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RESEARCH ARTICLE

Biochemical, Phytochemical Profile and Angiotensin-1 Converting Enzyme Inhibitory Activity of the Hydro-methanolic Extracts of *Tulbaghia acutiloba* Harv.

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Abstract

Phytotherapeutic treatments have been on the ascendancy over the years as the quest for alternate and easily accessible health care is on the rise. *Tulbaghia acutiloba* has been used by both traditional healers and the South African population for the management of chronic conditions, but lacks scientific authentication. Hydro-methanolic extracts (roots, rhizomes, leaves and flowers) of the plant were evaluated for their antioxidant activities, biochemical and phytochemical profile. Additionally, the heavy metal content and antihypertensive effects including Angiotensin 1-Converting Enzyme Inhibitory (ACEI) activities were investigated. The antioxidant ability of the hydro-methanolic extracts were determined by *2, 2-diphenyl-1-picryl hydrazyl (DPPH),* hydrogen peroxide and nitric oxide scavenging activities. Phytochemical profile was assessed using qualitative and quantitative methods whereas the heavy metal toxicity was determined by using Inductively-Coupled Plasma-Optical Emission Spectrometry (ICP-OES). *In vitro* ACEI activity was determined by the hydrolysis of the tripeptide, *hippuryl –L-Histidyl-L-leucine (Hip-His-Leu).* All the extracts showed potent antioxidant activities which was concentration dependent. Phytochemical analysis showed the presence of phenols, amino acids and alkaloids in all the extracts. The leaves showed a relatively higher total phenolic content of 43.26±1.15 mgGAE/g. Gas Chromatography–Mass Spectrometry (GC-MS) analysis showed the presence of major compounds such as *α-linolenic acid* in the leaves as well as *oleic acid* and *palmitic acid* in other parts of the plants. Toxicity of heavy metal was undetected in all extracts of the plant. All the extracts of the plants showed a >50% ACE inhibition at different concentrations with the leaves showing a relatively higher inhibitory activity (76.66 ± 1.65, *IC50;* 154.23 *µg/ml)* compared with the other parts of the plants. It is therefore concluded in this study that the biological activities and phytochemical component of the hydro-methanolic extracts of *T. acutiloba* is indicative of its possible use for the treatment as well as prevention of hypertension and oxidative stress-related diseases.

Keywords: Angiotensin 1-Converting Enzyme, Antioxidant, Heavy Metal Toxicity, *Tulbaghia acutiloba,* Phytochemical

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1. Introduction

Chronic lifestyle-related metabolic diseases such as Hypertension (HTN) cause double the number of deaths compared to infectious diseases[1]. Hypertension, as the major risk factor of cardiovascular diseases, continues to be on the ascendancy with increased numbers of patients with high blood pressure seeking herbal and naturopathic treatments, especially in developing countries such as South Africa[2]. Phytotherapy is an integral part of the traditional healing system in developing countries, a trend that is rapidly rising even in developed countries owing to the belief that herbal remedies and indigenous medicine are generally considered safer, affordable, easily accessible and with no or minimal side effect as compared to the drugs used in conventional medicine[3]. Moreover, several conventional drugs are derived from herbal medicines, but the major variation between the two is that herbal drugs mostly contain a number of bioactive compounds, whereas the conventional drugs mostly contain an isolated bioactive substance; therefore, the constituents of both herbal and conventional medicines may act on one another to augment or enhance an effect. In South Africa, it is estimated that between 12 and 15 million people still depend on traditional herbal medicine, from as many as 700 indigenous plant species[4,5]. New remedies discovered by traditional healers are increasingly being used (especially for cardiovascular diseases), increasing the need for scientific validation of mechanism of action and drug dosage formulations[6].

Bioactive compounds such as flavonoids, phenols, saponins, steroids and tannins obtained from medicinal plants, show high antioxidant activities that are key to the therapeutic management of chronic conditions such as hypertension, cancer and diabetes[7]. It is now widely reported that central to the pathology of these diseases is oxidative stress, caused by free radicals. Free radicals are either generated in the human body, *in situ* or from the external environment through air pollutants[8], however an increase in free radicals in the body could lead to oxidation of biomolecules[9], and other pathological conditions[10]. Prolonged oxidative stress leads to heart diseases, and in particular the risk factor, hypertension[11]. Moreover, the reducing capacity or scavenging activity of an extract or compound against free radicals may serve as a significant indicator of its potential antioxidant activity[12].

It is important to note that a drawback of phytotherapy may include heavy metal accumulation, absorbed from the environment by the plant. The continuous ingestion of toxic heavy metals such as lead, chromium, barium, arsenic and cadmium, into the human body may have genetic and epigenetic effects leading to numerous disorders[13]. Therefore, there is the need to evaluate medicinal plants for toxic heavy metal content.

