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In-Vitro Evaluation of Antioxidant, Anti-Inflammatory and Anti-Ageing Potentials of Some Common Anti-Ageing Medicinal Plants of Southwest Nigeria

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ABSTRACT:

Ageing is a multifactorial degeneration in body organs and physiological functions. *Adansonia digitata linn* (AD), *Bryophyllum pinnatum* (BP), *Vernonia amygdalina* (VA), and *Canna indica* (CI) are commonly used in folk medicine as anti-ageing remedies in Southwest Nigeria with little or no scientific bases. This study investigated *in-vitro* antioxidant, anti-inflammatory, and anti-ageing potentials of these medicinal plants. Ethylacetate Extracts of the powdered leaves of the plants were prepared by cold extraction and were subjected to *in-vitro* assays: Total Flavonoids content, Hydroxyl radical (OH^-) and 2,2- Diphenyl-1-picrylhydrazyl(DPPH) radical scavenging potentials, Inhibition of lipid peroxidation, Inhibition of Proteinase activity and Protein denaturation, while anti-tyrosinase and Anti-glycation potentials were also evaluated, using standard methods. Results showed that, ethylacetate extract of BP, at 400µg/ml, showed highest Total Flavonoids content Quercetin Equivalent (QE)(1.90mg/g/QE) compared with VA(1.52mg/g/QE), CI(0.80mg/g/QE) and AD(0.4mg/g/QE) respectively and highest OH- radical scavenging potential (66.57%) compared with CI(48.25%), VA(26.53%) and AD(26.43%) respectively. Furthermore, at 400µg/ml, VA exhibited the highest DPPH radical scavenging activity (62.02%) compare with BP(61.98%), AD(53.80%) and CI(45.20%), while AD was more potent at lower concentrations. Moreover, at 400µg/ml, Inhibition of lipid peroxidation was highest in CI(33.07%) compared with AD(26.60%), VA(24.98%), and BP(21.49%), although VA was more potent at lower concentrations. Furthermore, CI exhibited highest proteinase activity inhibition and Inhibition of protein denaturation at concentrations (100 µg/ml - 400µg/ml) while at 400 µg/ml, it elicited highest proteinase inhibition of 94.88% compared with AD(90.50%,), BP(85.89%) and VA(76.1%) respectively and also, highest protein denaturation inhibition of 71.82% compared with BP(59.60%), AD(36.40%) and VA(32.92%) respectively. At the highest concentration of 1mg/ml, VA exhibited the highest anti-tyrosinase activity (IC_{50} 0.28±0.004, 87.28%), while BP, AD, and CI were (IC_{50} 0.32±0.008mg/ml, 77.56%; IC_{50} 0.57±0.009mg/ml, 51.24%; IC_{50} 1.05±0.015mg/ml, 44.35%), respectively. Moreover, across all concentrations (0.3125mg/ml – 5mg/ml) from week 1- 4, VA also, exhibited highest anti-glycation potentials (71.23%, 87.54%, 86.20% and 90.06%) compare with AD(63.30%,75.98%,83.68% and 82.84%), CI (58.75%, 52.62%, 59.94%, and 78.29%) and BP(47.08%, 51.43%, 53.02% and 63.89%) respectively. *Vernonia Amygdalina* exhibited the highest DPPH radical scavenging, anti-tyrosinase, and anti-glycation properties, while CI showed the highest inhibition of lipid peroxidation, protein denaturation, and proteinase activity. BP exhibited the highest OH^- radical scavenging potential and total flavonoids content. The plants may be employed as anti-ageing remedies and also in the management and treatment of other oxidative stress related diseases.

Keywords: Ageing, *Adansonia digitata lin*, Antioxidant, *Bryophyllum pinnatum*, anti-tyrosinase, *Vernonia amygdalina*, anti-inflammatory, *Canna indica*, anti-glycation.

INTRODUCTION

Ageing is a functional deterioration that is associated with continuous decline in various physiological processes that will latter result to various health complications, diseases, and death in an organism (Adegoke *et al.*, 2021). Causes of ageing have been attributed to oxidative stress, glycation, telomere shortening, and mutation, while lifestyle choices that have been associated with ageing in man include unhealthy diets high in sugar or refined carbohydrate, regular consumption of alcohol, smoking, stress, medication, radiation, and diseases (Mandi *et al.*, 2023). Biomolecules like DNA, Lipids, and proteins are the most important constituents of living organisms. The above molecules have been suggested to accumulate damage, basically because of oxidation, and molecular oxidative damage have been traced to the etiology of ageing. Ageing occurs as a result of the buildup of damaged cellular constituents in terms of failure to remove these constituents, prevent the production of these constituents, or repair damages occasioned by them, and lastly, failure to replace lost cells due to accumulation (Lopez-otin *et al.*, 2013). Inability to handle wear and tear that occur in the human body, leading to ageing, has been recently associated with some factors, which include senescence of cells, epigenetic alteration, altered/reduced cell-cell communication, stem cell exhaustion, as well as deregulated nutrient sensing (Lopez-otin *et al.*, 2013). The above processes alone or in combination are the bases for age-related changes like muscle loss and loss of key functional hormones in the body. Maintenance mechanisms employed by living cells to protect themselves against oxidative damage include: replacement and repair of damaged molecule in the body, as well as an antioxidant defence system; hence, cellular protection against molecular damage will as well protect against ageing process. Oxidative damage theory of ageing predicts that, when the antioxidant defence system is boosted, the process of ageing is slowed down. Reactive oxygen Species are generated by the process of oxidative deterioration of polyunsaturated fatty acids (PUFA) from the membrane of the cells in a process called 'Lipid peroxidation' (Mladenov *et al.*, 2006). Intracellular reactive oxygen species (ROS) production through indirect formation of advanced glycation end products (AGEs) *in-vivo* is not limited to mitochondria of the cells, but mitochondria remain the target of reactive oxygen species (Liu *et al.*, 2009). Ageing and age-related diseases have been managed or treated by the use of orthodox drugs. Orthodox drugs are expensive, and have various degrees of toxic side effects; hence, the need to source for cheap, safer, and readily available natural agents with anti-ageing potential and the need to revert to natural remedies of plant origin. Healing with medicinal plants is as old as mankind itself. Plants are a natural and important part of human life, and various plant constituents have been employed in the development of various drug substances to combat various human diseases (Singh *et al.*, 2009).

Adansonia digitata, also called Baobab tree, bottle tree, monkey bread tree or upside down tree belongs to the family Malvaceae. It is a very massive tree with a very large trunk usually up to 10 m in diameter, which can grow up to the height of 25 m and may live for hundreds of years (De Caluwé *et al.*, 2010). It has a high content of vitamin C and well documented antioxidant capabilities (Vertuani *et al.*, 2002; Besco *et al.*, 2007: Brady, 2011), anti-inflammatory potential (Al-Qarawi *et al.*, 2003), antipyretic/anti-fever effect (Brady, 2011), antimicrobial potential (Afolabi and Popoola, 2005), Analgesic property (Masola *et al.*, 2007) as well as antiviral activity (Chadare *et al.*, 2009). *Bryophyllum pinnatum* (*B.pinnatum*), otherwise known as *Kalanchoe pinnatum* or *Bryophyllym Calycinum*, belongs to the family of Crassulaceae (Sadhana *et al.*, 2017). It is a perennial herb which is 3 to 5 meters high having opposed glabrous leaves (Kamboj and Saluja, 2017). *B. pinnatum* is sour to taste with sugary post digestive effect. It is made up of various valuable chemicals substances that could underlie its various pharmacological and medicinal effects. In various parts of the world, it is employed for the treatment of various pathological conditions like conjunctivitis, constipation, Epilepsy, Cholera, menstrual disorder, burns, cough suppression, insect bites, psychiatric disorders as well as abdominal discomforts (Sadhana *et al.*, 2017). Moreover, extracts from the leaves are useful for treatment of Jaundice, hypertension, and renal stones while slightly heated leaves are used as a tocolytic agent in southwest Nigeria to prevent premature labour and as well used for dropping of placenta (Gupta *et al.*, 2016; Latif *et al.*, 2019).

