ion-based diet, high table salt intake, and age are some factors that can predispose one to become hypersensitive. Scientific studies have also shown certain enzymatic actions in the progression of hypertension such as adenosine deaminase, arginase, acetyl cholinesterase and angiotensin converting enzyme (Agunloye et al., 2023).

Cardiac arginase metabolizes L-arginine to form two products L-ornithine and urea. The production of urea in mammals is disposed as ammonia which is very toxic (Li et al., 2022). Also, L-ornithine is metabolized by another protein ornithine decarboxylase to form polyamines which are responsible for cell proliferation, growth and transport (Kusano et al., 2008). Again L-ornithine is catabolized by ornithine amino transferase to form L-proline which is important in collagen production (Caldwell et al., 2018). L-arginine is catabolized by a competing enzyme known as nitric oxide synthase converting it into nitric oxide, in the blood vessels functions as regulator of vasodilation, inhibitor of platelet forming plug, adhesion, inhibition of leucocyte adhesion, inflammation and smooth muscle proliferation (Forstermann and Sessa, 2012). The competition of substrate leads to a decrease production of NO and an increase in production of L-ornithine. Polyamines or proline formation from ornithine promotes cell proliferation and collagen formation respectively leading to many health problems like hypertension, heart failure and atherosclerosis (Pudlo et al., 2017).

Also, uncontrolled activity of arginase leads to the deficiency of L-arginine leading to the uncoupling of NOS, where the enzyme produces superoxide ion instead of nitric oxide, this give rise to hypertension among other cardiovascular diseases (Forsterman and Sessa, 2012) Cardiac arginase is a promising target against hypertension (Caldwell et al., 2015). Two synthetic arginase inhibitors in market are derivatives of boronic acid and hydoxy-nor-L-arginine which are quite effective, but their toxicity and poor absorption, metabolism, excretion and distribution profile, makes researchers to look for alternatives which are plant rich polyphenols (Ivanen Kov and Chufarova, 2014; Girard-Thernier et al., 2015).

Heinsia crinita is a greenish vegetable mostly found in southern part of Nigeria, it is of the family Rubiaceae. Commonly called bush apple and in southern Nigeria known as “atama”. *H. crinita* leaves and fruits are edible, while the leaves mostly serve as vegetable in local cuisines. *H. crinita* is abound with many bioactives such as polyphenols, flavonoids, and alkaloids (Ozçelik et al., 2011). These compounds are responsible partly to the pharmacological activity of *H. crinita*. The leaves in alcohol extract are used for the management of many diseases like infertility, diabetes, bacterial disease and hypertension (Vladimir-Kneevuc et al., 2004, Ebong et al., 2014). Oboh et al. (2021) reported the anticholinesterase and antioxidant properties of *H. crinita* in *Drosophila melanogaster*. Okokon et al., 2009 also reported the antiplasmodial and antidiabetic effects of *H. crinita* extract. Recent studies have shown that *H. crinita* possesses anti-hyperglycemic, anti-cancer, anti-microbial, anti-inflammatory and antioxidant properties (Mgbeje et al., 2016; Boumba et al., 2022; Iwara et al., 2023). There are very few research reports on *H. crinita* activity against cardiac arginase related to hypertension, lipid peroxidation, phytochemicals and antioxidant properties that is why this research work was carried out. Despite promising and appealing pharmacological activities in *H. crinita*, elaborate analysis of molecular interaction between active constituents of *H. crinita* like tannins against cardiac arginase particularly with respect to amino acid on cardiac arginase interaction and inhibitory mechanisms remains to be elucidated. Many studies only involved preliminary screening solely on identifying inhibitory compounds. However, the present study utilizes an integrative approach that links computational studies with in vitro biochemical validation. In this context, the present study was undertaken to evaluate the cardiac

arginase-inhibitory potential of *H. crinita* extract.

2. Materials and Methods

2.1. Chemicals

Sodium dodecyl sulphate, ferrous sulphate, ascorbic acid, sodium acetate, acetic acid, 1, 10-phenanthroline, ferrous chloride ($FeCl_2$), sodium phosphate (Na_3PO_4) hydrogen peroxide (H_2O_2), sodium bicarbonate ($NaHCO_3$), manganese chloride ($MnCl_2$), arginine, perchloric acid ($HClO_4$), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), quercetin, gallic acid, sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), ammonium molybdate, Tris-HCl buffer, methanol, thiobarbituric acid (TBA), disodium hydrogen phosphate (Na_2HPO4), sodium dihydrogen phosphate (NaH_2PO4), trichloroacetic acid (TCA) were purchased from Sigma Aldrich (China) and Urea kit (Randox, UK).

2.2. *Heinsia crinita* collection and preparation

Heinsia crinita leaves (fresh) were obtained from Swali market in Yenegoa, Bayelsa State. The leaves were identified in Botany Department, Niger Delta University and deposited with the herbarium number NDUP/040. The leaves of *H. crinita* were washed clean and dried for 7 days; the dried leaves were grounded into fine powder. The grounded leaves were weighed 206.26 g and were transferred into 1000 ml amber colored jar holding 500 ml of methanol (Mlozi et al. 2022). The jar was agitated occasionally for 4 days. The extract was filtered, centrifuged at 4000 rpm; the clear supernatant was concentrated into paste. The paste weighed 13.5 g and was stored for further use.

2.3. Qualitative Phytochemical Test

The presence of phenols, flavonoids, alkaloids, tannins, steroids, and saponins were qualitatively determined based on the methods of Harbone (1973) and Nabi and Shrivastava (2017).

