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BIOACTIVE PROFILE, ANTIOXIDANT AND ANTIDIABETIC POTENTIALS OF *MAGNIFERA INDICA* AND *VERNONIA AMYGDALINA IN VITRO*

Temidayo Ogunmoyole[1*], Ruth Kehinde Oladele, Yusuff Adewale Akeem[3], Olaitan Daniel Johnson[4], Adenike Oluwaseun Adefila[5] and Aderonke Mary Ogundare[6]

[1*]Department of Medical Biochemistry, Faculty of Basic Medical Science, College of Medicine, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.
[2]Department of Science Laboratory Technology (Biochemistry Option), Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.
[3]Department of Biochemistry, Faculty of Science, University of Ilesa, Osun State, Nigeria
[4]Department of Biochemistry, School of Applied Sciences, Auchi Polytechnic, Auchi, Edo State, Nigeria.
[5]Department of Biochemistry, Faculty of Science, University of Ilesa, Osun State, Nigeria.
[6]Department of Biochemistry, Faculty of Science, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.

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***Corresponding Author: Temidayo Ogunmoyole**
Department of Medical Biochemistry, Faculty of Basic Medical Science, College of Medicine, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.
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ABSTRACT

Vernonia amygdalina and *Magnifera indica* are plants commonly cultivated for their nutritional and medicinal benefits. This study aims to profile the bioactive contents responsible for the folkloric usage of the two plants in the treatment of diabetes and identify the possible *in vitro* mechanisms involved. Antioxidant properties such as free radical scavenging potential against 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitric oxide and hydroxyl radicals were determined following established procedures. Ferric reducing antioxidant potential (FRAP), Fe^{2+} chelating ability, as well as the ability of the extracts to inhibit iron (II)-induced hepatic, renal and pancreatic lipid peroxidation in vitro, were assayed following standard protocols. Bioactive constituents of the extracts were also identified using gas chromatography coupled with mass spectrometry (GC-MS)). At the same time, their antidiabetic potentials were measured *in vitro* by their inhibitory effects on alpha-amylase and alpha-glucosidase. Results indicate that both plants demonstrated marked free radical scavenging activities, iron chelation as well as significant inhibition of lipid peroxidation. A mixture of both plants exhibited stronger inhibition of alpha-amylase and glucosidase than acarbose. On GC-MS chromatogram, 16 and 23 bioactive constituents were identified in *V. amygdalina* extract and a mixture of *M. indica* and *V. amygdalina* extracts respectively. A mixture of both plants' extracts enhanced their bioactive profile, antioxidant activity, and antidiabetic potentials *in vitro*. This study provides the scientific basis for the traditional usage of both plants in the management of diabetes particularly in low-income nations where the cost of conventional treatment is not affordable.

KEYWORDS: *M. indica; V. amygdalina*; mixture; diabetes; antioxidant.

1.0 INTRODUCTION

Leaves of Mango (*Magnifera indica* L. Anacardiaceae) has been known for its usage in the treatment of multiple diseases affecting the lungs, gallbladder, and kidney. Reports indicate that leaf extract of mango tree has been used as an antidiabetic, antimalaria, anticancer, and anti-inflammatory agent as well in the treatment of bronchitis (Wightman *et al*., 2020). The medicinal potential of mango leaf has been linked to mangiferin, a unique polyphenol, abundantly present in it (Wightman *et al*., 2020). It has been suggested that *M. indica* extract enhances physical activity by increasing cerebral aerobiosis, as well as improving ergogenic functions in apparently healthy individuals after ischaemia-reperfusion (Gelabert *et al.*, 2018).