Canna indica, otherwise known as indian shot, is the only genus in the family Cannaceae and has 19 species of flowering plants. It has large, eye–catching foliage, it is a horticultural plant and one of the richest starch sources,

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and used by horticulturists to form a large, flowered, bright garden (Sarje *et al.*, 2019). It is a coarse perennial herb, 90cm to 3m tall, and grows from edible underground rootstock. The flowers of the plant are brightly coloured reds, yellow, or orange (Al-Snafi, 2015; Pandey and Bhandari, 2021). According to Pandey and Bhandari (2021), it has anti-inflammatory, antibacterial, antioxidant, Molluscicidal, hepatoprotective, anti-diarrheal, as well as immumodulatory effects. *Vernonial amygdalina* is an angiosperm that belongs to the family Asteraceae. It is mostly cultivated and employed in folk medicine practices in Africa as well as Asia's tropical areas (Tekou *et al.,* 2018). *V. amygdalina* is a soft, woody shrub having several economic uses; it grows to a height of 1 meter to 6 meters (Ifedibaluchukwu *et al.,* 2020). It is commonly referred to as "Bitter leaf" because of its bitter taste, which may be due to its various anti-nutrient contents (Ifedibaluchukwu *et al*., 2020). The leaves' diameter is 6mm and 20cm long (Habtamu and Melaku, 2018), dark green, and making them an essential part of the human diet (Oyeyemi *et al.*, 2018). Cold water extract of the plant has been reported for suppression of cancer (Yedjou *et al.*, 2018), lowering of diet-induced obesity, typhoid treatment, and various other inflammatory diseases (Asante *et al*., 2019). *V. amygdalina* is also employed in the treatment of malaria (Okpe *et al.,* 2016).Hence, this study investigated antioxidant, anti-inflammatory and anti-ageing potentials of *Adansonia digitata, Bryophyllum pinnatum, Cana indica,* and *Vernonia amygdalina* as they were being employed in folk medicine as an anti-ageing remedy in southwest Nigeria (Oladele *et al.*, 2012).

Adansonia digitata linn leaves and fruit

Bryophyllum pinnatum Plant

Vernonia amygdalina leaves

Canna Indica Plant

MATERIALS AND METHODS

Materials

The materials used were: Measuring cylinders, spectrophotometer, micropipettes, centrifuge, water-bath, serum bottles, disposable gloves, thermometer, stopwatch, 2ml and 5ml syringes and needles, pH meter, cotton wool, conical flasks, test-tubes and racks, spatula, refrigerator, weighing balance, funnel, Pasture pipette, Filter paper, Beaker, rotary evaporator, and Incubator.

Reagents and salts

Trichloroacetic acid (TCA), Absolute Methanol (99.9%), Quercetin, Kojic acid, Amino guanidine hydrochloride, Thiobarbituric Acid (TBA), Bovine Serum Albumin (BSA), Glucose, Phosphate buffer, 3,4-dihydroxyphenylalanine (LDOPA), Nitro-blue tetrazolium (NBT), Phosphate buffer, Drosophila/mushroom tyrosinase, Ascorbic acid, 2-deoxyribose, Trypsin and Dimethyl Sulfoxide (DMSO), were all purchased from Sigma Chemical Company St. Louis, MO, USA. All other chemicals used for this experiment were of analytical grade.

Identification of plant materials

The plants, *Adansonia digitata lin*, *Bryophyllum pinnatum*, *Canna indica*, and *Vernonia amygdalina* leaves were obtained from the Oja Igbo area, Ogbomoso, Oyo state, and were identified and authenticated in the Botany section of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, with Herbarium Voucher numbers: *Bryophyllum pinnatum lin* (LHO 863), *Canna indica* (LHO 861), *Vernonia amygdalina Delile* (LHO 862), and *Adansonia digitata* (LHO 749) deposited. They

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were air-dried and blended into a powdery form to increase the surface area in order to facilitate the process of extraction.

Preparation of Ethylacetate Extracts

Two hundred and fifty grams (250g) of powdered leaves of *Adansonia digitata lin*, *Bryophyllum pinnatum*, *Cana indica,* and *Vernonia amygdalina* were each soaked in 2500ml of ethylacetate, left for 3days, filtered, and the filtrate was concentrated on a rotary evaporator at 35°C and later dried in a water bath (Wu *et al*. 2009). Percentage yield was 13.69% for *A. digitata*, 10.52% for *B. pinnatum*, 15.55% for *Cana indica,* 15.40% and *Vernonia Amygdalina,* and 17.25%, respectively.

METHODOLOGY

Determination of Total Flavonoid Content

Total flavonoid content of the plant extracts was determined by the method of Pekal and Pryzynska (2014). Flavonoids form complexes with Aluminium Chloride ($AlCl_3$), and the absorbance of the reaction was measured at a wavelength of 415nm. Total flavonoid content was extrapolated from the standard curve for Quercetin by plotting the graph of absorbance against concentration.

Determination of Percentage (%) Hydroxyl Radical (OH^-) Scavenging Activity (2-Deoxyribose Assay)

The hydroxyl radical scavenging activity of the extracts was determined by the method of Tijani (2018). 2-deoxyribose was degraded by the hydroxyl radical generated by the Fenton-type reaction. The amount of degradation was quantified spectrophotometrically at the wavelength 532nm.

Determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Potential

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging potential of the plant extracts was determined by the method described by Mensor *et al.* (2001). DPPH solution reacts with a substance that can donate a hydrogen atom, giving rise to the reduced form, losing violet colour to yield a pale yellow colour, which is absorbed at a wavelength of 518nm.

Determination of Percentage (%) inhibition of lipid peroxidation

The lipid peroxidation inhibition potential of the extracts was determined by the method of Sadighara (2012). Lipid peroxidation was induced in egg yolk with an oxidizing agent ($FeSO_4$), and the level of lipid peroxidation product was determined by the pink colour developed, which absorbed at a wavelength of 532nm.

Determination of Percentage (%) inhibition of Proteinase activity

Proteinase inhibition of the plant extracts was determined by the method described by Cotabarren *et al. (*2023) with slight modification. The extracts inhibit the activity of trypsin protease, preventing interaction with substrate, hence inhibiting its proteolytic activity. Trypsin reacts with the extract and egg white at room temperature. Trichloroacetic acid was added, and the supernatant was picked. Na_2CO_3 and Folin were added, and incubated until the colour changed, and read at 660nm. EDTA was used as a standard.

Determination of Percentage (%) inhibition of Protein Denaturation

Inhibition of protein denaturation was determined by the method described by Madhuranga and Samarakkon (2023). Egg albumin solution was exposed to heat in the presence and absence of plant extracts at 70ºC for 5minutes. The extent of protein denaturation was measured from the supernatant at the wavelength of 660nm. Non–steroidal anti-inflammatory drug (Aspirin) was used as the standard.

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Anti-glycation Assay

Antigycation assay was determined by the method of Rahbar and Figarola (2003). Glucose glycated material was prepared. Protein (BSA) and Sugar (glucose) were incubated in the presence and absence of plant extracts. The amount of advanced glycated end products (AGEs) formed was measured per week for the period of four weeks at the wavelength of 530nm. Aminoguanidine hydrochloride was used as a standard, while a mixture of BSA, glucose, phosphate buffer, and 1% DMSO was used as a control alongside the test samples.

Determination of Percentage (%) inhibition of Tyrosinase Activity

Anti-tyrosinase activity of the plant extracts was determined by the method of Ashraf *et al.* (2017) with slight modification. Drosophila/mushroom tyrosinase was incubated with varying concentrations of extracts, and 3,4-dihydroxyphenylalanine (LDOPA) was added. The absorbance of dopachrome formed was measured at the wavelength of 475nm. Kojic acid was used as a reference inhibitor, while phosphate buffer was used as a negative tyrosinase inhibitor.