The increasing use of plants therapeutically, with little scientific data to verify their safety profile and dosage could lead to side effects. One such plant is *Tulbaghia acutiloba. T. acutiloba* is commonly known as 'wild garlic", "*ishaladi lezinyoka*" in Zululand, "*sefothafotha*" in South Sotho and lisela in Swaziland. It is indigenous to the Eastern Cape, KwaZulu-Natal, Gauteng, Free State and Mpumalanga regions of South Africa[14]. Generally, It is used as a herbal treatment for a number of health conditions such as fungal infections, bruised skin, sores and also forms part of many delicacies in the Eastern Cape region[15]. Traditionally, the Basothos, cook the leaves, bulb and roots of *T. acutiloba* to make a lotion. This lotion is used to wash incisions to avoid bacterial contamination and aid in wound healing. A concoction of this plant is made and drunk for the treatment of high blood pressure as well as for diabetes[16,17]. They are also grown domestically by the Zulus and Xhosas around their homes as a protective charm to repel snakes and other harmful reptiles[17].

We have previously investigated *Tulbaghia violacea* or 'wild garlic' which is closely related to the *Tulbaghi acutiloba*. We were able to show antidiabetic, antihypertensive and reno-protective effects which could be attributed to its organosulfur compounds[18].

In spite of the therapeutic herbal properties and the wide use of *T. acutiloba*, there is a lack of scientific information concerning the pharmacological and biological activities of its extracts. This study investigated the antihypertensive properties, antioxidant activities, toxic heavy metal content and phytochemical profile of *T. acutiloba*.

2. Material and Methods

2.1 Preparation of Plant Material and Extract

Fresh rhizomes, roots, leaves and flowers of *T. acutiloba* were collected from Durban, KwaZulu-Natal and authenticated by Prof Himansu Baijnath (Botanist) from the University of KwaZulu-Natal (UKZN). The parts collected were washed thoroughly under tap water and was air dried at room temperature for 72 hours. Each part of the plant was crushed in a Waring blender. The powdered form (50g) of each part was immersed in separate 500 ml hydro-methanolic solutions (80% methanol; 20% water) and shaken at 80rpm for 48 hours. The crude extract for each part was filtered and concentrated in a rotary evaporator, thereafter air dried to form sludge. The remaining extract was weighed and the percentage of yield was determined for each part of the plant.

2.2 Antioxidant Activity

2.2.1 2, 2-Diphenyl-1-Picryl Hydrazyl (DPPH) Radical Scavenging Activity

The scavenging activity of each part of the plant on the stable radical DPPH was determined according to the method by Murthy *et al.*, (2002) with some modifications[19]. A volume of 150 µl of the hydro-methanolic solution of each extract at different concentrations (1000, 500, 250, 125, 62.5, 31.3 and 15.6 µg/ml) were mixed with 2.85 ml of a methanolic solution of DPPH (0.1 mM). Equal amount of MeOH and DPPH with no extract was used as control. The reaction was kept at room temperature for 30 minutes in the dark and thereafter the absorbance was measured at 517 nm against methanol as a blank using a UV spectrophotometer (SPECTROstar^{nano} BMG LABTECH, Germany). The positive control used in the study was Ascorbic acid. A triplicate run of all tests was performed. The free radical scavenging activity was expressed as percentage in accordance with the equation as follows:

% scavenging activity (DPPH) = Ac-As/Ac × 100

Where As = absorbance of sample and Ac = absorbance of control

2.2.2 Hydrogen Peroxide Scavenging Activity

The potential of the *T. acutiloba* extracts in the scavenging of hydrogen peroxide was evaluated using a method according to Ruch *et al.*, (1989) with some modifications[20]. Hydrogen peroxide solution of 40 mM was made in a phosphate buffer of pH 7.4. Briefly, 0.2 ml of the various concentrations of the extracts (1000, 500, 250, 125, 62.5, 31.3 and 15.6 µg/ml) were added to a hydrogen peroxide solution (0.6 ml, 40mM) for 10 minutes. The absorbance of hydrogen peroxide was determined at a wavelength of 560 nm after 10 minutes. A solution of phosphate buffer without hydrogen peroxide was used as a blank before each reading. The positive control used in the study was Ascorbic acid. The hydrogen peroxide scavenging activity of the extracts was expressed as percentage as follows:

% scavenging activity (H_2O_2) = Ac-As/Ac × 100

Where As = absorbance of sample and Ac = absorbance of control

2.2.3 Nitric Oxide Scavenging Activity

The scavenging activity of *T. acutiloba* against Nitric oxide was measured in accordance with the method developed by Govindarajan *et al.*, (2003) with some revisions[21]. A mixture of 0.5ml of a 10 mM sodium nitroprusside solution in a phosphate buffered saline was added to 1ml of different concentrations of the extracts (1000, 500, 250, 125, 62.5, 31.3, 15.6 µg/ml) and incubated at 25°C for 180 min. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner to test samples. After 180 min, the incubated mixture (1.5ml) was taken out. A solution of 1.5 ml Griess reagent made up of 1% sulphanilamide, 0.1% N-1-naphthylethylenediamine dihydrochloride and 2% phosphoric acid was used to dilute the incubated solution. The absorbance of the resulting chromophore formed after the addition of the Griess reagent to the incubated solution was measured at 546 nm. The positive control used in the study was Ascorbic acid. The radical scavenging activity of the extracts was expressed as a percentage using the formula as follows:

% scavenging activity (NO_2) = Ac-As/Ac × 100

Where As = absorbance of sample and Ac = absorbance of control

2.3 Determination of *In-vitro* Angiotensin-1 Converting Enzyme (ACE) Inhibition Activity

***Stock Substrate Solution*:** A 100ml stock substrate solution of 25mM hippury l –L-Histidyl-L-leucine (Hip-His-Leu) was freshly made by the dissolution of 1.12g of Hip-His-Leu (sigma Aldrich, schnelldorf, Germany) in 25mM sodium hydroxide (NaOH).

***Sodium Borate Buffer*:** Sodium borate buffer was freshly made up by the dissolution of 30.5g of boric acid (H_3BO_4) and 65.7g of sodium chloride (NaCl) in distilled water. The final volume prepared was 1L and this preparation gave a 1.25 –fold concentration (0.5M). The pH of the solution was adjusted to 8.3 using 13N NaOH.

***ortho-phthaldialdehyde* (*o*-phthaldialdehyde):** *Ortho-Phthaldialdehyde* (OPA) (sigma Aldrich) solution was prepared by dissolving 200mg of OPA in 10ml of methanol.

***Assay Buffer*:** An assay buffer used in the study was made by mixing one volume of stock substrate solution to four volumes of 0.5M sodium borate buffer

The activity of ACE was determined on the principle that the tripeptide hydrolysis is blocked at the amino terminal. The tripeptide (Hip-His-Leu) has a sequential terminus of Angiotensin I (Ang I) and its subsequent chemical breakdown due to the reaction with water could be identified by the resultant hippuric acid formation. The ACE inhibition activity of each plant extract was performed in accordance with the method developed by Santos *et al.* (1985) with amendments[22]. Briefly, 10µl of ACE from rabbit lung (Sigma Aldrich) was incubated in 480µl of assay buffer and 10µl of different concentrations of plant extracts (1000µg/ml, 500µg/ml, 250µg/ml and 125µg/ml) in a test tube for 15 mins at 37°C. The reaction was terminated by adjusting the pH with 1.2ml of 3.4N NaOH. *Ortho-phthaldialdehyde* (100µl) was then added to the reaction mixture and left for 10mins. In order to acidify the solution thereafter, 200µl of 3N hydrochloric acid was mixed to the solution. The measurement of the resulting chemical Histidine-Leucine (His- Leu) was made fluorometrically at 365nm excitation and 495nm emission wavelengths using a fluorescence spectrophotometer (F-2500/F-4500 FL solutions). A blank solution prepared by the addition of the ACE from rabbit lung after 3.4N NaOH without the test compounds was used. Ramipril, a conventional ACE inhibitor drug was used as control.

Percentage Inhibition was expressed as % Inhibition: = Ab-As/Ab x 100

Where As = absorbance of sample and Ab = absorbance of blank

2.4 Phytochemical Anlaysis

2.4.1 Primary and Secondary Metabolites

The presence and absence of alkaloids, tannins, terpenoids, steroids, flavonoids, saponins, phenols, glycosides, cardiac glycosides, amino acid and reducing sugars were detected as per reported methods[23-32].

2.4.2 Total Phenolic Content

The method developed and standardized by Singleton and Rossi (1965)[33] with minor modifications was used in the determination of the total phenolic content of each extract of the plant. The Folin and Ciocalteu reagent was used in the study. Each plant extract (0.2 ml) was dissolved in a 0.6 ml of distilled water and 0.2 ml of Folin-Ciocalteu's phenol reagent. The reaction was timed for 5minutes and thereafter, 1 ml of sodium carbonate solution saturated (8% w/v in water) was placed into the mixture and the resulting mixture volume was topped up to 3 ml with distilled water and kept in the dark for 40 min for the development of a colour. After incubation the absorbance of the resulting colour was determined at 750 nm wavelength. The phenolic content was expressed as Gallic acid equivalents (GAE/g) of dry plant material with reference to a Gallic acid standard curve with concentrations 25 - 200µg/l. All experimental procedures were conducted in triplicates. Readings for the various extracts and standards were carried out using a spectrophotometer