Bitter leaf (*Vernonia amygdalina*), is a perennial shrub belonging to the Vernonia genus in the family, Asteraceae. It is native to Africa but ubiquitously distributed across sub-Saharan Africa and parts of Asia (Wubayehu *et al.*, 2018; Nursuhaili *et al.*, 2019). It is one of the most widely used medicinal plants in the Vernonia genus. *V. amygdalina* leaf has been used as an antibiotic for controlling bacterial, and fungal infections. Its leaf extract has been used for treating hypertension and asthma and, as insect repellent (Sisay *et al.*, 2020). Besides their medicinal uses, *V. amygdalina* leaf is also used in the preparation of several Nigerian delicacies as appetizer and digestive tonic (Nursuhaili *et al.*, 2019; Olowoyeye *et al.*, 2022). The choice of *V. amygdalina* for human and animal nutrition is perhaps due to their high content of crude fibre, protein, dry matter, and ash. It is also rich in vital ions such as calcium, zinc, iron, sodium, potassium, and magnesium (Olumide *et al.*, 2019; Biru *et al.*, 2022).

Diabetes mellitus (DM), is a chronic metabolic derangement of carbohydrate metabolism commonly associated with unabatedly high blood glucose levels. Diabetes is a major threat to the attainment of Sustainable Development Goals on health particularly in Nigeria. It has been reported that an estimated 240 million individuals live with undiagnosed diabetes (Magliano *et al.*, 2021). Diabetes imposes huge financial stress on global healthcare systems. Reports have suggested that, worldwide, about 537 million (10.5%) people, between the ages of 20–79 years are managing diabetes (Magliano *et al*., 2021; Laraeni *et al*., 2021). Other reports have indicated that the prevalence of DM is expected to rise by 11.3% and 12.2% raising the number of diabetic patients to 643 and 783 million by 2030 and 2045 respectively (Onkyl *et al*., 2021; Magliano *et al*., 2021).

A cost-effective and potent panacea to the menace of diabetes is to leverage on medicinal plants. Since the leaf extracts of *M. indica* and *V. amygdalina* have been routinely used in treating diabetes in folkloric medicine, the present study is aimed at evaluating the possible synergistic effect that can be exploited when the two plant extracts are mixed and used as a regimen in the management of diabetes.

2.1 Collection of plant material

Leaves of *M. indica* and *V. amygdalina* were collected from Ekiti State University Campus, Ado Ekiti, Ekiti State Nigeria in March 2024. Taxonomic identification was made by a Senior Taxonomist, Mr Omotayo F. O. at the University Herbarium in the Department of Plant Science and Horticulture with voucher numbers 2024041 for *M. indica* and 2024040 for *V. amygdalina*.

2.2 Reagents and chemicals

2,2-diphenyl-1-picrylhydrazy (DPPH), Folin-ciocalteu reagent, sodium carbonate, sodium chloride, gallic acid, methanol, potassium acetate, Iron (III) chloride, sodium phosphate, potassium ferricyanide, trichloroacetic acid (TCA),

Ferrous sulphate Tris-HCl buffer, O-phenanthroline, sodium nitroprusside, saline phosphate buffer (PBS), sodium dodecyl sulphate (SDS), Griess reagent, thiobarbituric acid (TBA), and acetic acid were purchased from reputable commercial suppliers.

2.3 Preparation of Extracts

Leaves of *M. indica* and *V. amygdalina* collected were air-dried in a well-ventilated room and pulverised using a warring blender. The equal weight of each powdered leaf was mixed and extracted in methanol for 72 h with gentle shaking. In another determination, only *V. amygdalina* powder was extracted with methanol. Decantation was done to obtain two supernatants (from *V. amydalina* only and a mixture of *V. amygdalina* and *M. indica*) which were well-labelled and left to dry on the bench. The weight of the supernatants was monitored daily until they were fully dried, after the complete evaporation of methanol from the extract. Complete evaporation was confirmed by constant weight for three consecutive days. The extracts obtained were then weighed, refrigerated and reconstituted in distilled water for subsequent *in vitro* analysis (Ogunmoyole *et al*., 2023).

2.4 Antioxidant Assays

2.4.1 Free Radical Scavenging Ability

The free radical scavenging ability of *V. amygdalina* and a mixture of *M. indica* and *V. amygdalina* extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined according to an established protocol (Gyamfi *et al.,* 1999). One millilitre (1 ml) aliquot of each extract was mixed with 1 ml of methanolic DPPH (0.4 mM) radical. Incubation of mixture was done for 30 min in the dark at 25 ^{0}C. The absorbance of the resulting mixture was read at 516 nm. The blank test tube contained DPPH solution mixed with distilled water to replace the extract. The percentage DPPH radical scavenging potential of the extract was then estimated relative to the control.