2.4. Quantitative Phytochemical Analysis

Estimation of phenol

The amounts of phenols in *Heinsia crinita* extract was according to the described method of Singleton et al. (1999) and Demiray et al. (2009). The plant extract was mixed with diluted (1:10) Folin and kept for 5 minutes, afterwards 4 ml of sodium carbonate (7.5%w/v) was added and the solution incubated at 25 ℃ for 60 minutes with intermittent shaking. The color was read at 765 nm. Total phenol was calculated as milligram GAE/g extract.

2.4.1. Flavonoid content

The spectrophotometric procedure of Zhishen et al. (1999) was used to determine flavonoid content in extract of *Heinsia crinita*. An aliquot of filtrate 0.4 ml was added to 0.6 ml H_2O and 0.06 ml of 5% $NaNO_2$. The mixture was kept at 25 ^{0}C for 5 minutes with occasional agitation. Later 10% $AlCl_3$ was added followed by 0.4 ml of 1M NaOH and 0.4 ml of H_2O sequentially. This mixture stood for 30 mins and thereafter absorbance read at 510 nm. Quercetin was used for the calibration of standard curve. Values of total flavonoid were calculated and reported as milligram quercetin equivalent per gram (mgQE/g).

2.4.2. Flavonol content

The procedure of Millauskas et al. (2004) was adopted in evaluating flavonol, in *Heinsia crinita*. Both rutin and *H. crinita* were prepared and were added to 1 ml of $AlCl_3$ (20g/L) and 6 ml of Sodium acetate (50g/L). The mixture was

incubated at 25 ℃ for 4 hours. Thereafter optical density was measured at 440 nm. Total flavonol was extrapolated and reported as mg rutin equivalent/g (mgRE/g).

2.4.3. Alkaloid content

The procedure of Unuofin et al. (2017) was applied in the determination of alkaloids in *Heinsia crinita*. The grounded leaves (5g) were dissolved in 10% acetic acid (0.1 L). The mixture was kept at 25 ºC for 4 hours. The mixture was concentrated at 55 ºC to one fourth of the volume. Then concentrated NH_4OH was added in drops until alkaloid were precipitated. Alkaloids were filtered and dried; the dried weight was calculated and reported in %.

2.5. Total condensed tannins

Condensed tannins were evaluated in line with the procedure of Sun et al. (1998). Diluted sample of *Heinsia crinita* was mixed with 3 ml of 4% vanillin prepared in methanol and 1.5 ml of hydrochloric acid was added. The solution was incubated for 15 min at 25 ºC and absorbance was read at 500 nm using methanol as blank. The concentration of condensed tannins in *H. crinita* was expressed as milligram tannic acid equivalent per gram extract (mgTAE/g).

2.6. Gas chromatography flame ionization detection (GCFID) for quantification of tannins in *Heinsia crinita* extract

The quantification of tannins in *Heinsia crinita* was carried out on an Agilent 6890 Gas chromatography equipped with a flame ionization detector. The injector temperature was 280 ºC and a velocity of 30 cms^{-1} and Helium was the carrier gas with 2 µl of sample. Tannins were determined by the ratio between the area and mass of internal standards injected into the GC-FID (punicalagin, praecoxin a, castalin, granatin a, granatin b, punicacortein c) and the area of the identified phytochemicals. The concentrations of tannins were express as µg/ml (Kumar and Rajakumar, 2016).

2.7. Total antioxidant capacity (TAC)

The phosphomolybdenum procedure of Prieto et al. (1999) was adopted. Graded concentrations of ascorbic acid were mixed with the solution of 0.6 M H_2SO_4, 28mM Na_3PO_4 and 4mM ammonium molybdate. The tubes were corked and heated at 95 ºC for 90 min. The tubes were cooled and the absorbance was determined at 695 nm using a spectrophotometer. Total antioxidant of *Heinsia crinita* extract was also determined accordingly and calculated from a standard curve of ascorbic acid. The TAC was calculated and reported as µgAAE/g extract.

2.8. DPPH antiradical assay

Diluted extract of *Heinsia crinita* was added to 3 ml of ethanolic solution of DPPH (0.9mg/10ml). The reacting solution was incubated at 25 ºC in the dark accompanied by intermittent shaking. After 20 minutes absorbance was read at 517nm. Ascorbic acid served as reference antioxidant, results were calculated as percentages and IC_{50} (Sanchez-Moreno et al, 1998).

2.9. Hydrogen peroxide scavenging

Heinsia crinita's ability to scavenge H_2O_2 was evaluated according to Nabavi et al. (2009). Hydrogen peroxide 0.04 M was prepared in phosphate buffer pH 7.4. The extract at different concentration was incubated with 600 µl of H_2O_2 at 25 ºC in the dark. The ability of *H. crinita* to scavenge H_2O_2 was read at 230nm using UV-Vis spectrophotomer. Values were reported as percentages and IC_{50}.

2.10. Hydroxyl radical scavenging

The ability of *Heinsia crinita* extract to scavenge hydroxyl radicals was evaluated in line with Yu et al. (2004). In a reaction

containing 0.06 ml, 1.0 mM ferrous chloride, 0.09 ml of 1 mM 1, 10-phenanthroline, 2.4 ml of 200 mM phosphate buffer (pH 7.8) 0.15ml of 170 mM hydrogen peroxide and 1 ml of different concentrations of *H. crinita* extract. The reaction was incubated for 5 minutes at 25 ℃ and absorbance was read at 560 nm. Results were calculated and reported as percentages and IC_{50}.