Statistical Analysis

The results were analysed using graphpad prism 5 and were expressed as Mean ± Standard error of Mean (SEM) of duplicate tests, One-way analysis of variance (ANOVA) was used for comparison of relative expression levels of different groups. Value of $p<0.05$ was considered statistically significant.

RESULTS

Antioxidant Potential

Table 3.1a: Result showing Total Flavonoids content (Quercetin Equivalent(QE)) of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Concentration (µg/ml) (QE)	Total Flavonoids content of *Adansonia digitata* Ethylacetate Leaves extract (mg/g)(QE) Mean ± SEM	Total Flavonoids content of *Bryophyllum pinnatum* Ethylacetate Leaves extract (mg/g)(QE) Mean ± SEM	Total Flavonoids content of *Vernonia amygdalina* Ethylacetate Leaves extract (mg/g)(QE) Mean ± SEM	Total Flavonoids content of *Canna indica* Ethylacetate Leaves extract (mg/g)(QE) Mean ± SEM
50	0.080 ± 0.003^{a}	0.250 ± 0.001^{b}	0.272 ± 0.002^{b}	0.107 ± 0.002^{c}
100	0.128 ± 0.005^{a}	0.350 ± 0.001^{b}	0.336 ± 0.002^{b}	0.160 ± 0.005^{c}
150	0.160 ± 0.002^{a}	0.501 ± 0.008^{b}	0.640 ± 0.008^{c}	0.187 ± 0.005^{a}
200	0.192 ± 0.001^{a}	0.800 ± 0.003^{b}	0.800 ± 0.002^{b}	0.240 ± 0.001^{c}
250	0.256 ± 0.002^{a}	1.250 ± 0.003^{b}	0.960 ± 0.004^{c}	0.320 ± 0.002^{d}
300	0.288 ± 0.003^{a}	1.50 ± 0.003^{b}	1.09 ± 0.005^{c}	0.533 ± 0.001^{d}
350	0.320 ± 0.001^{a}	1.800 ± 0.00^{b}	1.220 ± 0.016^{c}	0.667 ± 0.001^{d}
400	0.400 ± 0.002^{a}	1.900 ± 0.009^{b}	1.520 ± 0.003^{c}	0.800 ± 0.001^{d}

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The values were expressed as Mean ± SEM of duplicate tests. Means with different alphabets at the same concentration are significantly different at (P< 0.05), Means with the same alphabets at the same concentration are not significantly different.

Table 3.1b: Result showing Percentage Hydroxyl radical (OH^-) scavenging activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Concentration (µg/ml)	Percentage (%) Hydroxyl radical (OH^-) scavenging activity of *Adansonia digitata* Ethylacetate Leaves extract	Percentage (%) Hydroxyl radical (OH^-) scavenging activity of *Bryophyllum pinnatum* Ethylacetate Leaves extract	Percentage (%) Hydroxyl radical (OH^-) scavenging activity of *Vernonia amygdalina* Ethylacetate Leaves extract	Percentage (%) Hydroxyl radical (OH^-) scavenging activity of *Canna indica* Ethylacetate Leaves extract
50	66.57[a]	84.38[b]	62.66[c]	86.29[b]
100	58.26[a]	84.18[b]	55.26[a]	85.49[b]
150	54.45[a]	81.68[b]	47.95[c]	83.68[d]
200	53.35[a]	77.08[b]	46.55[c]	83.08[d]
250	44.34[a]	68.57[b]	36.44[c]	77.27[d]
300	37.24[a]	59.66[b]	39.24[a]	61.06[b]
350	33.03[a]	71.87[b]	28.53[c]	57.66[d]
400	26.43[a]	66.57[b]	26.53[c]	48.25[d]

values with different alphabets at the same concentration are significantly different at (P< 0.05), values with the same alphabets at the same concentration are not significantly different.

Table 3.1c: Result showing Percentage DPPH radical scavenging activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Concentration (µg/ml)	Percentage (%) DPPH scavenging activity of *Adansonia digitata* Ethylacetate Leaves extract	Percentage (%) DPPH scavenging activity of *Bryophyllum pinnatum* Ethylacetate Leaves extract	Percentage (%) DPPH scavenging activity of *Vernonia amygdalina* Ethylacetate Leaves extract	Percentage (%) DPPH scavenging activity of *Canna indica* Ethylacetate Leaves extract
50	44.00[a]	48.08[a]	54.71[b]	28.51[c]
100	58.88[a]	54.47[b]	58.63[a]	29.79[c]
150	64.53[a]	54.63[b]	60.80[c]	30.83[d]
200	64.62[a]	55.83[b]	61.82[a]	34.10[c]

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250	63.00[a]	56.07[b]	61.50[c]	39.29[d]
300	63.50[a]	59.03[b]	62.14[a]	21.17[c]
350	60.62[a]	60.62[a]	62.30[a]	27.87[c]
400	53.80[a]	61.98[b]	62.06[b]	45.20[c]

values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabets at the same concentration are not significantly different.

Table 3.1d: Result showing Percentage Inhibition of lipid peroxidation of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Concentration (µg/ml)	Percentage (%) inhibition of lipid peroxidation of *Adansonia digitata* Ethylacetate Leaves extract	Percentage (%) inhibition of lipid peroxidation of *Bryophyllum pinnatum* Ethylacetate Leaves extract	Percentage (%) inhibition of lipid peroxidation of *Vernonia amygdalina* Ethylacetate Leaves extract	Percentage (%) inhibition of lipid peroxidation of *Canna indica* Ethylacetate Leaves extract
50	18.41[a]	20.01[b]	23.76[c]	21.45[d]
100	18.53[a]	20.29[b]	24.08[c]	22.52[d]
150	18.73[a]	20.57[b]	24.08[c]	22.88[d]
200	19.41[a]	20.69[b]	24.24[c]	23.00[d]
250	19.61[a]	20.77[b]	24.28[c]	23.32[c]
300	21.65[a]	21.21[a]	24.52[b]	23.36[c]
350	28.68[a]	21.29[b]	24.84[c]	23.84[c]
400	26.60[a]	21.49[b]	24.96[b]	33.07[c]

values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabets at the same concentration are not significantly different.

Antioxidant potential

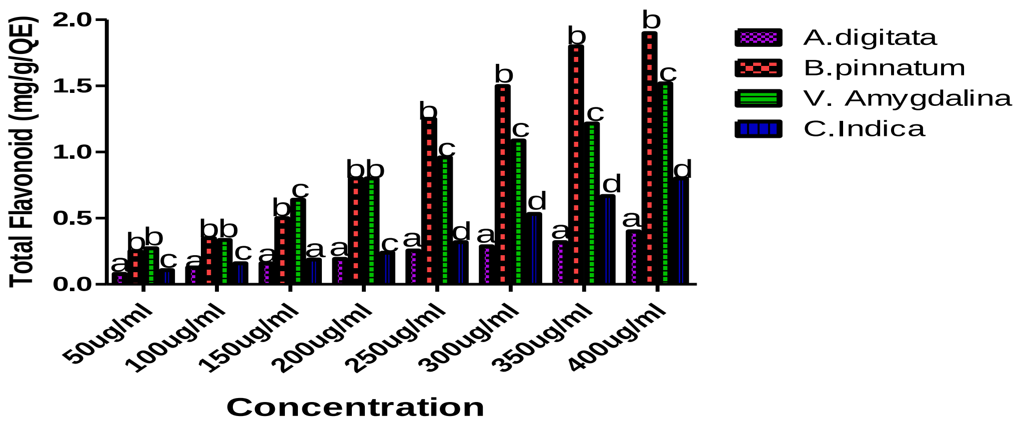

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Figure 3.1a: Total Flavonoids content (Quercetin Equivalent) of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