2.4.2 Estimation of Ferric Reduction

The ability of the extracts to reduce $FeCl_3$ was measured according to the method of Oyaizu (1986). Sample of the extract was mixed with 2.5 ml each of 0.2 M sodium phosphate buffer at pH 6.6 and potassium ferricyanide (1% w/v). Incubation of the mixture was performed at 50 ^{o}C for 20 min followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v). Centrifugation of the mixture was done at 801 × *g* for 10 min. Five millilitres each of the supernatant and water were mixed and 1 ml of ferric chloride (0.1% w/v). The optical density of the final mixture was read at 700 nm and ferric reducing power was estimated as ascorbic acid equivalent.

2.4.3 Fe^{2+} Chelation Assay

The ability of the extracts to chelate Fe^{2+} was measured following the modified method of Minotti and Aust (1987), and Puntel *et al.* (2005). One hundred and fifty microliters of $FeSO_4$ (500 µM) was transferred to a reaction mixture consisting of 100 mM Tris-HCl (168 µl, pH 7.4), saline (218 µl) and the extracts (0 – 25 µl). Incubation of the reaction mixture was done in the water bath at 25 ^{0}C for 5 min, followed by the introduction of 13 µl of 1,10 phenanthroline (0.25%, w/v). The absorbance was monitored at 510 nm and Fe^{2+} chelating activity (%) was estimated relative to the control.

2.4.4 Determination of nitric oxide radical scavenging ability

The method of Igbinosa *et al* (2011) was adopted in determining the ability of the extracts to scavenge nitric oxide. One millilitre of sodium nitroprusside (25 mM) in 0.5 mM phosphate buffered saline (PBS) at pH 7.4 was transferred to 0.5

ml of plant extract or standard (100–400 µg/ml) and thoroughly mixed and transferred to a water bath for 2h at37ºC for 2 h. One millilitre of the mixture was measured and added to Griess reagent (1 ml) and transferred to an incubator at 25 ^{0}C for 30 min. The absorbance was read at 546 nm and the percentage nitric oxide scavenging ability of the extract was estimated.

$$\% \text{ nitric oxide radical} = \frac{A_{(control)} - A_{(extract)} * 100}{A_{(control)}}$$

2.4.5 Inhibition of lipid peroxidation

The method described by Okhawa *et al.* (1979) was followed in the determination of lipid peroxidation. One hundred microliter of liver, kidney and pancreas homogenates was added separately to test tubes containing 100 mM Tris-HCl at pH 7.4, plant extracts (0 - 100 µl) and Iron (II) sulphate (25 µM). Three hundred microliter of water was added and the test tubes transferred to the water bath for 2 hours at 37^{0}C. After incubation, the following were added in sequence: Sodium dodecyl sulphate, (300 µl 8.1% w/v), 600 µl each of acetate buffer at pH 3.4 and thiobarbituric acid (TBA, 0.8% w/v). Another round of incubation was then performed for 1 h at 100°C. The optical density of the products (thiobarbituric acid reactive species (TBARS)) was monitored at 532 nm in a UV-Visible spectrophotometer. Malondialdehyde (MDA) produced was presented as a percentage of the blank.

2.5 Assay of α-Amylase Activity

Extracts of both plants were assayed for amylase inhibition following the CNPG3 method (2-chloro-p-nitrophenyl-D-maltotrioside) using acarbose as standard. The method described by Gella *et al*. was adopted with little modifications. The enzyme solution contained 0.032 mg/ml of amylase in 40 mM potassium phosphate buffer mixing 3.20 mg of amylase enzyme in 100 ml of 0.4 M phosphate buffer at pH 6.9. Acarbose (positive control) contained 2.5 mg/ml in 0.4 M phosphate buffer. Serial dilutions of the samples were prepared to give 10, 50, and 100 µg/ml of acarbose in the final concentration. In separate determinations, acarbose and the extracts were separately mixed with 125 µl of 2-chloro-4-nitrophenol-D-maltotrioside (CNPG3) followed by incubation for 8 min at 37°C. The optical density was read at 405 nm using a UV-visible spectrophotometer.