2.11. Lipid peroxidation assay

The anti-lipid peroxidation property of *Heinsia crinita* was carried out by the procedure of Sato and Bremner (1993). 1ml of ten percent cardiac homogenate was mixed with Tris-Cl buffer 0.15 M, pH 7.2 (0.1ml), ascorbic acid 50 µl, 1% (w/v), 0.07 M $FeSO_4$ (0.05 ml) and varying concentration of the extract. The mixture was incubated at 37 ℃ for 60 minutes. Thereafter 500 µL of 0.1 M HCl, 200µL of 9.8% SDS, 0.9 ml of H_2O and 2 mL of 0.67% TBA were added in sequence, later the mixture was heated at boiling temperature for 30 minutes, cooled and butanol was added (2 ml) and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was read at 532 nm. Quercetin served as reference.

2.12. Cardiac arginase *in vitro*

Wistar albino rats were obtained from the Department of Pharmacology; Niger Delta University. The rats (N=5) were maintained for two weeks according to standard guidelines and procedures. After two weeks of acclimatization rats were sacrificed to extract cardiac arginase for *in vitro* inhibitory studies. The heart tissues were dissected out cleaned in rinsing buffer (KCl) and were weighed. The cardiac tissues were homogenized in cold phosphate buffer. The homogenate was later centrifuged at 4 ºC at 1000 x g for 20 minutes, supernatant used as enzyme source and for anti-lipid peroxidation (Bordage et al. 2017).

2.13. Inhibition of *Heinsia crinita* against cardiac arginase

In a reaction mixture consisting of 0.05 mM $NaHCO_3$ buffer pH 9.5, substrate (arginine) 0.02 mM, 0.0005 mM manganese chloride, 0.2 ml of *Heinsia crinita* at different concentrations and 0.79 ml of enzymes source (arginase). The solution was incubated for 1 hour at 37 ℃. At the expiration of 1 hour 0.5 M $HClO_4$ was added to halt the enzymatic activity of arginase. The solution was centrifuged and the supernatant containing urea was determined using urea kits according to Campbell (1961). The results of % inhibition of cardiac arginase were presented as percentages.

2.14. Molecular docking of bioactives in *Heinsia crinita* against cardiac arginase (PDB ID:4HWW)

Cardiac arginase protein (PDB ID: (4HWW) with very high percentage homology to that of rat (Ohtake et al. 1998) was downloaded from (www.rcsb.org) and also the six selected bioactives from *Heinsia crinite:* punicacortein, granatin b, granatin a, castalin, praecoxin a and punicalagin, were downloaded from PubChem website. Afterwards the protein cardiac arginase (PDB ID: 4HWW) was prepared by deleting unwanted steric hindrances using the Maestro Suite (Maestro 2023). Molecular docking of cardiac arginase protein (PDB ID: 4HWW) and punicacortein, granatin b, granatin a, castalin, praecoxin a and punicalagin were done using the Maestro software of OPLS3, 2018 Force field (Maestro 2023) and Pymol software (Seeliger and de Groot, 2010). The best docked positions were selected and results presented in tables and figures.

2.15. Statistical analysis

All experimental data were carried out in triplicate. Data are presented as mean ± standard deviation (SD). SPSS version 17.0

(New York, USA) was utilized, running on Windows. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussions

Percentage yield of *Heinsia crinita* was 6.58%. Hypertension is a cardinal risk factor for all diseases linked to the cardiovascular system, such as stroke, and a major cause of death (Whelton, 1994). Secondary metabolites are major constituents of plants and they can be revealed through screening and quantitative analysis. The qualitative phytochemical detection of *Heinsia crinita* revealed the presence of phenols, saponins, alkaloids, flavonoids, and steroids (**Table 1**). These metabolites act as antioxidant, antilipid peroxidation and protection of DNA damage (Kumar et al., 2013) antimicrobial (Thompson et al., 2024) and antihypertensive (Ademiluyi et al., 2016).

Phenolics, flavonoids tannins and flavanols are potent antioxidants, anti-inflammatory, antidiabetic, antitumor, and antiaging properties (Zhang et al., 2017). The antioxidant mechanisms of secondary metabolites include neutralization of radical and transfer of hydrogen atom to a radical (Valko et al., 2007). Flavonols and flavonoids are aromatic secondary metabolites with a ketone group. In our present investigation the presence of flavonol was 22.0±0.3 mgRE/g while of flavonoid was 18.6±0.5 mgQE/g which act as antioxidant and anti-inflammatory molecules. Flavonoids structurally are C_3–C_6 bridge linked to a phenol with hydroxyl group (Cowan, 1999 and Lafay and Gil-Izquierdo, 2008). Phenolics present in *H. crinita* was 10.0±0.9 mgGAE/g as depicted in **figure 1**.

Table 1. Qualitative phytochemical screening in *Heinsia crinita*

Phytochemicals	Result
Flavonoids	+++
Saponins	+
Phenols	+++
Steroids	++
Tannins	++
Alkaloids	+