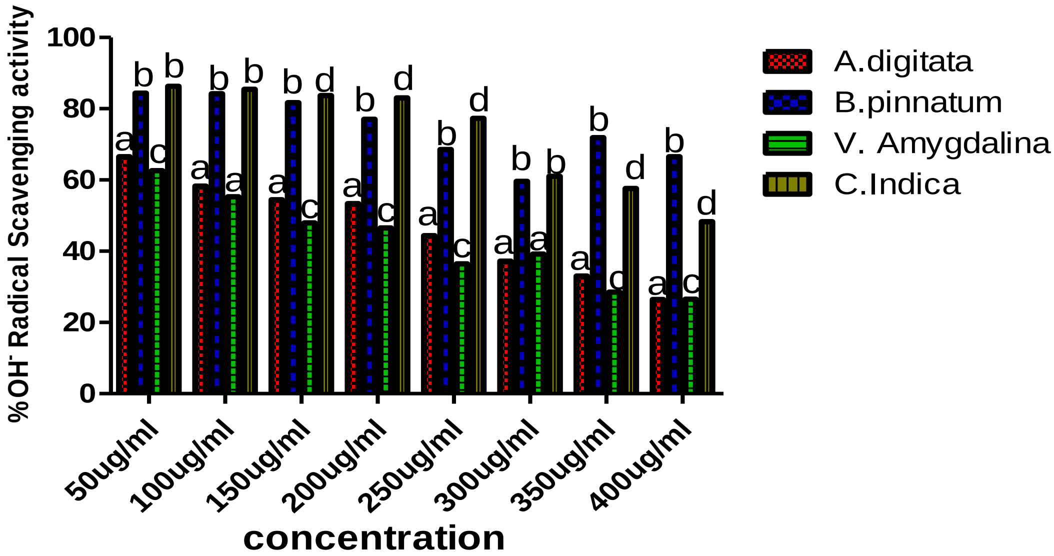

Figure 3.1b: Percentage Hydroxyl radical (OH^-) scavenging activity of Ethylacetate leaves extract of *Adansonia* digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica.

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

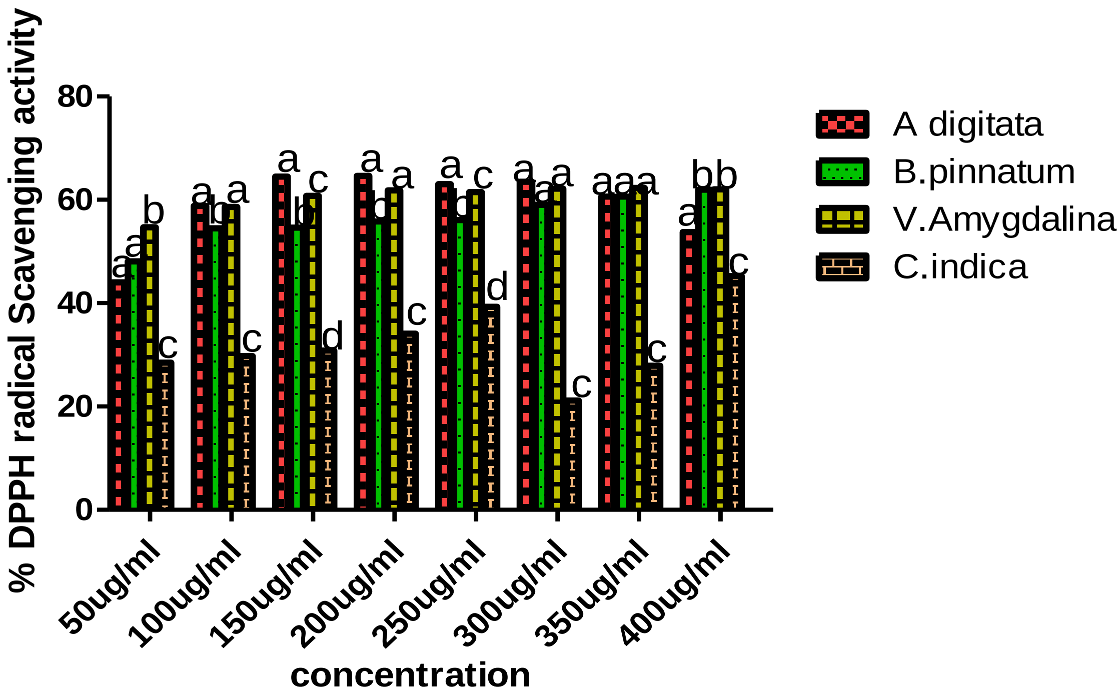

Figure 3.1c: Percentage DPPH radical scavenging activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

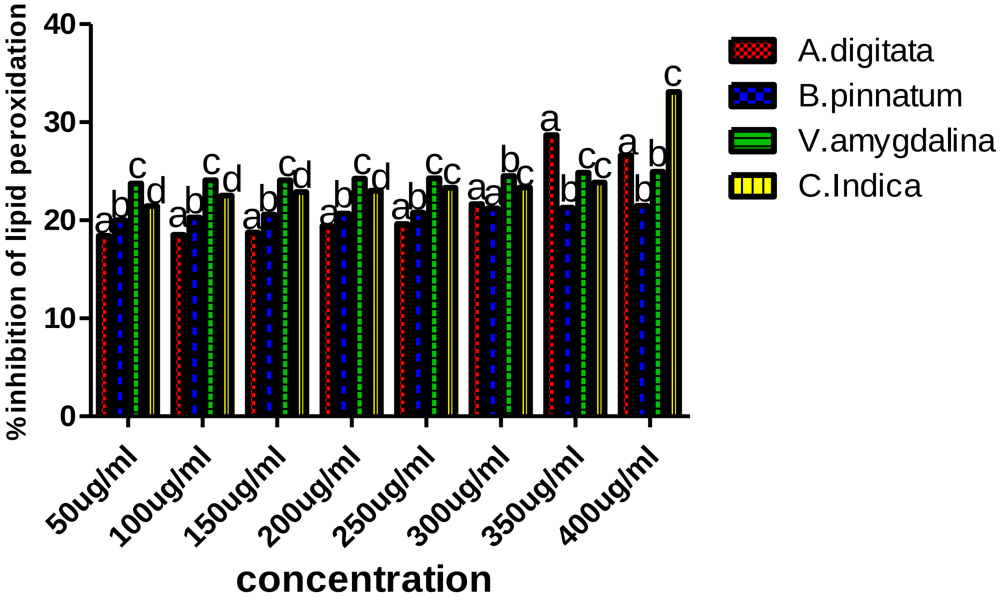

Figure 3.1d: Percentage Inhibition of lipid peroxidation of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

Anti-inflammatory potentials

Table 3.2a: Result showing Percentage inhibition of Proteinase activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Concentration (μg/ml)	Percentage (%) inhibition of proteinase activity of *Adansonia digitata* Ethylacetate Leaves extract	Percentage (%) inhibition of proteinase activity of *Bryophyllum pinnatum* Ethylacetate Leaves extract	Percentage (%) inhibition of Proteinase activity of *Vernonia amygdalina* Ethylacetate Leaves extract	Percentage (%) inhibition of Proteinase activity of *Canna indica* Ethylacetate Leaves extract
50	82.36^{a}	91.71^{c}	91.71^{c}	90.65^{c}
100	84.83^{a}	91.36^{b}	88.89^{c}	92.59^{a}
150	86.24^{a}	91.18^{b}	87.30^{a}	92.96^{b}
200	87.48^{a}	90.48^{b}	84.83^{c}	93.12^{d}
250	87.83^{a}	90.12^{b}	82.72^{c}	93.30^{d}
300	88.36^{a}	89.07^{a}	81.13^{b}	93.65^{c}

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350	89.42^{a}	87.30^{b}	77.60^{c}	94.18^{d}
400	90.50^{a}	85.89^{b}	76.19^{c}	94.88^{d}

values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabets at the same concentration are not significantly different.

