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Phyto-Constituents in White Butterfly (*Clerodendrum volubile* P. Beauv) Leaf Extract Demonstrated Antioxidant Activity and Inhibited Key Enzymes linked to Hyperglycemia and Hypertension

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Abstract

Chlorogenic acid is bioactive phenolic compound found in medicinal and nutritional plants, linked to several therapeutic relevance, making it a highly commercialized phenolic compound responsible for the acceptability of the popular green tea. The aim of this research is to isolate chlorogenic acid from the white butterfly leaf and determine the antioxidant, hypoglycemic and angiotensin-1 converting enzyme (ACE-1) inhibiting potentials as a rider to further studies on the chlorogenic acid fraction. The chlorogenic acid isolate of white butterfly leaf (MLECS) was evaluated using biochemical analyses to determine its antioxidant properties, and inhibition of α-glucosidase and α-amylase activities, and ACE-1 activity. The characterization of the polyphenolic compounds revealed the presence of chlorogenic acid as the most predominant compound, while quercetin, ellagic acid, salicylic acid, p-hydroxybenzoic acid, and caffeic acid are in high amount. The chromatogram of the chlorogenic acid fraction showed a single high peak and the purity level of the chlorogenic acid isolate. The chlorogenic acid isolate demonstrated antioxidant properties, inhibited α-glucosidase, α-amylase and ACE-1 activities in a concentration dependent manner with varying IC50 values. The white butterfly leaf is nutritional and therapeutic with respect to the starch hydrolysis and angiotensin-II anabolism. The predominance of chlorogenic acid could increase the economic value as a commercial source of chlorogenic acid.

Keywords: *Chlorogenic acid;* White butterfly leaf; α-glucosidase; α-amylase; Angiotensin-1 Converting Enzyme

1.0 Introduction

Trado-medicinal alternatives rely on phytochemicals which are active agents against numerous diseases in humans. This alternative is dated back to several centuries before civilization, and neo-civilization era presented therapeutic plants which are undergoing laboratory surveys relying on previous and partially documented purposes. Leafy vegetables are affordable, cooked easily, and are recognized sources of antioxidants,

minerals and vitamins that are vital to the growth and development of tissues and the replacement of worn-out tissues (Okaiyeto *et al.,* 2021). Not limited to these, they constitute the defence systems against pathogens and endogenous reactive oxygen or nitrogen species.

Clerodendrum volubile also called white butterfly is an underutilized vegetable consumed for both its nutritional and medicinal importance. It is called "Marugbo" or "Eweta" among the Ikale, Ilaje and Apoi people of southwestern Nigeria (Ogunwa *et al.,* 2015). The leaf is mostly consumed as a blend with other vegetables to have a soup that is fortified for nutritional and therapeutic purposes. Undocumented reports have portrayed the white butterfly leaf as useful in attenuating various ailments, and even more potent in blending with other leaves such as *Vernonia amygdalina* and *Occimum gratissimum*.

Chlorogenic acid is an ester of caffeic acid and quinic acid that is predominantly found in coffee, prunes and plums (Clifford, 1999; Nakatani *et al.,* 2000; Fang and Prior, 2002). It is reported as a major polyphenol attributable to its importance as a natural antioxidant and commercial relevance in cosmetics, medicine and food. Its ability to scavenge free radicals is due to its potency as a proton donor (Clifford, 2000). It is also a bioactive compound known for its roles as an anti-inflammatory, anti-carcinogenic, anti-diabetic, analgesic, antipyretic, and antitumor activities (dos Santos *et al.,* 2006; Xiang *et al.,* 2008; Zhang *et al.,* 2008).

Type-II diabetes mellitus is characterized by insulin resistance, hyperglycemia and hyperlipidemia (Falusi *et al.,* 2021). Systemic hyperglycemic conditions can eventually result in elevated intracellular glucose. Elevated intracellular glucose concentrations progressed into the actuation of preference pathways of metabolism, which include hexosamine and the aldose reductase reaction mechanisms, which are both implicated in the pathophysiology of chronic consequences of diabetes (Matheus *et al.,* 2013). These pathways set off an elevation in the generation of reactive oxygen species, and depletion in the substrate required by antioxidant enzymes. Furthermore, increased intracellular glucose also elevates the formation of advanced glycation end-product (AGES) and the actuation of protein kinase C. All these mechanisms result in an increased oxidative stress condition (Matheus *et al.,* 2013) necessitating the need for antioxidant compounds to arrest the condition and bring it under normal physiologic control. The study reveals the effects of chlorogenic acid isolated from white butterfly leaf (MLECS) on the activities of α-glucosidase, α-amylase, and ACE-1, as well as the radical scavenging potentials of the chlorogenic acid isolate.