Key, + = present, - = not present

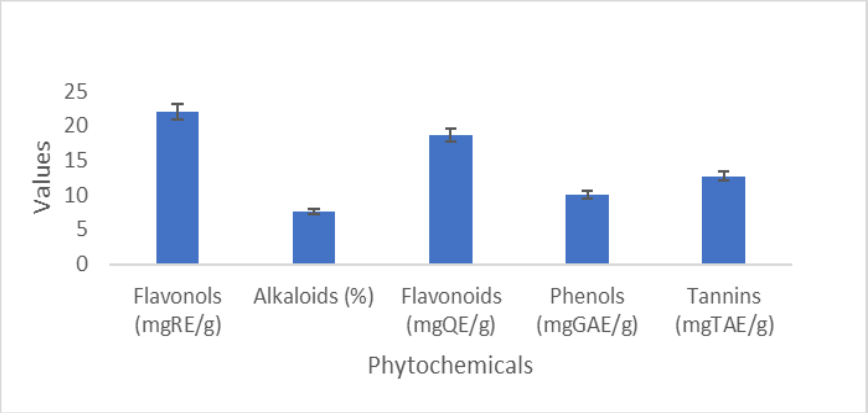

Fig. 1. The amounts of phytochemicals in *Heinsia crinita* determined by various methods: Flavonol milligram rutin equivalent per gram extract, alkaloids %, flavonoids milligram quercetin equivalent per gram extract, phenols milligram gallic acid equivalent per gram extract and tannins milligram tannic acid equivalent per gram extract, n = 3 determinations

Tannins are compounds described as polymers of phenols (hydrolysable and condensed). Gallic acid linked to D-glucose forms the monomers of hydrolyzable tannins and flavonoids served as monomers of condensed tannins or proanthocyanidins (Cowan, 1999). Tannins found in *H. crinita* was 12.7±0.8mgTAE/g. Alkaloids are nitrogen containing compounds found in plants (Cushine et al., 2014). The quantitative results revealed the presence of alkaloid 7.64±0.5%. The report of quantitative phytochemicals is similar to that reported by Kumar et al. (2013) and Eboh et al. (2024).

The gas chromatography flame ionization detection analysis of tannins revealed a total of seventeen tannins according to the table and the chromatogram depicted in **figure 2** and **table 2**.

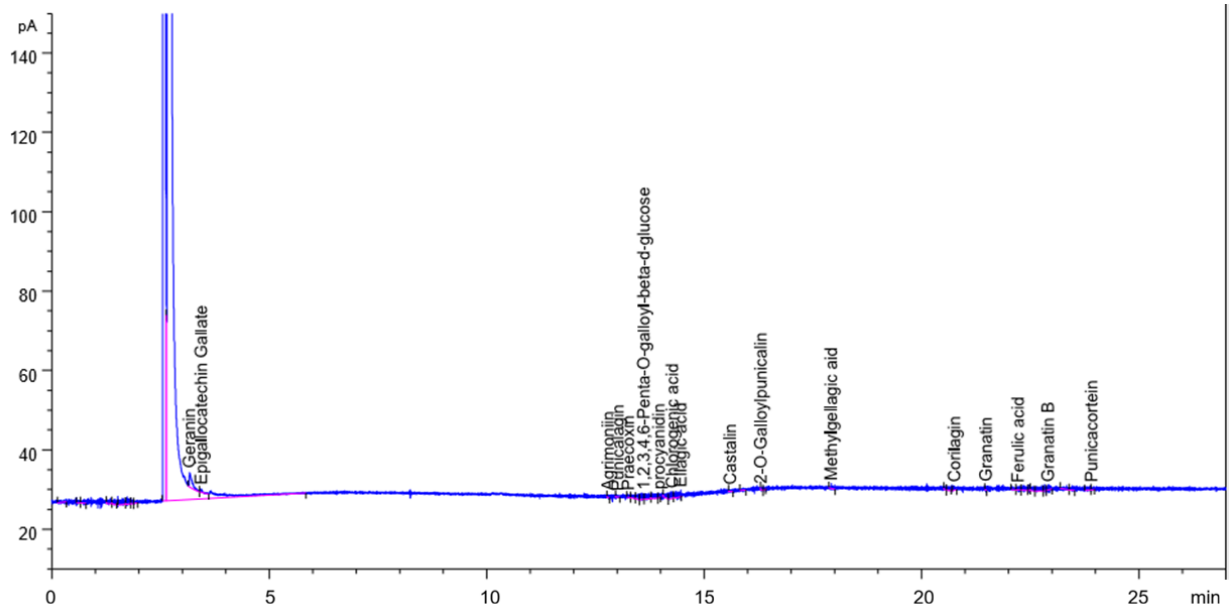

Fig. 2. The GCFID chromatogram of *Heinsia crinita* extract revealing different tannins

Table 2. The retention time, area, amount and names of tannins detected in *Heinsia crinita*

S/N	Ret Time (min)	Area (pA∗s)	Amount (µg/ml)	Name
1	3.166	16.39	4.44	Geranin
2	3.433	2.36	4.82×10^{-1}	Epigallocatechin gallate
3	12.79	1.10	2.15×10^{-1}	Agrimoniin
4	13.0	1.14	1.99×10^{-1}	Punicalagin
5	13.25	1.26	2.46×10^{-1}	Praecoxin
6	13.60	5.07	2.05	1,2,3,4,6-penta-o-galloyl-β-D-glucose
7	13.94	1.46	2.8×10^{-1}	Procyanidin
8	14.26	5.92	2.22	Chlorogenic acid
9	14.46	6.65	2.38	Ellagic acid
10	15.60	1.18	2.29×10^{-1}	Castalin
11	16.3	1.52	2.79×10^{-1}	2-O-galloyl-punicalin
12	17.93	2.47	5.01	Methylellagic acid
13	20.74	2.92	5.96×10^{-1}	Corilagin
14	21.47	1.09	2.09×10^{-1}	Granatin A
15	22.22	2.48	5.03×10^{-1}	Ferulic acid
16	22.90	2.22	4.49×10^{-1}	Granatin B
17	23.90	1.67	3.16×10^{-1}	Punicacortein