Table 3.2b: Percentage Inhibition of Protein denaturation of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Concentration (µg/ml)	Percentage (%) inhibition of Protein denaturation of *Adansonia digitata* Ethylacetate Leaves extract	Percentage (%) inhibition of Protein denaturation of *Bryophyllum pinnatum* Ethylacetate Leaves extract	Percentage (%) inhibition of Protein denaturation of *Vernonia amygdalina* Ethylacetate Leaves extract	Percentage (%) inhibition of Protein denaturations of *Canna indica* Ethylacetate Leaves extract
50	79.30^{a}	87.28^{b}	78.05^{a}	86.78^{b}
100	74.31^{a}	82.79^{b}	72.82^{a}	86.28^{c}
150	67.58^{a}	78.80^{b}	66.83^{a}	84.54^{c}
200	62.84^{a}	76.06^{b}	59.60^{c}	81.79^{d}
250	56.11^{a}	72.07^{b}	53.37^{a}	82.04^{c}
300	46.31^{a}	68.83^{b}	46.13^{b}	79.30^{c}
350	44.14^{a}	65.59^{b}	42.37^{a}	79.55^{c}
400	36.40^{a}	59.60^{b}	32.92^{c}	71.82^{d}

values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabets at the same concentration are not significantly different.

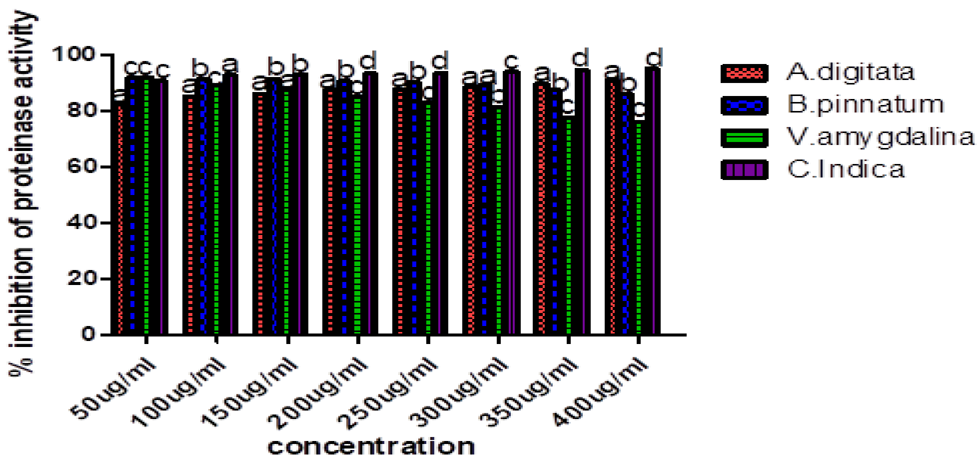

Figure 3.2a: Percentage inhibition of Proteinase activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

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Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

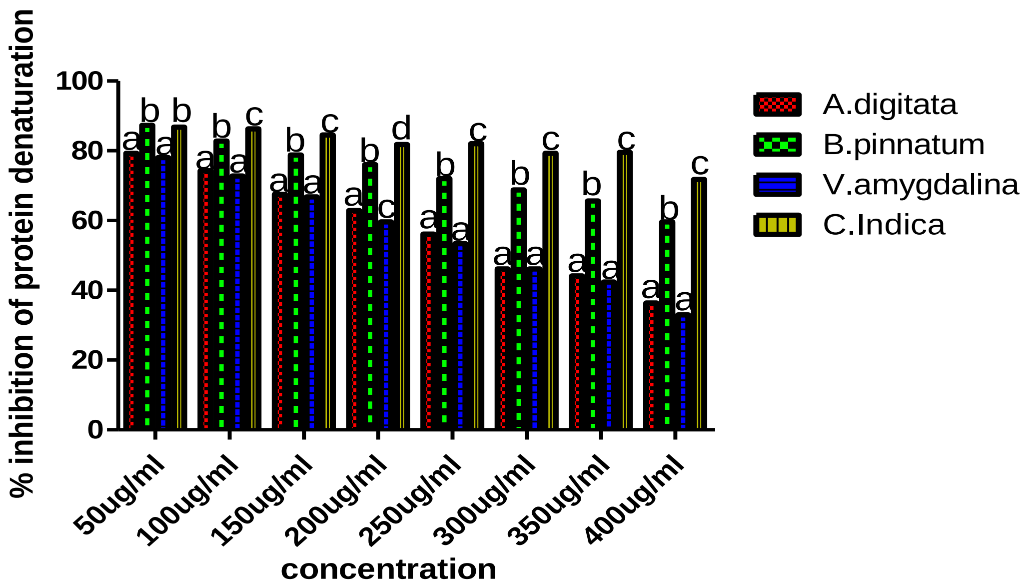

Figure 3.2b: Percentage Inhibition of protein denaturation of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina and Canna indica*.

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

Anti-ageing potential

Table 3.3a: Result showing Percentage Anti-tyrosinase activity of Ethylacetate leaves extract of *Adansonia digitata*, *Bryophyllum pinnatum*, *Vernonia amygdalina*, *Canna indica and* Kojic acid.

Concentration (mg/ml)	Percentage (%) Anti-tyrosinase activity of *Adansonia digitata* Ethylacetate Leaves extract	Percentage (%) Anti-tyrosinase activity of *Bryophyllum pinnatum* Ethylacetate Leaves extract of	Percentage (%) Anti-tyrosinase activity of *Vernonia amygdalina* Ethylacetate Leaves extract	Percentage (%) Anti-tyrosinase activity of *Canna indica* Ethylacetate Leaves extract	Percentage (%) Anti-tyrosinase activity of Kojic acid
0.03125	13.25[a]	23.85[b]	5.48[c]	9.54[c]	23.49[d]
0.0625	19.43[a]	32.16[b]	24.56[c]	14.66[d]	27.21[d]
0.125	22.14[a]	44.52[b]	30.92[c]	15.90[d]	30.57[e]
0.25	31.45[a]	46.29[b]	48.94[c]	27.56[d]	53.00[e]
0.50	43.99[a]	67.49[b]	75.97[c]	34.63[d]	75.79[e]
1.00	51.24[a]	77.56[b]	87.28[c]	44.35[d]	82.16[e]

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IC_{50}	0.57± 0.013[a]	0.32± 0.011[b]	0.28± 0.006[c]	1.05 ± 0.02[d]	0.03±0.00[e]

values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabet at the same concentration are not significantly different.

Table 3.3b (week 1): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and standard; Amino guanidine*

Concentration (mg/ml)	Percentage (%) Anti-glycation activity of *Adansonia digitata* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Bryophyllum pinnatum* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Vernonia amygdalina* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Canna indica* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of Aminoguanidine
0.3125	50.00[a]	1.09[b]	42.53[c]	30.27[d]	39.30[e]
0.625	50.79[a]	4.25[b]	52.82[c]	45.99[d]	43.20[e]
1.250	52.27[b]	9.69[b]	65.48[c]	49.46[d]	49.85[d]
2.500	60.29[a]	36.89[b]	69.14[b]	51.04[d]	50.55[d]
5.000	63.30[a]	47.08[b]	71.22[c]	58.75[d]	59.25[d]

values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabet at the same concentration are not significantly different.