2.0 Materials and Methods

2.1 Materials

2.1.1 Chemicals

The chemicals used in this study include thiobarbituric acid, tris-buffer, sodium azide, dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), hydrochloric acid, hydrogen peroxide, potassium chloride, tris-hydrochloric acid, sodium chloride, ethylene diamine tetra acetic acid (EDTA), ammonium molybdate, tetra methylbenzidine, sodium dihydrogen phosphate, thiobarbituric acid, sulphosalicylic acid, acetylcholine iodide, ferrous chloride, potassium ferricyanide, ascorbic acid, tannic acid, quercetin, trichloracetic acid (TCA), glacial acetic acid, folin-ciocalteu reagent, sodium carbonate and potassium acetate were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used including the solvents were obtained from standard chemical suppliers and were of analytical grade.

2.1.2 Sample Collection

The leafy part of *Clerodendrum volubile* was collected from a farm garden located in Akure, Ondo State (Nigeria). The plant was identified by Dr. O. P. Aiyelari, Crop Soil and Pest Department, the Federal University of Technology, Akure, Nigeria.

2.1.3 Method of Extraction of Ethanol Extract of *Clerodendrum volubile* and Chlorogenic Acid Fraction

After authentication, the leafy part of *Clerodendrum volubile* was collected in bulk and washed under running tap water to remove adhering dirt, followed by rinsing with distilled water, air drying and grinding by a mixer grinder. After grinding, 1000 g of the powdered leaf was soaked in 5.0 l of 80% ethanol at 40˚C for 24 hours orbital shaker. The solvent solution was filtered through Cheese cloth and filter paper (Whatman Number 1) till a clear solution was obtained. The solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 80˚C, concentrated and lyophilized with a Bioevopeak Benchtop Freeze Dryer (LYO06B-1S, Seattle, United States). The ethanol extract was stored in a light-proof, airtight and moisture-proof container at 4°C for further use.

The ethanol crude extract was subjected to column chromatography. The extract was dissolved in 80 % ethanol and chromatographed over a 10 g Sephadex LH-20 (Sigma) column. There was elution with 200 ml of 80% ethanol and the fractions collected were dried in a rotary evaporator at 50 ^{o}C prior to analysis (Chan *et al.,* 2011).

2.1.4 Characterisation of Polyphenolic Compounds with HPLC in *Clerodendrum volubile* Extract and Chlorogenic acid isolate

The phenolic acid standards used were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, WI). The phenolic compounds were characterized by HPLC (Agilent 1200 series), with a detector Agilent 1260 at a wavelength of 320 nm. The column used was CHROMSPHER5, C18 at a temperature of 40 ^{o}C. The dimension adopted was 5 µm, 3 mm x 250 mm with a Hamilton microliter syringe. The injector volume was 100 µl and the flow rate of 0.7 ml/min, pressure 180 x 105 Pa and isocratic elution of 2 % acetic acid in the water-methanol mixture (82:18, v/v).

While the chlorogenic acid content was determined by HPLC (Agilent Technologies 1200). The Mobile phase used was methanol, acidified with 0.1% trifluoroacetic acid for better resolution. A loop which was 20µl in length was used for injection and elusion was monitored at 280 nm.

2.2 *In Vitro* Evaluation of Antioxidant Activities

2.2.1 DPPH Radical Scavenging Activity

The DPPH scavenging activity of the extracts was estimated according to the method described by Leong and Shui (2002). One millilitre (1 ml) of 0.3 mM DPPH prepared in methanol was added to 1 ml of extract of various concentrations. The reaction mixture was swirled thoroughly and bivouac in the dark at room temperature for 30 min. The absorbance of the chromophore was measured using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States) at 517 nm and the scavenging activity was calculated.