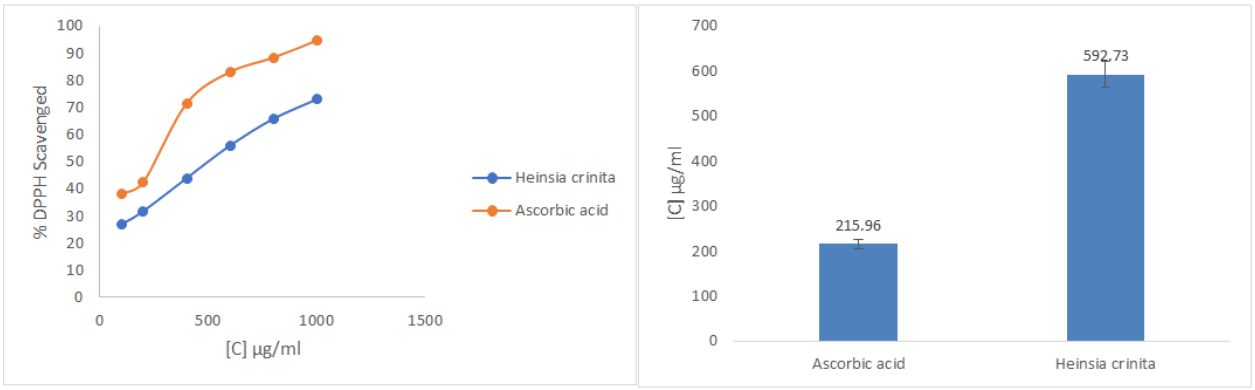

Fig. 3. Percentage inhibition of DPPH and IC_{50} values of ascorbic acid and *Heinsia crinita* extract, values are mean±S.D, triplicate determination

Out of these six (punicacortein c, granatin b, granatin a, castalin, praecoxin a, and punicalagin) were selected for the molecular docking studies against cardiac arginase.

DPPH is a non-biological stable free radical that is used to detect the radical scavenging ability of antioxidants. In the present study *H. crinita* at concentrations of 100-1000 µg/ml inhibited DPPH as compared to ascorbic acid. However, the IC_{50} values of *H. crinita* and ascorbic acid are depicted in **figure 3**. Our report is in line with the works of Atere et al. (2018) and Eboh et al. (2024).

The TAC assay is based on the plant extract reducing molybdenum (VI) to (V) which is greenish with maximum absorption of 695nm. The antioxidant capacity of *H. crinita* was 25.33 ± 0.57 µgAAE/g extract. Hypertension is linked to lower levels of antioxidants in the system or increased oxidative stress. In this study the total antioxidant of *H. crinita* shows that it is due to the availability of reducing agents like flavonoids and phenols present as secondary metabolites. Our findings are in consonant with the works of Attia et al. (2019) and Atere et al. (2018).

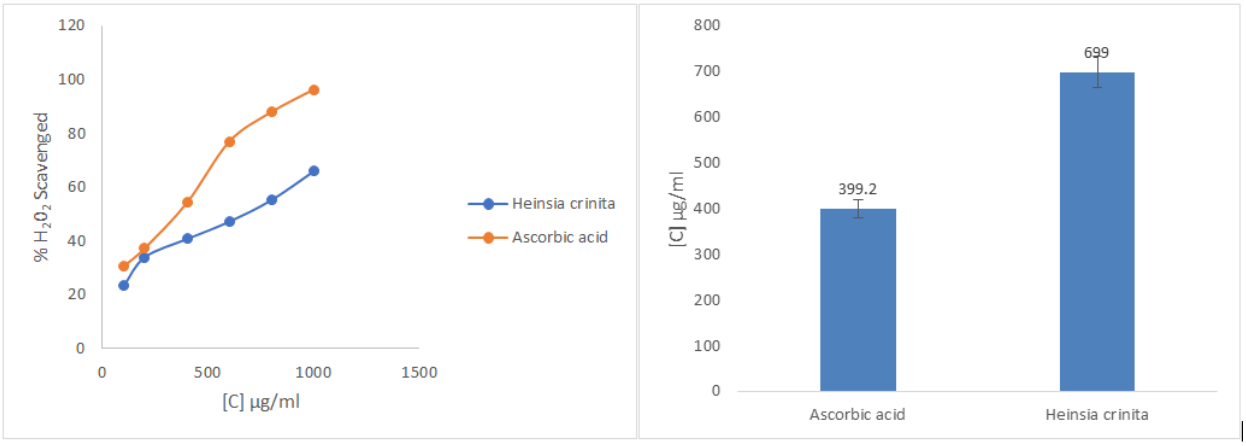

Fig. 4. Percentage inhibition of H_2O_2 and IC_{50} values of ascorbic acid and *Heinsia crinita* extract, values are mean±S.D, triplicate determination