Table 3.3b (week 2): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and standard; Amino guanidine*

Concentration (mg/ml)	Percentage (%) Anti-glycation activity of *Adansonia digitata* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Bryophyllum pinnatum* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Vernonia amygdalina* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Canna indica* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of Aminoguanidine
0.3125	58.56[a]	24.73[b]	64.59[c]	27.20[d]	72.80[e]
0.625	66.52[a]	38.48[b]	77.25[c]	28.68_{d}	78.30[e]
1.250	72.35[a]	42.04[b]	78.73[c]	35.80[d]	80.55[e]
2.500	74.93[a]	49.46[b]	82.39[c]	46.88[d]	81.20[c]
5.000	75.96[a]	51.43[b]	87.54[c]	52.62[b]	82.20[d]

Values with different alphabets at the same concentration are significantly different at ($P< 0.05$), values with the same alphabet at the same concentration are not significantly different.

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Table 3.3b (week 3): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and standard; Amino guanidine*.

Concentration (mg/ml)	Percentage (%) Anti-glycation activity of *Adansonia digitata* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Bryophyllum pinnatum* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Vernonia amygdalina* Ethylacetate leaves extract	Percentage (%) Anti-glycation activity of *Canna indica* Ethylacetate leaves extract of	Percentage (%) Anti-glycation activity of Aminoguanidine
0.3125	75.47[a]	34.12[b]	71.51[c]	39.96[d]	75.80[e]
0.625	76.56[a]	44.51[b]	82.19[b]	43.82[b]	76.05[a]
1.250	81.16[a]	49.06[b]	85.36[c]	50.45[b]	83.05[d]
2.500	82.99[a]	51.04[b]	85.60[c]	51.14[b]	83.10[d]
5.000	83.68[a]	53.02[b]	86.20[c]	59.94[d]	85.10[e]

values with different alphabets at the same concentration are significantly different at (P< 0.05), values with the same alphabet at the same concentration are not significantly different.

Table 3.3b (week 4): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and Amino guanidine*

Concentration (mg/ml)	Percentage (%) Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata*	Percentage (%) Anti-glycation activity of Ethylacetate leaves extract of *Bryophyllum pinnatum*	Percentage (%) Anti-glycation activity of Ethylacetate leaves extract of *Vernonia amygdalina*	Percentage (%) Anti-glycation activity of Ethylacetate leaves extract of *Canna indica*	Percentage (%) Anti-glycation activity of Aminoguanidine
0.3125	75.37[a]	49.46[b]	70.72[c]	27.00[d]	74.20[e]
0.625	80.81[a]	52.42[b]	84.27[c]	42.28[d]	77.05[e]
1.250	81.36[a]	59.05[b]	87.64[c]	73.44[d]	83.80[e]
2.500	81.60[a]	61.91[b]	88.92[c]	78.04[d]	85.25[e]
5.000	82.83[a]	63.89[b]	90.06[c]	78.29[d]	87.95[e]

values with different alphabets at the same concentration are significantly different at (P< 0.05), values with the same alphabet at the same concentration are not significantly different.

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(a) Anti-tyrosinase activity

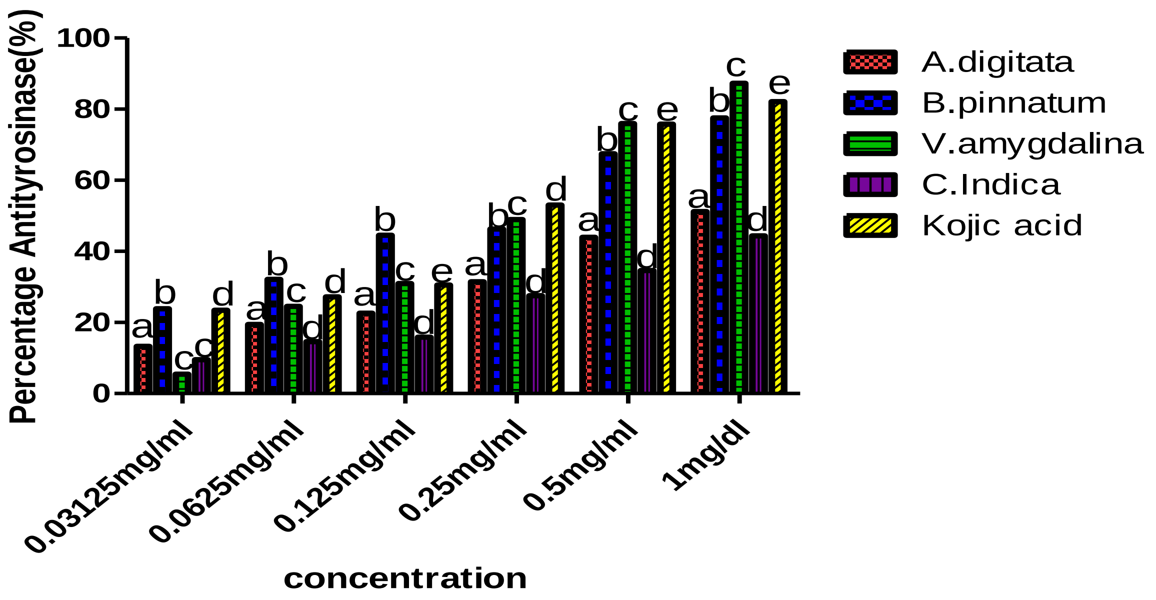

Figure 3.3a: Percentage Anti-tyrosinase activity of Ethylacetate leaves extract of *Adansonia digitata*, *Bryophyllum pinnatum*, *Vernonia amygdalina*, *Canna indica and* Kojic acid

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

(b) Anti-glycation potential (Week 1 – week 4)

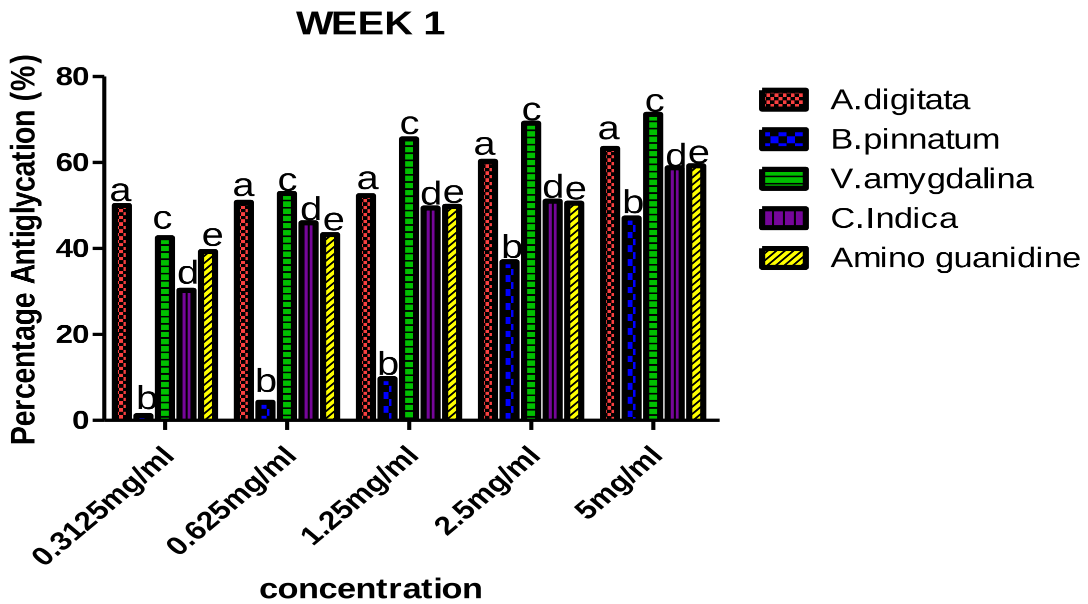

Figure 3.3b (i): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata*, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and standard; Amino guanidine (Week 1)