% scavenging activity = ((Ac – As)/Ac) x 100

Where Ac is the absorbance of the control and the absorbance of the extract.

2.2.2 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was estimated according to the method of Halliwell and Gutteridge (1990). The reaction mixture consists of 30µl of 1 mM $FeCl_3$, 45µl of 1 mM Phenanthroline, 1.2 ml of phosphate buffer (pH 7.8), 0.5 ml of extract of various concentrations and 75 µl of 0.17 M H_2O_2. The addition of H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance at 560 nm using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States) and hydroxyl radical scavenging activity was calculated thus:

OH scavenging activity (%) = ((Ac – As)/ Ac) x 100

2.2.3 Nitric Oxide Radical Scavenging Activity

Nitric oxide scavenging activity was assessed by mixing sodium nitroprusside (5 mM), pre-dissolved in phosphate-buffered saline, with various concentrations of the extract. After incubation at room temperature for 30 min, 1.5 mL of the diluted solution was collected and diluted with 1.5 mL of Griess reagent, pre-dissolved in 1 % sulphanilamide, 2 % phosphoric acid and 0.1% N-1-naphthyl ethylenediamine dihydrochloride. During this reaction, diazotization of nitrite with sulphanilamide and subsequent N-1-naphthyl ethylene diamine

dihydrochloride, resulted in the formation of the active chromophore, the value of which was estimated using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States) at 546 nm, against the reagent blank value (Bhalodia *et al.*, 2013).

% scavenging activity = ((Ac – As)/Ac) x 100

Where Ac is the absorbance of the control and As is the absorbance of the extract.

2.2.4 Lipid Peroxidation Inhibition Activity

The inhibition of Fe^{2+}-induced lipid peroxidation was determined according to the method described by Ohkawa *et al*. (1979). Wistar albino rats were sacrificed by cervical dislocation. The liver was carefully excised and washed in ice-cold 1.15 % potassium chloride solution, smudge with filter paper and weighed. The liver was homogenized in four volumes of phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 3000 x g in a high-speed refrigerated centrifuge (CFGR-16DR, Seattle, United States) for 10 min to obtain the supernatant. A 200 µl aliquot of each supernatant was mixed with 60 µl of 0.1M Tris HCl buffer (pH 7.4) and extracts of various concentrations followed by adding 50 mM $FeSO_4$ and 240 µl of distilled water. The resultant mixture was incubated at 37 ^oC for 1 h. 600 µl of 8.1 % SDS was added followed by the addition of 1200 µl of 1.3 M Acetic acid buffer (pH 3.4) and 1200 µl of 0.8 % TBA. The mixture was heated at 100 ^oC for 1 h to complete the reaction. Then the samples were cooled and centrifuged at 3000 x g in a high-speed refrigerated centrifuge (CFGR-16DR, Seattle, United States) for 10 min. The intensity of pink coloured complex was measured using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States) at 532nm and the inhibition of lipid peroxidation was calculated thus:

Inhibition (%) = ((Ac – As)/Ac) x 100

2.2.5 Ferric Reducing Antioxidant Potential

The reducing power of the extracts was determined as reported by the method of Oyaizu (1986). 0.5 ml of the extracts were mixed with 1.25 ml each of phosphate buffer and potassium ferricyanide ($C_6N_6FeK_3$). The mixture was incubated at 50 ^0C for 20 min. Trichloroacetic acid (1.25 ml) was added, and the mixture was centrifuged at 3000 x g in a high-speed refrigerated centrifuge (CFGR-16DR, Seattle, United States) for 10 min. Thereafter, 1.25 ml of the upper surface of the solution was mixed with 1.25 ml of distilled water and 0.25 ml of $FeCl_3$. The absorbance was read at 700 nm using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States). Higher absorbance of the reaction mixture indicates greater reductive potential.