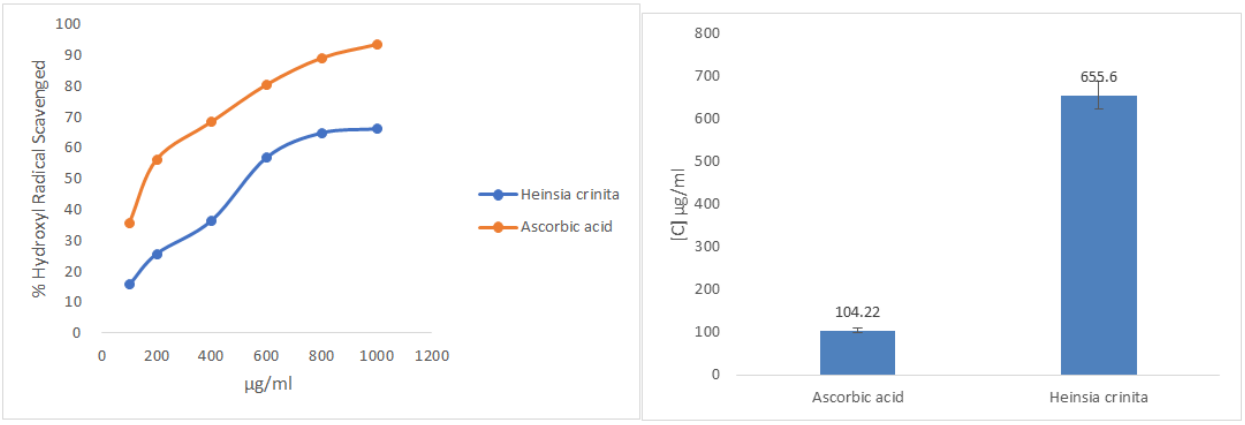

Fig. 5. Percentage inhibition of ˙OH radical and IC_{50} values of ascorbic acid and *Heinsia crinita* extract, values are mean±S.D, triplicate determination

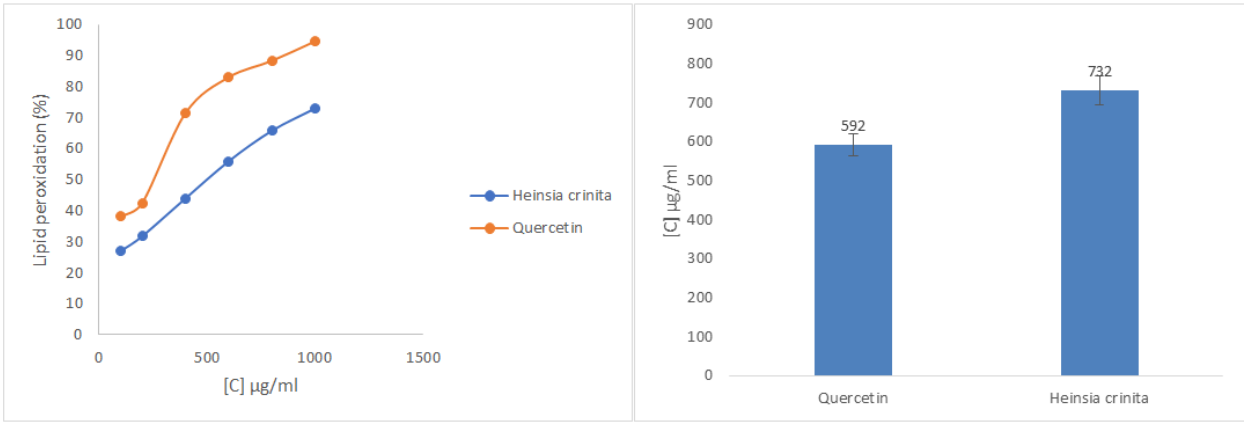

Fig. 6. Percentage inhibition of lipid peroxide and IC_{50} values of ascorbic acid and *Heinsia crinita* extract, values are mean±S.D, triplicate determination

Table 3. The percentage of *Heinsia crinita* inhibition of cardiac arginase

Conc. (mg/mL)	**% Inhibition**
0.1	26.17± 0.98
0.2	39.8± 0.81
0.4	47.73± 0.34
0.6	61.9± 0.76
0.8	77.5± 0.79
1	80.2± 1.18
IC_{50}	0.41±0.78 µg/ml

Values are mean±S.D, triplicate determination

When a radical reacts with hydrogen atom from unsaturated lipid, the lipid becomes a free radical which later reacts with oxygen to forming peroxyl. Upon rearrangement peroxyl radical break down to form malondialdehyde (MDA) and 4-hydroxynonenal (HNE), which cause damage to macromolecules (Marnett, 1999). This process can worsen hypertensive case. Therefore, our report reveals that *H. crinita* inhibits lipid peroxidation in a concentration dependent pattern as shown in **figure 6**.

Hydrogen peroxide present in biological systems can generate free radicals through the enzyme myeloperoxidase/chloride/H_2O_2 reaction. Myeloperoxidase in neutrophils converts H_2O_2 and Cl^- ions into hypochlorous acid which is an oxidant. Hydroxyl radical can also be generated from $O_2^{-\cdot}$ and H_2O_2 or H_2O_2 and Fe^{2+} given rise to $^{\cdot}OH$ radical (Nimse and Palb, 2015). This dangerous radical hydroxyl radical is better prevented by neutralizing H_2O_2. The results of the present study show that *Heinsia crinita* inhibited H_2O_2 in a concentration dependent fashion as shown in **figure 4**.

Cardiac arginase competes with the substrate L-arginine with nitric oxide synthase. Elevated levels of cardiac arginase are leading to endothelial nitric oxide synthase uncoupling, followed by very low levels of NO and increased formation of ONOO. This imbalance in low levels of NO plays a role in endothelial dysfunction leading to hypertension (Mahdi et al., 2020). Our results (**Table 3**) show that *H. crinita* inhibited cardiac arginase in vitro in a concentration dependent manner, this report is similar to the works of Attia et al. (2019) and Oboh et al. (2021).

The hydroxyl radical generated is very reactive can attack DNA, protein and lipids leading to hypertension (Touyz, 2004a) because free radicals like $^{\cdot}OH$, $O_2^{\cdot-}$ and lipid peroxidation products react with NO forming ONOO, thereby reducing its availability for vasorelaxation and myocardial contractility resulting in hypertension (Touyz, 2004a). Depletion of NO and the presence of various ROS are seen in hypertensive patients (Touyz, 2004b). The reports of our study show how *H. crinita* scavenged hydroxyl radical in a concentration dependent way as depicted in **figure 5**.