(Cary 50 Bio UV-Vis Spectrophotometer, Varian) at 725 nm with a reagent blank.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Plant Extracts

The gas chromatography- mass spectrometry analysis of each part of *T. acutiloba* extract was performed using the Perkin- Elmer Gas Chromatography (Clarus 580) which has an MSD spectrometer (Clarus SQ8S) instrument with in-built auto sampler. Samples were analyzed on Elite 5MS columns (30m × 0.25mm id × 0.25µm). The temperature of the oven was programmed from 37°C to 320°C at an 18-25°C/min rate and held for 0.5; 1.85min and 18 at 320°C, respectively. The temperature of the injector was 250°C with an MS Ion source temperature of 280°C with a full scan and a solvent delay of 0 – 2.3min. The scan range of the MS scan was m/z 35-500 in 0.10s. Each sample (1µl) was introduced into a helium carrier gas with a split flow of 20ml/min.

2.6 Heavy Metal Toxicity Testing

Heavy metal toxicity analysis was performed using the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The test is based on the principle that when energy from a plasma source is transferred to a sample, the component elements (atoms) get excited and they move to a higher energy state. Once these stimulated atoms come back to a low energy state, rays are emitted. There is therefore the measurement of the released emitted rays that characterises the wavelength of the photons. The constituent elements are identified by their characteristics emission lines and quantified by the intensity of the same lines[34]. The following heavy metals were analysed; Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Lead (Pb), and Nickel (Ni). Each extract (0.5g) was weighed out. The digestion was carried out on a hot plate (60-70 °C) using 10 ml of Aqua Regia. The mixture was then filtered into a 10ml volumetric flask. The solution was made up to the mark with double distilled water. The solution was then filtered into ICP vials with a 0.45 micro-filter and analysis was performed using ICP-OES. Analysis was performed in triplicate.

2.7 Data Analysis

All experimental observations were performed in triplicate and were expressed as an average of three analyses ± Standard Error of the Mean (SEM). One sample t-test was used in the statistical analyses for comparing two observations and one-way ANOVA for multiple comparisons. The p-value<0.05 were regarded as significant.

3. Results

3.1 Plant Extraction and Physicochemical Properties

The percentage yield and the physicochemical properties of the parts of *T. acutiloba* are shown in Table 1. The plant is well known for its sweet-scented nature that is inherent in the flowers and leaves.

Table 1. Physicochemical properties and percentage yield of extracts of *Tulbaghia acutiloba*

Plant Part	Percentage yield (%)	Physical appearance	Odour
Roots	2.62	Pale brown	Garlic-like odour *(alliaceous odour)*
Rhizomes	8.00	Brown	Garlic-like odour *(alliaceous odour)*
Flowers	3.62	Green with fleshy orange to reddish brown ring	Sweet scented
Leaves	12.8	Dark green	Sweet scented

3.2 Antioxidant Activities

3.2.1 2, 2-diphenyl-1-picryl hydrazyl (DPPH) Radical Scavenging Activity

It is observed from the results that there is a concentration dependent activity for the DPPH radical scavenging activity for the various parts (roots, rhizomes, leaves,

flowers) of *T. acultiloba*. Interestingly, the rhizomes showed a relatively higher antioxidant activity at the various concentrations than all the other parts of the plants with an IC50 value of 201.99µg/l and a peak scavenging activity of 95.18± 0.20. However, there was no significant difference in the activities of the various parts of the plants as compared to the positive control, ascorbic acid (p>0.05) as shown in Figure 1.