$$\text{Inhibition (\%)} = \frac{Abs_{405}\text{ (control)} - Abs_{405}\text{ (extract)} * 100}{Abs_{4105}\text{(control)}}$$

2.6 α-Glucosidase Assay

The p-NPG was used as a substrate in the α -α-glucosidase inhibition assay performed using methods previously described by Telagari and Hullatti, (2015), with minor changes. Twenty microliters (20 µL) of plant extracts (in 30% DMSO) was added to 20 µL of 0.5 U/mL glucosidase and 120 µL of buffer and incubation was done in a water bath for 15 min at 37 °C. The reaction was then stopped by adding 40 µL of p-NPG and another round of incubation at 37 °C 15 min was performed. The absorbance of the mixture was monitored at 405 nm using a UV-visible spectrophotometer with acarbose serving as the reference α -glucosidase inhibitor.

Percentage α-glucosidase inhibition was determined using the following formula:

$$\text{Inhibition (\%)} = \frac{Abs_{405}\text{ (control)} - Abs_{405}\text{ (extract)} * 100}{Abs_{410}\text{ (control)}}$$

2.7 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis of the extract was performed using a Varian 3800/4000 gas chromatograph mass spectrometer equipped with an Agilent fitted with a capillary column DB5ms (30.0m x 0.25mm, 0.25µm film thickness) The carrier gas used is Nitrogen with 99.9995% purity and at low of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min as Sparkman et al. (2011) described. The identification of components was based on Willey and NIST libraries and a comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated (NIST, 2011).

All the samples and replicates were continuously injected as one batch in random order to discriminate technical from biological variations. Additionally, the prepared pooled samples were used as quality controls (QCs), which were injected at regular intervals throughout the analytical run to provide a set of data from which the repeatability can be assessed.

2.8 Statistical analysis

All experimental data were expressed as mean ± S.D. Data were analysed using One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using the SPSS 21 version for Windows. The level of significance was at $p < 0.05$.

3.0 RESULT AND DISCUSSION

Table 1 shows the inhibition of α-amylase by acarbose and *V. amygdalina* leaf. Acarbose steadily increased α-amylase inhibition with increasing concentration, reaching a maximum inhibition of 38.73% at 100 µg/ml. In contrast, *V. amygdalina* leaf demonstrated a higher inhibition percentage across all concentrations, peaking at 60.23% inhibition at 100 µg/ml. The IC_{50} values indicate that *V. amygdalina* leaf (9.74 µg/ml) is less potent than acarbose (1.39 µg/ml) in inhibiting α-amylase. Specifically, *Vernonia amygdalina* leaf extract demonstrated potent inhibitory effects on α-amylase, reaching a maximum inhibition of 60.23% at 100 µg/ml, and on α-glucosidase with a maximum inhibition of 36.18% at the same concentration. Table 2 presents the α-glucosidase inhibition data. Acarbose exhibited higher inhibition levels than *V. amygdalina* leaf, achieving 56.21% inhibition at 100 µg/ml. On the other hand, *V. amygdalina* leaf showed a maximum inhibition of 36.18% at the same concentration. The IC_{50} for *V. amygdalina* leaf (5.96 µg/ml) was higher than that for acarbose (3.08 µg/ml), indicating that *V. amygdalina* leaf was less effective in inhibiting α-glucosidase.