2.3 *In vitro* Evaluation of Anti-diabetic and ACE-1 Inhibition Activities

2.3.1 Inhibition of α-Amylase Activity

The inhibition of alpha-amylase activity was carried out by the starch-iodine method. The α-amylase solution (0.025 mg/ml) was pipetted (10 µl) and mixed with 390 µl of 0.02 M phosphate buffer saline (0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µl of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 ml of 1% iodine solution was added, and after adding 5 ml distilled water, the absorbance was taken at 565 nm using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States). Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and

C= absorbance of the control (without starch). (Sheikh *et al.*, 2008)

2.3.2 Inhibition of α-Glucosidase Activity

The final volume of the reaction mixture was 100 µl, which contained 70 µl of phosphate buffer saline (50 mM, pH 6.8), 10 µl of test extracts, and 10 µl (0.057 U) enzyme. The content was mixed, pre-incubated at 37 ^oC for 10 min, and pre-read against the reagent blank value by spectrophotometry using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States) at 400 nm. The reaction was initiated using 10 µl of 0.5 mM substrate (i.e., p-nitrophenol glucopyranoside). Acarbose was the standard inhibitor. After incubation at 37^oC for 30 min, optical absorbance was measured against the

reagent blank value by spectrophotometry using a UV VIS spectrophotometer (SP-LUV7500, Seattle, United States) at 400 nm. The percentage of enzyme inhibition was calculated using the Equation.

% scavenging activity = ((Ac – As)/Ac) x 100

Where Ac is the absorbance of the control and As the absorbance of the extract (Tiwari *et al.,* 2011).

2.4 Angiotensin-I Converting Enzyme Inhibition

The angiotensin-1-converting enzyme inhibitory activity was carried out by using n-furnacryloyl-phenylalanylglycyl glycine (FAPGG) as the substrate (Umamaheswari *et al.,* 2012). The extract and the standard drug Lisinopril (1 mg/ml) were prepared by dissolving in reaction buffer (HEPES 25mM, NaCl 293mM, pH 8.3). The assay mixture (750µl) consisted of 530µl of FAPGG (3mM in reaction buffer) and 200 µl of extract at different concentrations and was incubated for 3 min at 37°C. The reaction was initiated by adding 20 µl of ACE solution (0.05U/ml) to the test reaction and the samples were incubated for 1 h at 37 ºC. The reaction was then stopped by adding 80 µl of 5% trifluoroacetic acid solution and samples were centrifuged at 9000 x g in a high-speed refrigerated centrifuge (CFGR-16DR, Seattle, United States) for 5 min. The enzymatic activity was calculated by quantifying the decrease in FAPGG concentration by recording the decrease in absorbance at 345 nm using RP-18 column (50 mm × 7 mm, 3 µm pore size) with isocratic elution using acetonitrile and 1.1% trifluoroacetic acid in Milli-Q in the ratio of 75:25 v/v; it was filtered through 0.45 µL filter (Sartorius, Germany) and using an ultrasonic bath was degassed before use. The column temperature was ambient and the total running time was 10 min using a flow rate of 1.5 ml/min with a retention time of 2.7 min for FAPGG, the injection volume was 20µl and the detection wavelength was 345 nm. Percentage enzyme inhibition was calculated by comparing the enzymatic activity with, and without inhibitor using the following formula,

% ACE inhibition = (1−a) ×100 where a is the activity with inhibitor and activity without inhibitor

2.5 Statistical Analysis

The results were pooled and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis. The significance level was set at $p < 0.05$.