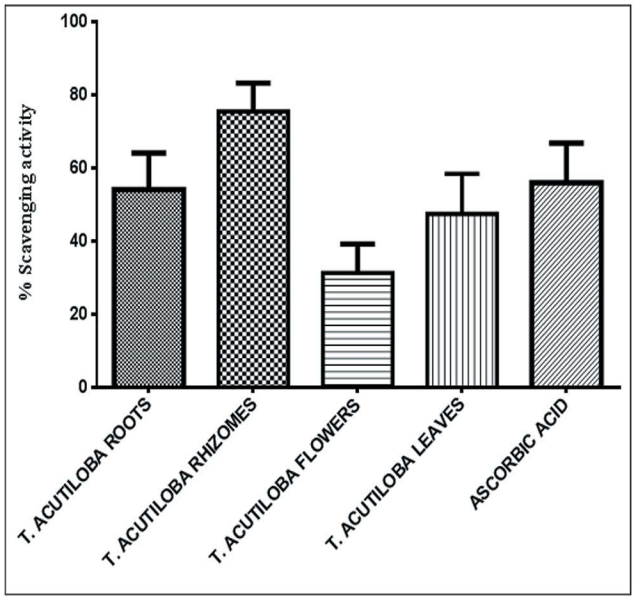
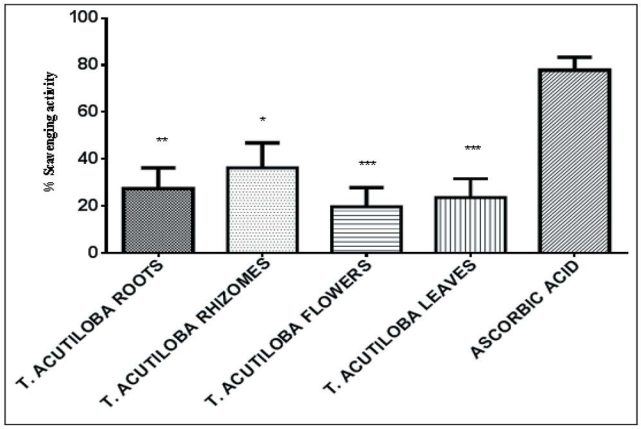


Figure 1. DPPH scavenging activity.

3.2.2 Hydrogen Peroxide Scavenging Activity

Figure 2 shows the hydrogen peroxide scavenging activities of each part of the plant and the positive control. The result indicated a dose dependent inhibition of hydrogen peroxide with the rhizomes once again showing a relatively higher scavenging activity than the other parts of the plants with a peak value of 72.42±0.35 occurring at the concentration of 1000µg/l and an IC50 value of 518.16µg/l. Interestingly, there was no scavenging activity at 15.6µg/l for all the parts Moreover, all the parts of the plants showed a significantly lower scavenging activities (Leaves (p<0.001), flowers (p<0.001), roots (p<0.01), rhizomes (p<0.05)) when compared with the scavenging activities of Ascorbic acid.

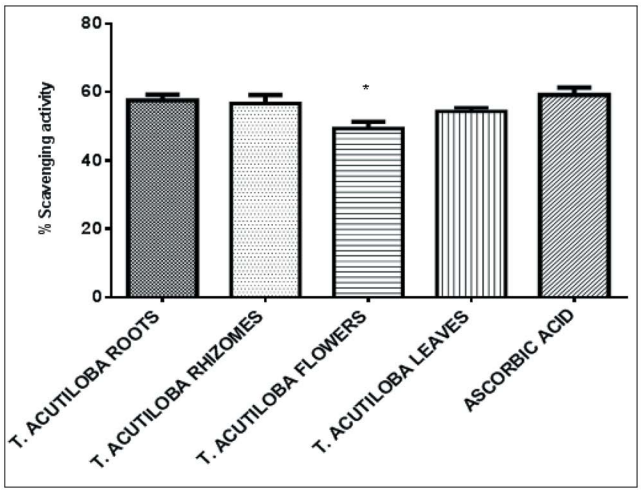


*significant different (p<0.05) from the value of standard compound; **significant different (p<0.01) from the value of standard compound; ***significant different (p<0.001) from the value of standard compound

Figure 2. H_20_2 scavenging activity

3.2.3 Nitric Oxide Scavenging Activity of *T. acutiloba*

The outcome of the free radical scavenging potential of each extract tested is presented in Figure 3. There was a generally good scavenging activity for all the extracts though the results did not show a strong dose-dependent activity. Interestingly, the lowest concentration of 15.6µg/l, produced an effective antioxidant activity. The leaves, rhizomes, and roots did not show any significant difference (p>0.05) from the activity of Ascorbic acid with the exception of the flowers that showed a significantly lower activity (p<0.01).

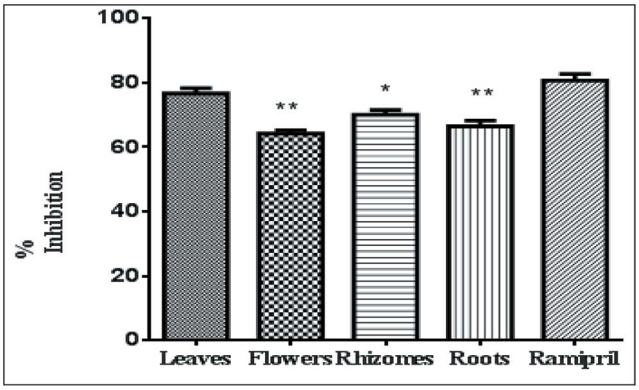


*significant different (p<0.05) from the value of standard compound

Figure 3. Nitric oxide scavenging activity.