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

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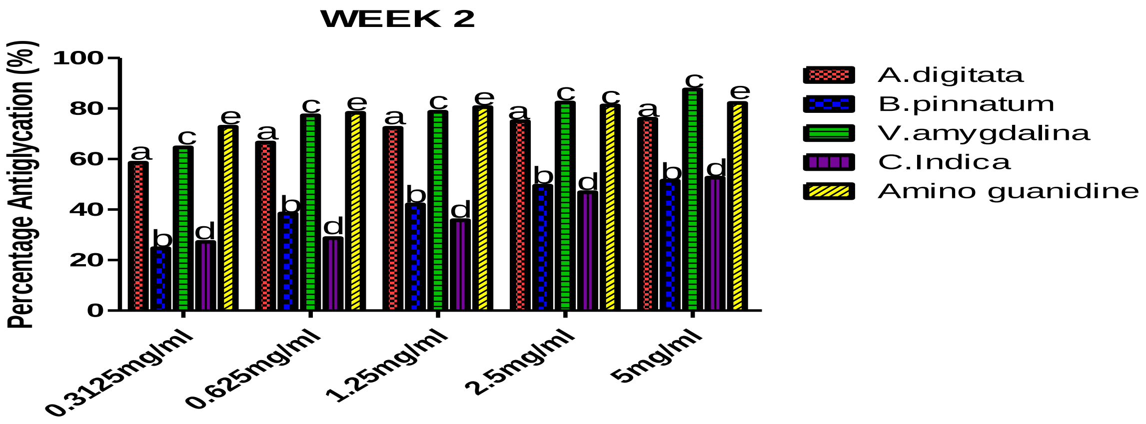

Figure 3.3b (ii): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and standard; Amino guanidine* (Week 2)

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

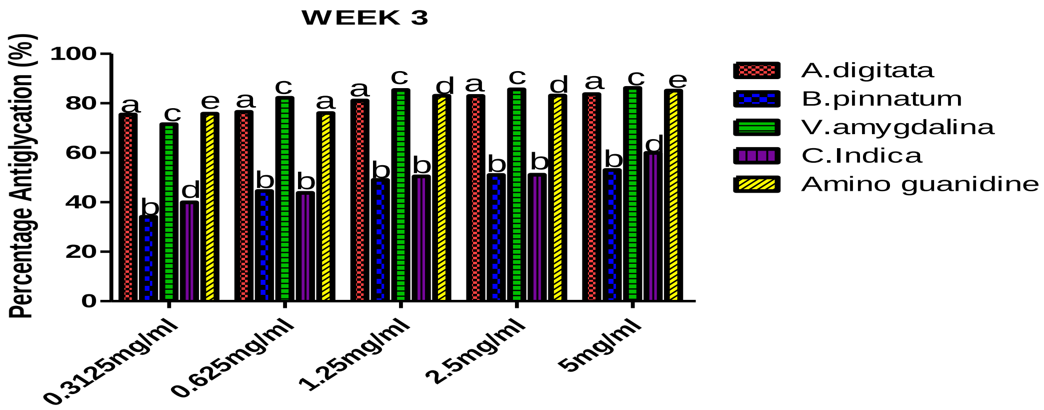

Figure 3.3b (iii): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and standard; Amino guanidine* (Week 3)

Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

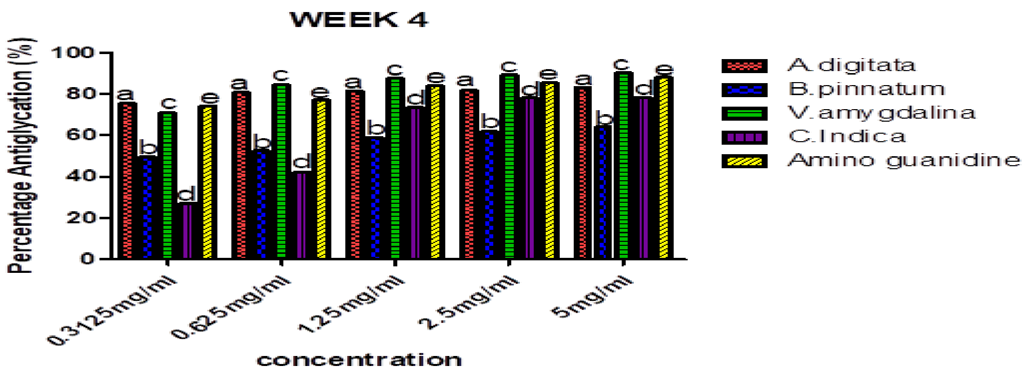

Figure 3.3b (iv): Percentage Anti-glycation activity of Ethylacetate leaves extract of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina, Canna indica and Amino guanidine* (Week 3)

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Bars with different alphabets at the same concentration are significantly different at (P< 0.05).

Bars with the same alphabet at the same concentration are not significantly different from each other.

DISCUSSION

Reactive Oxygen species (ROS) have been implicated in the etiology of ageing, and the antioxidant system has been reported to control ageing effects occasioned by ROS by decreasing the rate of free radical-mediated oxidative damage, thereby increasing the life expectancy of an organism (Kazeem *et al.*, 2012). Ageing is a complex and pleiotropic phenomenon, a natural and irreversible process that affects living organisms by adversely impacting the tissue and cells' functionality and morphology, and is responsible for various disease conditions like hypertension, atherosclerosis, Dementia, Diabetes, Osteoporosis, and Cancer, and have ever increasing economic impact on the health of people worldwide (Parmark *et al.*, 2022).

Flavonoids are a ubiquitous group of polyphenolic secondary metabolites in plants with a number of medicinal benefits, including antioxidant and anti-inflammatory properties (Ullah *et al.*, 2020). Flavonoids are present in various fruits and vegetables and are also responsible for their colours and defense mechanism (Miao *et al.*, 2022). Flavonoids function as antioxidants by neutralizing free radicals that can damage cells, hence, preventing or reducing free radical-mediated cellular damage, which can result in ageing (Jin *et al.*, 2022). Total flavonoid content was highest in *Bryophyllum pinnatum* (1.90 mg/g/Quercetin Equivalent) compared to the other three plants across various concentrations in a concentration-dependent manner. Moreover, *Vernonia amygdalina* (1.50mg/g/Quercetin Equivalent) also showed significant total flavonoid contents next to *Bryophyllum pinnatum,* while *Canna indica* (0.80mg/g/Quercetin Equivalent) has total flavonoid contents lower than both *Bryophyllum pinnatum and Vernonia amygdalina. Adansonia digitata* showed the lowest total flavonoid content (0.40 mg/g/Quercetin Equivalent). The total flavonoid content of these plants may reflect their individual ability to scavenge free radicals and also, a pointer to their therapeutic abilities to prevent, manage, or cure various oxidative stress-related and degenerative diseases.

Hydroxyl radical (OH-) is recognized as the most potent but short-lived of the reactive oxygen species radicals. It is a powerful oxidant produced via the Fenton reaction in the biological system (Richard *et al.*, 2015). Hydroxyl radicals have been reported to be implicated in the ageing process by causing oxidative damage to various cells and tissues of the body (Gurgoze *et al.*, 2007). The hydroxyl radical scavenging potential of the four plants was concentration dependent, decreasing with increasing concentration. *Canna indica* displayed the highest scavenging ability from (100 µg/ml to 300 µg/ml) with the percentages (86.29%, 85.49% and 83.68). *Bryophyllum pinnatum,* at the last two concentrations (350µg/ml and 400µg/ml), exhibited the highest hydroxyl radical scavenging ability (66.57% and 67.37%). *Adansonia digitata* also exhibited good scavenging potential across all the concentrations, while *Vernonia*

amygdalina showed the lowest scavenging ability across all concentrations. The plant extracts ability to scavenge hydroxyl radical also corroborated their potential to eliminate biological cells generated free radicals that may lead to ageing and other oxidative stress-related diseases (Adedosu *et al.*, 2018).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical is a stable compound used to ascertain the antioxidant activity of various substances while in solution (Njoya *et al.*, 2021). It is a free radical used to study antiradical activity (Bouabid *et al.*, 2020). It comprises an unpaired electron located on one of the atoms of the nitrogen bridge responsible for its characteristic violet colour. The efficacy of plants with antioxidant potentials is measured by a decrease in violet colouration. The liberation of the unpaired electron as a result of the trapping of radicals by antioxidant molecules correlates with their antiradical potentials (Bouabid *et al.*, 2020). 2,2-Diphenyl-1-picrylhydrazyl free radical Scavenging ability of the ethylacetate extracts of the plants showed that they exhibited concentration-dependent inhibition up to 200µg/ml. *Adansonia digitata* exhibited the highest DPPH scavenging potential from 100µg/ml to 300µg/ml, while *Vernonia amygdalina* showed the highest inhibition at 400µg/ml (62.06%). *Bryophyllum pinnatum* also, at 400µg/ml, showed a significant scavenging ability (61.98%) while *Canna Indica* showed the lowest DPPH scavenging potential across all the concentrations. Scavenging potential of these plant extracts might be attributed to a significant amount of phenolics present in them and a pointer to their possible antioxidant, anti-inflammatory, and anti-ageing abilities (Kazeem *et al.*, 2012; Chu *et al.*, 2002).