3.0 Results and Discussion

3.1 In vitro Antioxidant, Anti-diabetes and Antihypertensive Activities of MLECS

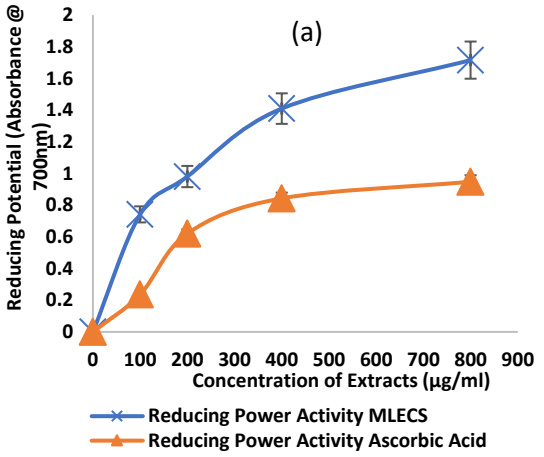
The ferric-reducing antioxidant property of chlorogenic acid isolate (MLECS) and ascorbic acid was presented in Figure 1a. A concentration-dependent effect was recorded, MLECS demonstrated higher ferric-reducing activity than ascorbic acid. The ability to reduce DPPH radical by MLECS was compared with ascorbic acid (Figure 1b). The EC50 of MLECS was 463.82 µg/ml and ascorbic acid was 521.38 µg/ml. The NO* radical scavenging activity of MLECS and rutin against nitric oxide was revealed in Figure 1c. The result showed concentration-dependent effects of MLECS and rutin. The EC50 value of MLECS was 517.06 µg/ml while rutin had 507.61 µg/ml. Figure 1d revealed the percentage inhibition activity of iron (II) induced lipid peroxidation by MLECS. MLECS demonstrated a concentration-dependent effect which increased inhibition of lipid peroxidation with an EC50 of 526.87 µg/ml. The EC50 value for quercetin was 695.41 µg/ml, showing a significantly higher concentration of quercetin was required to achieve effectively 50 % inhibition of lipid peroxidation. Figure 1e revealed the hydroxyl radical scavenging percentage activities of MLECS and mannitol. The 50% radical scavenging activity of MLECS would require a concentration of 612.0 µg/ml while EC50 for mannitol was 397.46 µg/ml. MLECS and mannitol exhibited concentration-dependent activities which were significantly higher for mannitol than MLECS.

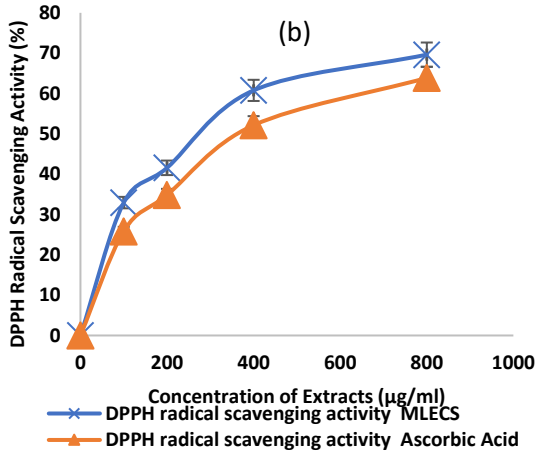
The α-glucosidase inhibition activity of MLECS was evaluated and revealed in Figure 2a. The inhibition activities increased with the concentration increase. The IC_{50} value for MLECS was 499.0 µg/ml while acarbose had an

IC_{50} value of 478.47 µg/ml. The α-amylase inhibition activity of MLECS was evaluated and revealed in Figure 2b. The IC_{50} value for MLECS was 796.18 µg/ml while acarbose had an IC50 value of 492.13 µg/ml.

Percentage ACE-1 inhibition activity of MLECS was evaluated and revealed in Figure 3. The inhibition activities increased with the concentration increase.

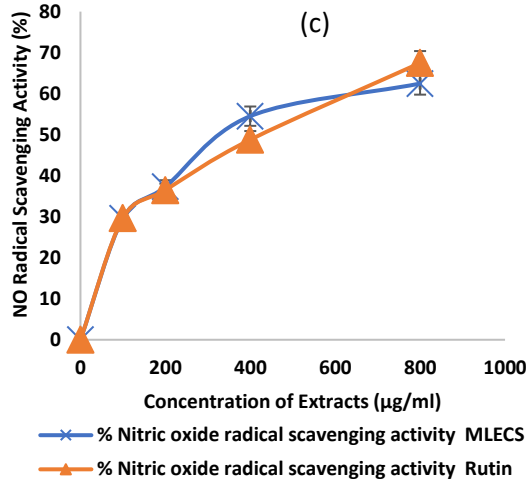
The standard ACE-1 inhibitor used was lisinopril which manifested significantly higher inhibitory activity than MLECS. The IC50 value for MLECS was 634.52 µg/ml while lisinopril had IC50 value of 353.86 µg/ml.