3.3 *In vitro* Angiotensin-1 Converting Enzyme (ACE) Inhibitor Activity

Figure 4 shows the percentage ACE inhibition of the hydro-methanolic extract of the various parts of the plants. All extracts showed a percentage inhibition of >50% within a concentration range of 125 - 1000µg/ml. The conventional ACEI drug used as a positive control in the study, showed a mean percentage inhibition of 80.67% with an IC50 value of 112.91µg/ml. There was no significant difference from the activity of the leaves (76.6% ± 1.65; IC50:154.23) as compared to the value of the conventional drug, Ramipril. Moreover, the roots and flowers showed a significant lower activity when compared to both the conventional drug and the leaves. Conversely, the rhizomes showed a significant lower activity as compared to Ramipril but no significant difference when compared to the activity of the leaves. This observation from the study indicates a comparable activity of the leaves to the activity of the conventional drug, Ramipril.



*Significant different (P<0.05) from the value of Ramipril; **Significant different (p <0.05) from the value of both Ramipril and Leaves.

Figure 4. ACE inhibition activity of *T. acutiloba.*

3.4 Phytochemical Analysis and Total Phenolic Content of *T. acutiloba*

The phytochemical analysis of the extracts of the plants is shown in Table 2. Our findings indicate the presence of tannins, phenols, alkaloids and amino acids in all parts of the extract of the plant used in the study. Moreover, saponins were found only in the roots of the plants. The rhizomes and leaves showed the presence of terpenoids. However, there was an absence of glycosides, cardiac glycosides, flavonoids and reducing sugars in all parts of the plant. The presence of steroids was identified in the rhizomes only.

Table 2. Phytochemical analysis of *T. acutiloba*

Phytochemical	Rhizomes	Roots	Flowers	Leaves
Tannins	+	+	+	+
Saponins	-	+	-	-
Terpenoids	+	-	-	+
Phenols	+	+	+	+
Glycosides	-	-	-	-
Cardiac Glycosides	-	-	-	-
Steroids	+	-	-	-
Alkaloids	+	+	+	+
Flavonoids	-	-	-	-
Amino Acids	+	+	+	+
Reducing sugars	-	-	-	-

'+' indicates the presence; '-'Indicates the absence

3.5 Total Phenolic Content of *T. acutiloba*

The total phenolic content was expressed as mgGAE/g extract as y =0.0072x + 0.0819 (R^2 = 0.9981). The total phenolic content of the various extract is represented in Table 3. The leaves showed a relatively higher phenolic content of 43.26 ± 1.15 mg/g as shown in Table 3.

Table 3. Total phenolic content of parts of *T. acutiloba*

Sample	Total Phenolic content (mg/g)
Rhizomes	28.04 ± 0.36
Roots	31.54 ± 8.28
Flowers	20.80 ± 1.22
Leaves	43.26 ± 1.15

3.6 GC/MS Analysis of Hydro-Methanolic Extracts of *T. acutiloba*

Gas Chromatography–Mass Spectrometry (*GC-MS*) was performed to determine the individual constituents in each extract. The various chemical components were detected by corresponding their retention time and mass spectra with that of an inherent citations library. The outcome of the analysis is shown in Table 4. A total of 14 compounds were isolated in the extracts. Major compounds identified include acetic acid, *α-linolenic acid,* palmitic acid, oleic acid, *9,12- octadecadienoic acid* and decanoic acid among others.

Table 4. Compounds identified in the gas chromatographic analysis of *T. acutiloba*

Sample/compound name	Chemical formula	Retention time (mins)	Relative abundance height (%)
4a. Leaves			
Acetic Acid	CH3COOH	9.89	10.07
α-linolenic acid	$C_{18}H_{30}O_2$	21.87	4.24
Palmitic acid	$C_{16}H_{32}O_2$	22.61	5.92
Oleic Acid	$C_{18}H_{34}O_2$	24.22	54.56
4b. Roots			
Acetic Acid	CH3COOH	9.73	5.75
Decanoic acid	$C_{10}H_{20}O_2$	20.16	3.12
1-Tetradecyne	$C_{14}H_{26}$	21.80	3.22
9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	21.81	3.22
Hexanamide	$C_6H_{13}NO$	22.61	8.37
Oleic Acid	$C_{18}H_{34}O_2$	24.22	61.54
4c. Rhizomes			
1-methylcyclo-propanemethanol	$C_5H_{10}O$	10.14	3.10
Dodecanamide	$C_{14}H_{29}NO_2$	22.60	7.86
Lauramide	$C_{12}H_{25}NO$	22.61	8.78
9-octadecanamide	$C_{18}H_{37}NO$	24.21	69.44
Oleamide	$C_{18}H_{35}NO$	24.22	71.50
4d Flowers			
Acetic acid	CH3COOH	9.76	8.32
myristimide	$C_{14}H_{29}NO$	22.60	8.15
Palmitic acid	$C_{16}H_{32}O_2$	22.61	6.41
Oleic acid	$C_{18}H_{34}O_2$	24.21	65.79

3.7 Heavy Metal Toxicity Analysis

Table 5 shows the analysis of heavy metals that are of great concern to public health. From the results, it is evident that there were no toxic heavy metals detected in *T. acutiloba*. Moreover, the levels of barium, chromium and nickel were insignificant.