Lipid peroxidation refers to oxidation of polyunsaturated fatty acids, by free radicals which has been linked to various disease pathologies, as a result of oxidation products formed during such process, which includes reactive aldehydes such as malondialdehyde which can form adducts with proteins as well as DNA, resulting in alteration of their functions leading to ageing and various free radicals mediated diseases (Nam, 2011). Inhibition of lipid peroxidation of the four plant extracts was not concentration dependent, but seemed to be more pronounced at the last two concentrations (350µg/ml and 400µg/ml). *Vernonia amygdalina* showed the highest inhibition across various concentrations up to 300 µg/ml (24.52%), followed by *Canna indica* (23.36%). At the highest concentration (400 µg/ml), *Canna indica* exhibited the highest inhibition (33.07%), followed by *Adansonia digitata* (26.60%). However, *Adansonia digitata* exhibited the lowest inhibition up to 250 µg/ml. This also corroborates the abilities of these plant extracts; they may be utilized in the management and treatment of oxidative stress diseases. This effect may be traced to active secondary metabolites embedded in the leaves of these plants. (Adedosu *et al.*, 2017).

Proteinase, otherwise called protease or peptidase, is an enzyme that performs the function of catalyzing proteolysis as well as breaking down proteins into smaller polypeptides or individual amino acids (Lopez-Otin and Bond, 2008). They play important roles in various biological processes, like cell migration, immunity, wound healing, as well as cell death (Morohashi and Tomita, 2013). Protease, when activated, triggers the release of mediators of inflammation, and protease-mediated inflammation has been reported to promote insulin resistance, resulting in diabetes and other oxidative stress-related diseases (Lin *et al.*, 2020). Inhibition of proteinase activities of the extracts of the four plants was not concentration-dependent. However, *Canna indica* exhibited the highest proteinase inhibition across all concentrations. *Bryophyllum pinnatum* exhibited significant inhibition at concentrations below 400µg/ml than *Adansonia digitata*, while at the highest concentration (400 µg/ml), *Adansonia digitata* inhibition of proteinase activity (90.50%) was higher than *Bryophyllum pinnatum* (85.89%). *Vernonia amygdalina* inhibition decreases as the concentration increases, and exhibits the lowest inhibition of proteinase activity. Protease inhibitors have been reported to serve as anti-inflammatory agents that can terminate inflammation by modulating cytokine expression, tissue remodeling, as well as signal transduction (Shigetomi *et al.*, 2010 ; Machado *et al.*, 2022).

Proteins are large biological molecules that comprise various amino acid residues joined together by a peptide bond. They have a vast array of functions in living organisms, which include: catalyzing various metabolic reactions, Structural roles, and transportation of molecules from one location to another in the body system (Anyazor *et al.*, 2019). Protein denaturation results in loss of structural and biological functions. Denatured proteins have been reported to trigger an inflammatory response, and also, inflammation can result in the denaturation of proteins (Anyasor *et al.*, 2019). Agents that can inhibit protein denaturation can function as anti-inflammatory substances (Bachheti *et al.*, 2023). The anti-inflammatory activities of the four plant extracts were tested by the egg albumin denaturation assay. Most age-related disorders have been linked to inflammation, which is a significant factor for morbidity as well as mortality in old age (Ameena *et al.*, 2023). Inhibition of protein denaturation was concentration-dependent, as it decreased with increasing concentration across all concentrations. *Canna indica* showed the highest inhibition of protein denaturation across all concentrations, followed by *Bryophyllum pinnatum*. *Adansonia digitata* showed a slight increase in inhibition than *Vernonia amygdalina*, but not statistically significant ($P< 0.05$). All the four plant extracts showed notable abilities to inhibit protein denaturation, reflecting their anti-inflammatory activities and their potential to stabilize or maintain the integrity of structural and functional proteins, as well as their therapeutic abilities, which might be traceable to phenolic compounds present in them (Zeb, 2020).

Tyrosinase, a key enzyme in Melanogenesis, protects the skin against ultraviolet (UV) radiation exposure that may result in pathological skin conditions. Excessive production or abnormal accumulation of Melanin can result in age spots and hyperpigmentation (Majeed *et al.*, 2020). Suppression of activities of tyrosinase results in down-regulation of melanogenesis and reduces skin hyperpigmentation, which can prevent premature ageing as well as abnormal pigmentation of skin (Saeedi *et al.*, 2019; Majeed *et al.*, 2020; Mostafa *et al.*, 2021). All the four plant extracts exhibited good anti-tyrosinase activity in a concentration-dependent manner. *Bryophyllum pinnatum*, at the first three concentrations (0.03125mg/ml, 0.0625mg/ml and 0.125mg/ml) exhibited highest anti-tyrosinase potentials (23.85%, 32.16% and 44.52%) compared with other three plants extracts and standard (Kojic acid), while *Vernonia amygdalina*, displayed highest anti-tyrosinase activity at concentrations 0.25mg/ml

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(48.93%), 0.5mg/ml (75.97%), and 1mg/ml (87.28%)) respectively. *Adansonia digitata* also exhibited good anti-tyrosinase activity across all the concentrations, while *Canna indica* showed the lowest anti-tyrosinase activity throughout all the concentrations. These plant extracts possess various polyphenols having potential to suppress or inhibit tyrosinase activity, hence, may be employed as anti-ageing remedies.

Glycation refers to a non–enzymatic condensation reaction that occurs between the amino group of protein and reducing sugars, which latter undergo various rearrangements resulting in stable Ketoamines, leading to the formation of Advanced Glycation End products (AGEs) implicated in ageing (Kazeem *et al.*, 2012). All the four plant extracts displayed good anti-glycation potential in a concentration-dependent manner from week 1 to week 4. *Vernonia amygdalina* exhibited the highest antiglycation potential across all concentrations throughout the four weeks. Moreover, the antiglycation potential of *Vernonia amygdalina* was higher than that of Aminoguanidine (standard) throughout the four weeks. *Vernonia amygdalina* and *Adansonia digitata* displayed higher antiglycation activity than *Canna indica,* while *Bryophyllum pinnatum* displayed the lowest antiglycation potential. Glycation as well as AGE-induced-Toxicity have been reported to be associated with increased free radical production, hence, agents with good antioxidant potentials, mopping up free radicals, can also inhibit AGE formation. (Nakagawa *et al.,* 2002; Ahmad and Ahmed, 2006).

CONCLUSION:

The extracts of *Adansonia digitata, Bryophyllum pinnatum, Vernonia amygdalina*, and *Canna indica* exhibited remarkable antioxidant, anti-inflammatory, as well as anti-ageing activities, hence, may be employed in the treatment and management of ageing, related oxidative stress phenomenon such as inflammation and tissue degeneration, and as a possible template for drug design in the management and treatment of other oxidative stress-related diseases. The results also validated the medicinal uses of these plants as anti-ageing agents in Southwest Nigeria.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this work.

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