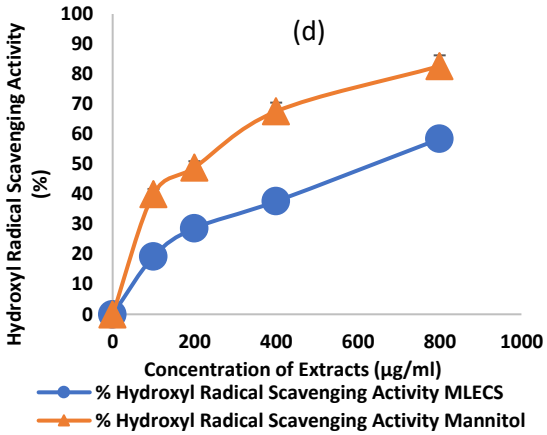




3.2 Phytochemical Constituents in White Butterfly Leaf

The HPLC characterization of the phytochemicals as revealed in Table 1 revealed predominantly the following phytochemicals and their respective amounts: chlorogenic acid (19.17 mg/100g), quercetin (5.75 mg/100g), ellagic acid (5.23 mg/100g), sinapipic acid (5.71 mg/100g), caffeic acid (4.42 mg/100g), kaemferol (5.01 mg/100g), luteolin (4.79 mg/100g),myrecetin (2.20 mg/100g), naringenin (1.55 mg/100g), syringic acid (3.58 mg/100g), apigenin (3.24 mg/100g), ferrulic acid (5.88 mg/100g), gallic acid (5.13 mg/100g), p-hydroxybenzoic acid (7.00 mg/100g), protocatechuic acid (4.30 mg/100g), salicylic acid (5.80 mg/100g).





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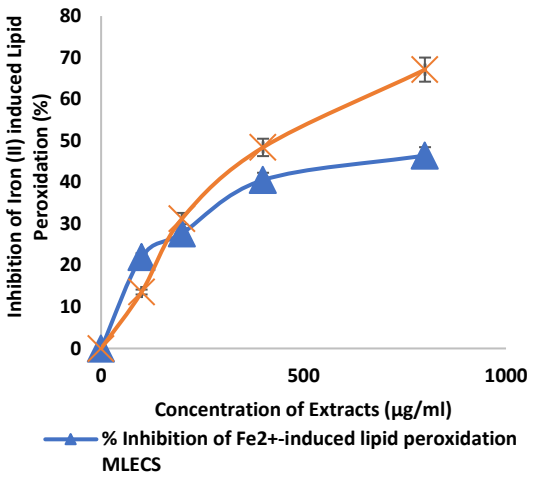
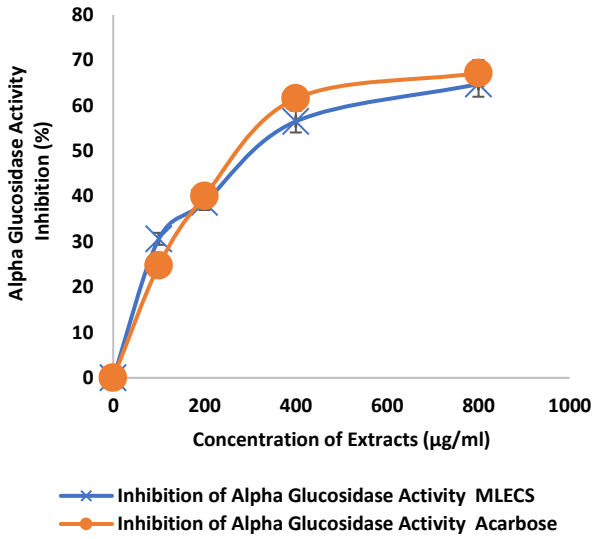


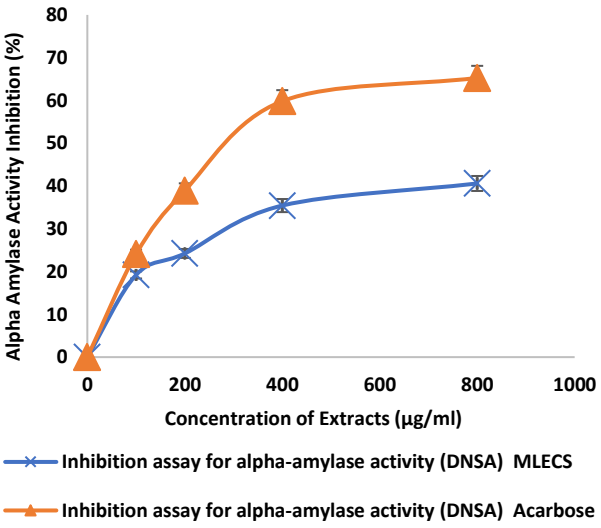
Figure 1: Antioxidant properties of chlorogenic acid isolate (MLECS) (n=3; p≤0.05)