Molecular docking is a technique used for screening potential druggable candidates carried out by modeling and simulation of the interactions between a protein's receptor and a ligand. In the present study selected bioactives punicacortein c, granatin b, granatin a,castalin, praecoxin a and punicalagin in *Heinsia crinita* extract were docked into the active site of cardiac arginase (PDB ID: 4HWW). These compounds interacted favorably with the active site of cardiac arginase (PDB ID: 4HWW) thereby affording binding affinities of -6.48, -6.29, -4.94, -5.16, -5.53 and -5.16 kcal/mol for punicacortein c, granatin b, granatin a,castalin, praecoxin a and punicalagin respectively. These favorable interactions were made possible through H-bonding, hydrophobic, polar, pi-cation and salt bridge interaction between the ligands and cardiac arginase (PDB ID: 4HWW). These results (**Table 4, figure 7 and 8**) reveal that *Heinsia crinita* extract inhibits cardiac arginase and reduces hypertension *in vitro*. Therefore, all the bioactives docked against cardiac arginase (PDB ID: 4HWW), punicacortein c, granatin b, granatin a,castalin, praecoxin a and punicalagin possess atomic properties favorable to interact with the active site of cardiac arginase inhibiting and lowering the activity. The report is similar to the works of Eboh et al. (2025) and Mahdi et al. (2024).

Table 4. Docking score and protein residues-ligand interactions: PDB ID: 4HWW

S/N	Name		Type of Protein-Ligand Interaction				
		(kcal/ mol)	H-bonding	Hydrophobic	Polar	Pi-cation	Salt bridge
1	Punicacortein_C	-6.48	Asn-139, Hip-141, Asp-181, Asp-128, Glu-186	Pro-20, Ile-129, Pro-184, Val-182, Val-248	Thr-136, Ser-137, Asn-130, Asn-139, Thr-246	Arg-21	Hip-126,
2	Granatin_B	-6.29	Asn-139, Hip-141, Asp-181, Asp-183, Arg-21	Pro-20	Asn-139, Ser-137, Asn-130, Thr-136, Thr-246	-	-
3	Granatin_A	-4.94	Asp-128, Arg-21, Asn-139, Thr-136, Glu-186, Asp-181	Pro-20, Pro-184, Val-182	Asn-130, Thr-246, Asn-139, Ser-137, Thr-136	Hip-126	-
4	Castalin	-5.16	Asn-139, Hip-141, Gly-245, Asp-183, Asp-181	Pro-20, Pro-247, Val-248	Asn_130, Ser-137, Asn-139, Thr-126	Arg-21	Arg-21
5	Praecoxin_A	-5.53	Asp-183, Asp-128, Hip-141, Thr-136	Pro-20, Val-248, Pro-247	Thr-246, Thr-136, Ser-137, Asn-139, Asn-130	Arg-21	-
6	Punicalagin	-5.16	Asp-183, Asp-128, Hip-141, Arg-21, Thr-136, Lys-68	Val-182, Pro-20	Thr-246, Thr-136, Asn-139, Ser-137	-	Arg-21