Table 5. Toxic heavy metal analysis of *T. acutiloba*

Metal	Root	Leaves	Rhizomes	Flowers
Arsenic	Not Detected	Not Detected	Not Detected	Not detected
Barium	**0.25ng/ml***	**0.29ng/ml***	**0.131ng/ml***	**0.20ng/ml***
Cadmium	Not Detected	Not Detected	Not Detected	Not detected
Chromium	**0.12ng/ml***	Not Detected	Not Detected	Not detected
Lead	Not Detected	Not Detected	Not Detected	Not detected
Mercury	Not Detected	Not Detected	Not Detected	Not detected
Nickel	**2.06ng/ml***	**0.22ng/ml***	**0.424ng/ml***	**0.15ng/ml***

*values are far below the minimum toxicity levels

3.8 Correlation between the Antioxidant Activites, Total Phenolic Content and ACE Inhibition Activities of *T. acutiloba*

Table 6 indicates the correlation between the antioxidant activities, Total phenolic content and ACE inhibition activities of *T. acutiloba* using the Pearson correlation coefficient analysis. From the results obtained, there was generally a weak positive correlation between the antioxidant activities and ACE inhibition activities. However, a moderate positive correlation was observed between the Total Phenolic content and ACE inhibition activities (r=0.464).

Table 6. Pearson correlation coefficient between antioxidant activities, total phenolic content and ACE inhibition activities of *Tulbaghia acutiloba*

	ACE Inhibition Activity	Total Phenolic Content
DPPH scavenging activity	0.092	0.168
Nitric Oxide scavenging activity	0.035	0.033
Hydrogen peroxide scavenging activity	0.077	0.226
ACE Inhibition activity		0.464

4. Discussion

The prevention and treatment of hypertension involves several mechanisms in the body that contributes significantly to the regulation of blood pressure[35]. These mechanisms include the reduction of oxidative stress in the body, inhibition of Angiotension-I Converting Enzyme (ACE), the presence of bioactive compounds as well as the regulation of nitric oxide in the human body[36].

Since most diseases are related to increased free radicals or reactive oxygen species, the potential scavenging activity will reduce excess free radicals which may otherwise lead to disease[37]. Our current findings indicate an active scavenging activity of the various parts of *T. acutiloba*, as seen in the DPPH assay. Our findings in the scavenging activities of the DPPH is in corroboration with other studies done on similar plant species of the same family of *Tulbaghia* where effective scavenging activity was observed[38,39].

Additionally, though our findings indicate a significantly lower scavenging activity as compared to Ascorbic acid, there was generally a good scavenging

activity of hydrogen peroxide especially at the highest concentration of 1000µg/L of each part of the plant. This finding too seems to be in line with other studies performed on similar species of plants within the family of *Tulbaghia*[38,40]. Hydrogen peroxide is known to be a weak oxidizing agent that incapacitates some enzymes in the body directly, usually through the oxidation of essential thiol (-SH) groups. It is permeable to cells and can cross cell membranes rapidly and once inside the cell, it could form a bond with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this could account for the basis of many of its toxic effects[41,42]. Therefore, the reducing capacity of an extract or compound may serve as a significant indicator of its potential antioxidant activity[12].

With respect to Nitric Oxide scavenging activities, it was generally observed that there were no significant differences between the activities of the parts of the plants and Ascorbic acid with the exception of the flowers. This findings seems to be in agreement with other studies conducted on *Tulbaghia violacea* which was observed to have a good scavenging activity against Nitric oxide[43,44]. There is a substantial evidence that Nitric Oxide (NO) is a potent pleiotropic mediator of physiological processes such as neuronal signaling, smooth muscle relaxation, regulation of cell mediated toxicity and inhibition of platelet aggregation[45]. It is known to have a biphasic action at the cellular level; optimal concentrations of NO are known to safeguard the tissues against oxidative killing whereas higher doses promote cell death, and these dual effects occur through distinct mechanisms[46-48]. At higher doses, it is a permeable free radical which has been proven to play a crucial role in the pathogenesis of inflammation and pain. Nitric oxide inhibitors that could reduce excessively high dose of nitric oxide to optimal levels, have been shown to have anti inflammatory effects on inflammatory tissues and tissue changes are seen in models of inflammatory bowel disease[49,50]. This may be one of several reasons for its usage by traditional healers in the hypertensive and wound healing process; as anti-inflammatory agents are very crucial in wound healing and the management of high blood pressure.