Table 2 showed the amount of chlorogenic acid present in the chlorogenic acid fraction extracted from the white butterfly leaf. Plate 1 revealed the HPLC chromatogram showing chlorogenic acid peak of fractionated sample. The purity of the fraction was demonstrated by the presence of a single chlorogenic acid peak.

This study revealed the presence of the following bioactive compounds in the leaf of the white butterfly plant (*Clerodendrum volubile*): quercetin, epicatechin, luteolin, apigenin, caffeic acid, ferulic acid, chlorogenic acid, resveratrol, kaemferol, butein, catechin, gallic acic, salicylic acid, syringic acid, ρ-hydroxybenzoic acid, naringenin, epigallocatechin-3-gallate, sinapic acid, hesperidin, silymarin, baicalein, myricetin, diadzein in significant amounts which are mostly phenolic compounds and flavonoids that are important as bioactive agents in the treatments and management of life-threatening diseases such as cardiovascular diseases, cancer and neurodegenerative disease. Chlorogenic acid is the most predominant phytochemical present in the *Clerodendrum volubile* leaf. Chlorogenic acid had been implicated in the reduction of the relative risk of cardiovascular disease, type 2 diabetes mellitus and Alzheimer's disease (Farah *et al.*, 2018). A study had provided evidence that sinapic, gallic, p-hydroxybenzoic, and dihydrocaffeic acids are major metabolites of CGA in humans (Farah *et al.*, 2018).



(a)



(b)

Figure 2: Effects of chlorogenic acid isolate on α-glucosidase (a) and α-amylase (b) activities (n=3; p≤0.05).

Table 1: The HPLC summary of the quantities of polyphenols in leaf extract of white butterfly leaf

Phytochemicals	Amount (mg/100g)
Salicylic acid	5.78895
Protocatechuic acid	4.29645
p-hydroxybenzoic acid	6.59498
Gallic acid	5.12978
(+)-Catechin	0.843359
Ferulic acid	5.88196
Resveratrol	0.00000381
Genistein	0.000010734
Daidzein	0.00001223
Apigenin	3.2427
Syringic acid	3.57665
Butein	0.000005353
Biochanin	8.41132E-06
Naringenin	1.55343
Luteolin	4.78912
Kaemferol	5.00923
Caffeic acid	4.42813
(-)- Epicatechin	0.00317034
Sinapinic acid	5.71056
(-)- Epigallocatechin	0.00056851
Ellagic acid	5.23194
Gallocatechin	0.000053518
Quercetin	5.75166
(-)-Epicatechin-3-gallate	0.000283663
(-)-Epigallocatechin-3-gallate	0.000267249
Chlorogenic acid	19.17438
Isorhamnetin	1.89814E-06
Robinetin	1.01378E-05
Myrecetin	2.20351
Baicalein	4.59732E-06
Nobiletin	7.11397E-06
Baicalin	4.51817E-06
Isoquercetin	0.014944
Tageretin	2.78827E-06
Artemetin	4.11569E-06
Silymarin	2.63527E-06
Resperidin	5.53418E-06

There were strong correlations between chlorogenic acid with radicals scavenging antioxidant activities (Chan *et al.*, 2011; Rai *et al.*, 2018). Although the release of reactive oxygen species by macrophages is a mechanism of the body defense (Hensley and Floyd, 2002), the depletion of the antioxidant system leads to increased oxidative burden. This indicates that chlorogenic acid can protect endogenous molecules against pro-oxidant attack through chelation of free Fe2+ and Cu2+, radical scavenging, and inhibition of lipid peroxidation. All these are possible through available hydrogen and electron donation.