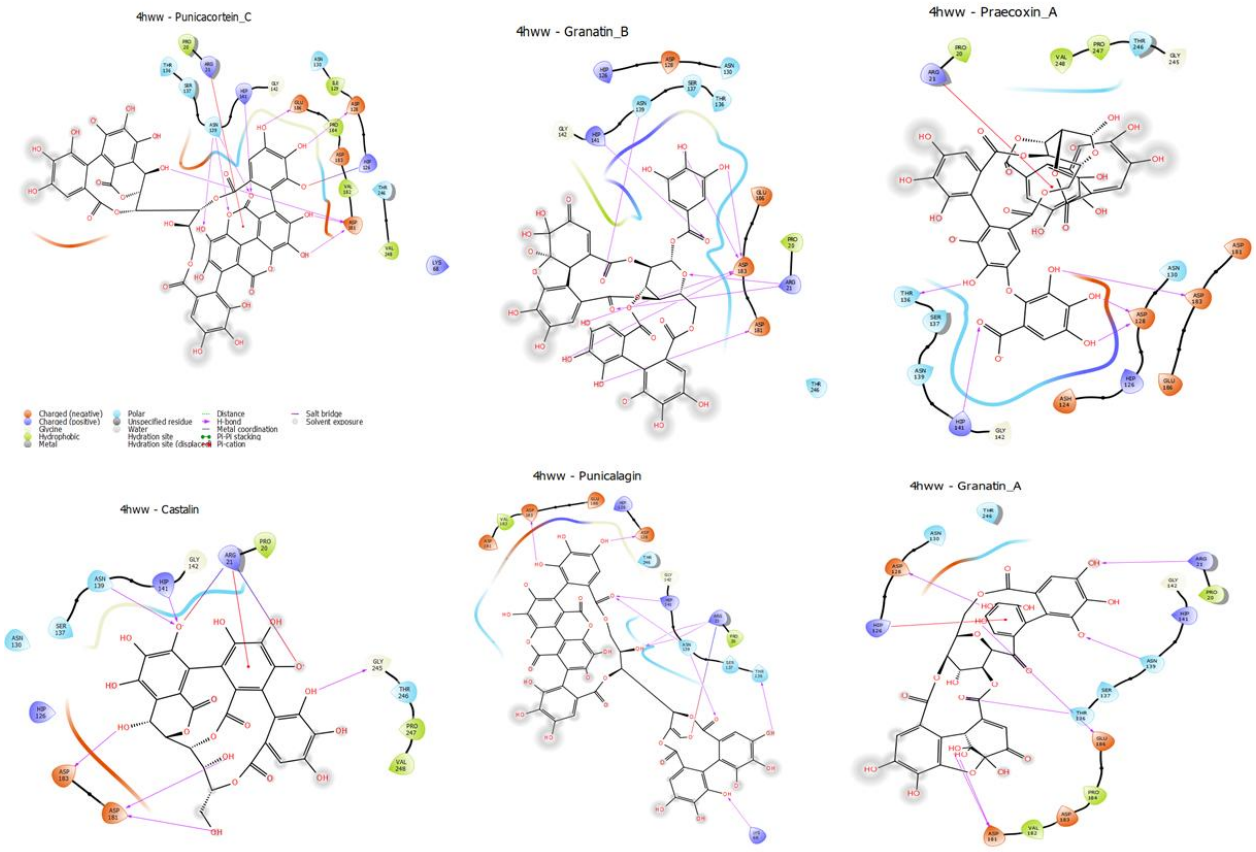

Fig. 7. 2D interaction between punicacortein c, granatin b, granatin a,castalin, praecoxin a and punicalagin and the active site of cardiac arginase (PDB ID: 4HWW)

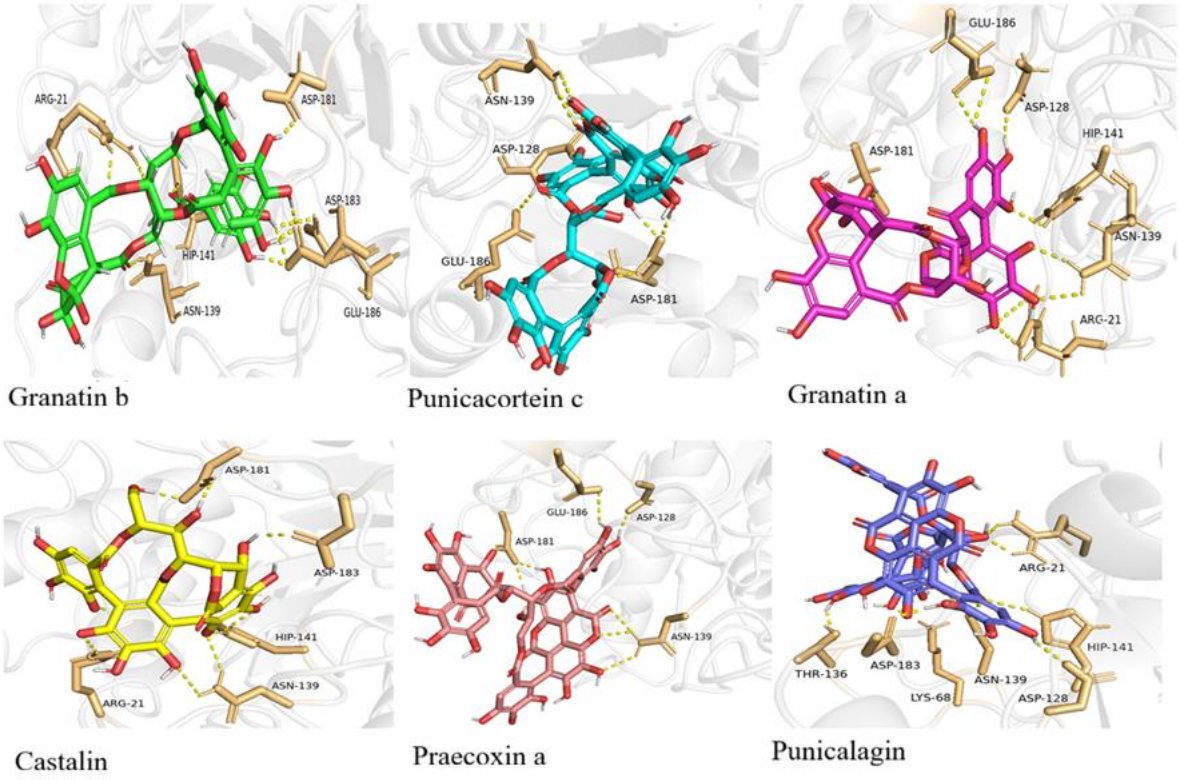

Fig. 8. 2D interaction between punicacortein c, granatin b, granatin a, castalin, praecoxin a, and punicalagin and the active site of cardiac arginase (PDB ID: 4HWW)

Conclusions

Hypertension is a disease that kills silently and has become a problem in the healthcare system. Therefore, our study has revealed that the methanolic extract of *H. crinita* contains phytochemicals that possess diverse roles. Tannins detected in the plant also afforded the plant antioxidant properties by quenching radicals like DPPH, peroxides, H_2O_2 and hydroxyl radicals. The extract also inhibited cardiac arginase *in vitro* and *in silico.* Therefore *H. crinita* possess the potential to inhibit cardiac arginase linked hypertension.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualization: A.S.E and E.W.; Methodology: B.M.A and A.S.E; Software: S.B.; Validation: B.P.S. and E.W.; Formal analysis: B.M.A., A.S.E and E.W; Data curation, writing-original draft preparation: A.S.E; Writing-review and editing, visualization and supervision: A.S.E.

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Acknowledgments

The authors express their gratitude to the Laboratories of Biochemistry and Medicinal and Pharmaceutical Chemistry, Niger Delta University, Bayelsa State, Nigeria.

Fundings

No external funding was received

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