The phytochemical analysis showed the presence of phenols in all parts of the plant. Phenolic compounds are well known for their antimicrobial and antioxidant protection against cancer and heart diseases. The high concentrations of phenolic compounds may account for the medicinal value of *T. acutiloba* in the management and treatment of oxidative stress induced disorders. Moreover, tannins are water soluble polyphenols that bind and precipitate proteins and other organic compounds[51,52]. There are controversial views of the role of tannins in the human body. In the past years, tannins were known to be anti-nutritional because it was suggested that they bind to proteins and amino acids, hence reducing their absorption in the body[53,54]. However recent studies have supported their potential benefit in inhibiting the ACE and hence reducing blood pressure in the vessels[55-57]. Studies have also suggested that low doses of tannins in the human body may be very beneficial[58].

T. acutlioba also has alkaloids. The presence of alkaloids in plants is known for their numerous health benefits and therapeutic functions. Various studies has confirmed the effective role of alkaloids as anti-infectious, antipyretic, aphrodisiac, vasodilator, antihypertensive and anticarcinogenic[59,60]. Other studies conducted on similar species of the plants indicates an active role of such plants in the management of hypertension and infectious diseases[61,62].

Heavy Metal analysis of *T. acutiloba* also revealed that all metals analysed were either undetected or were within the safe limit, therefore, we can deduce that different parts of the plant are safe for human consumption provided it is sampled from a non-contaminated geographical location.

Heavy metals are naturally occurring elements with a high atomic weight and density[63] and are considered toxic based on their dose and route of exposure in the human body[8]. Due to the irreversible effects that heavy metals could pose on the health of human beings, this study confirms the validity of the safe usage of *T. acutiloba*[45].

In this study we also found that all parts of the plants at different concentrations produced more than 50% ACE inhibition. Additionally, the activities of the leaves were comparable to the conventional ACEI. Similar studies conducted on the parts of plants in the same family of *Tulbaghia* and related species, also exhibited more than 50% inhibition of ACE[64-66]. Importantly, unlike other studies conducted on other species of

Tulbaghia and related families, *T. acutiloba* showed very promising ACE inhibition that is comparable to the conventional ACEI, Ramipril. The significance of this is multifold; including the possible modification of these plants derived novel compounds to avoid the debilitating side effects of conventional ACEIs.

Furthermore, it was observed that the correlation between the antioxidants and ACE inhibitory effect was positively associated, although a weak correlation was recorded. However, since a moderately strong positive correlation was observed between the total phenolic content of the plant and ACE inhibitory effect, it suggests that the ability of the plant to inhibit ACE could be due to the synergistic effect of the antioxidant potential, total phenolic content and the presence of other bioactive compounds indentified in the GCMS analysis. Key among these bioactive compounds is the presence of α-Linolenic Acid (ALA), which was found mainly in the leave extract and absent in the other parts of the plant extracts. Palmitic acid and oleic acid were also identified as bioactive compounds which have supporting literature confirming their therapeutic effect especially in regards to cardiovascular diseases[67,68]. Interestingly, the higher concentration of total phenolic content, antioxidant potential and the presence of ALA and other bioactive compounds in the leaves may account for the comparable ACE inhibition activity of the leaves to the conventional drug, Ramipril. This is worth noting since none of the parts of the plants exhibited a comparable activity to Ramipril. Several studies conducted on ALA (also known as omega-3-fatty acids) have proven to be an effective therapeutic agent especially in relation to cardiovascular diseases such as hypertension[69-71]. Studies conducted on other species of plants of Tulbaghia and similar plants revealed compounds such as palmitic acids, oleic acids, octadecanoic acids among others which were also identified in our studies[72-74].

Though there are several studies to suggest that isolated compounds may produce therapeutic effects, current studies has also confirmed that bioactive compounds in certain plants may produce superior therapeutic effects when they act synergistically with other bioactive compounds[75-78].

5. Conclusion

The current study has so far shown that *T. acutiloba* contains bioactive compounds that are essential for therapeutic effects. It further shows that *T. acutiloba* may have an antihypertensive effect since it showed a comparable ACE inhibition activity to Ramipril. This finding therefore, support the ethnopharmacological use of *T. acutiloba* Harv. (Alliaceae) in the management and regulation of essential hypertension particularly in areas of the world where access to conventional ACEI drugs are limited for several reasons. *T. acutiloba* is a nutraceutical that can easily be harvested and obtained for its therapeutic effects. However, further studies are needed to explicate its effect on body organs, biochemical profile and relevant gene expressions *in vivo* in order to ascertain its comprehensive effect on hypertension.

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