In addition to the inhibition of oxidative stress through its antioxidant properties, chlorogenic acid also inhibited ACE-1, which mediates the formation of angiotensin II, which is a vasoconstrictor and reactive oxygen species initiator. The ACE-inhibiting potentials of p-coumaric acid, caffeic acid and procyanidin C1 (polyphenol flavonoid trimer of (–)-epicatechin) (Actis-Goretta *et al.*, 2013) have been reported except for chlorogenic acid.

Chlorogenic acid has been reported has an inhibitor of α-amylase and α-glucosidase, although the effects was not as low as described by Oboh et al. (2015) compared to acarbose, whose allergic effects have been described to include stomach discomfort, gas, bloating, mild diarrhea or skin rash or itching, excluding some other disturbing side effects that requires clinical remedies . Therefore chlorogenic is a better alternative that has antioxidant, anti-inflammatory, hypoglycemic, and inhibition of angiotensin-1 converting enzyme properties.

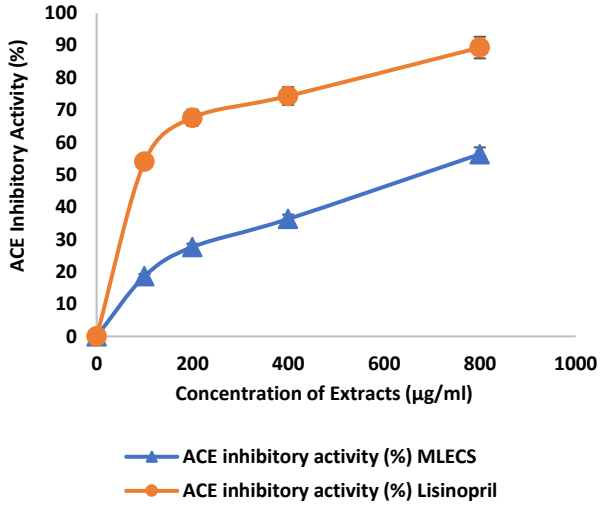


Figure 3: Effects of chlorogenic acid isolate on angiotensin-1 converting enzyme activities (n=3; p≤0.05).

4.0 Conclusion

The white butterfly leaf is a nutritional plant with few proven effects on human ailments. It cannot be acclaimed to be of high economic value. However, the high quantity of chlorogenic acid present in the leaf could raise its economic value as a commercial source of chlorogenic acid.

Table 2: The amount of chlorogenic acid isolate

RetTime (min)	Type	Area [pA*s]	Amt/Area	Amount [mg/100g]	Grp	Name
25.047	PV	43.71908	2.35316	102.8781	1	Chlorogenic acid

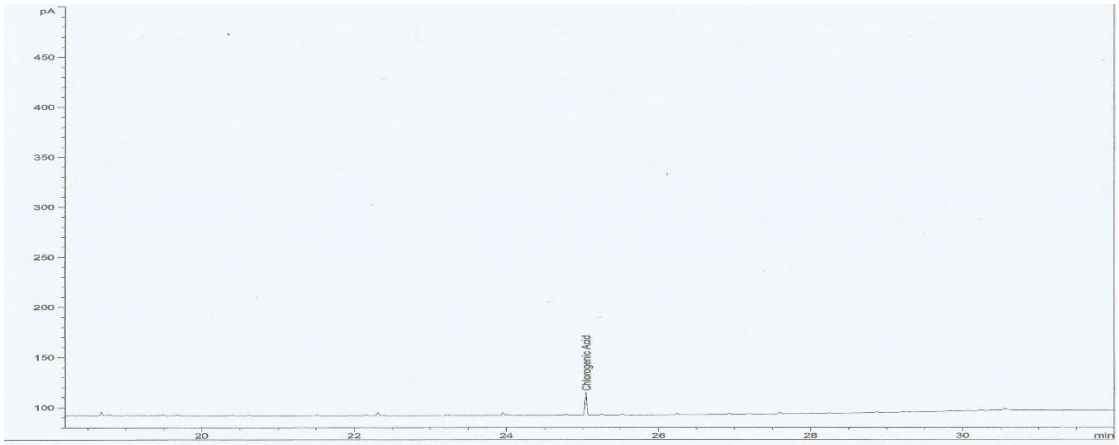


Plate 1: HPLC of chlorogenic acid fraction, isolated from the White butterfly leaf.

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