Table 3 shows the glycemic index of *V. amygdalina* and a mixture *M. indica* and *V. amygdalina*. Table 4 details the α-amylase inhibition results for the mixture of *M. indica* and *V. amygdalina* leaf. Acarbose maintained higher inhibition levels at lower concentrations, yet the *M. indica* and *V. amygdalina* leaf combination demonstrates slightly higher inhibition (63.56%) at 100 µg/ml compared to acarbose (61.05%). The IC_{50} for the mixture of *M. indica* and *V. amygdalina* leaf extracts (3.15 µg/ml) is slightly higher than for acarbose (2.71 µg/ml). Table 5 shows the inhibitory effect of the mixture of *M. indica* and *V. amygdalina* extracts on α-glucosidase activity. It showed an IC_{50} value of 3.15 and 2.71 for mixture of the two extracts and acarbose respectively. Inhibition of alpha-amylase and alpha-glucosidase is a critical strategy for managing diabetes by controlling postprandial hyperglycaemia. These enzymes play pivotal roles in carbohydrate metabolism by facilitating the breakdown of complex carbohydrates into simpler sugars and the subsequent absorption of glucose in the gastrointestinal tract. *V. amygdalina* leaf extracts exhibited significant

inhibition of both α-amylase and α-glucosidase enzymes across various concentrations. This inhibition pattern suggests promising antidiabetic effects of the extract perhaps by slowing down the digestion of dietary starches and reducing glucose absorption rates into the bloodstream (Oboh and Aluyor, 2019). On the other hand, a mixture of *M. indica* and *V. amygdalina* leaf extracts showed an enhanced inhibitory effect on α-amylase and α-glucosidase compared to *Vernonia amygdalina* leaf alone. This synergistic effect suggests that the bioactive compounds present in both plants may act synergistically to potentiate their antidiabetic properties (Olatunde *et al*., 2020). The *M. indica* + *V. amygdalina* leaf combination achieved a maximum inhibition of 79.24% for α-amylase and 63.56% for α-glucosidase at 100 µg/ml, with IC_{50} values indicating effective inhibition even at lower concentrations.

Table 6 shows the bioactive compounds in *V. amygdalina* leaf extracts. The primary compounds detected include 2,3-butanediol (4.79% peak area), benzaldehyde, 4-ethyl- (2.66% peak area), and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.05% peak area). Notably, n-hexadecanoic acid (14.89% peak area) and Oleic acid (24.47% peak area) are present in significant quantities. The presence of these fatty acids, along with other compounds like caryophyllene (8.51% peak area) and phenol derivatives, indicates a diverse range of bioactive molecules in the extract. The bioactive compounds present in the *V. amygdalina* leaf extract showed significant quantities of several key bioactive principles including n-hexadecanoic acid, oleic acid, caryophyllene, and various phenol derivatives (Table 6). The broader spectrum of bioactive compounds in the combined extracts results in enhanced antioxidant activity and potential therapeutic effects, thereby underscoring the potential of *M. indica* and *V. amygdalina* leaf extracts as natural sources of antioxidants and bioactive molecules. These extracts can be utilized in the development of functional foods or pharmaceuticals aimed at combating oxidative stress-related diseases and promoting overall health.

These compounds are recognized for their potent antioxidant properties, which are crucial for neutralizing free radicals and reducing oxidative stress. Palmitic acid is a saturated fatty acid that plays a vital role in maintaining cellular integrity and function. Oleic acid, a monounsaturated fatty acid found abundantly in olive oil, is known for its cardioprotective effects and its ability to enhance the body's antioxidant defences by neutralizing free radicals. Caryophyllene, a bicyclic sesquiterpene, is notable for its anti-inflammatory, analgesic, and antioxidant properties, contributing significantly to the therapeutic potential of *V. amygdalina*. The phenol derivatives present in the extract exhibit strong antioxidant activities by scavenging free radicals, chelating metal ions, and inhibiting oxidative enzymes, thereby protecting cells from oxidative damage. A study conducted by Ezejiofor *et al*. (2020) reported similar findings, highlighting the antioxidant capabilities of *V. amygdalina*. Collectively, these compounds contribute to the plant's health-promoting properties, making it a valuable natural resource for combating oxidative stress-related diseases.

Table 7 shows bioactive compounds in the combined extracts of *M. indica* and *V. amygdalina* leaf. Significant compounds include dihydroxyacetone (12.31% peak area), 3-furaldehyde (5.23% peak area), and 1,2-benzenediol (8.31% peak area). Additionally, n-hexadecanoic acid (14.46% peak area) and oleic acid (10.15% peak area) were also present. Other prominent compounds such as eugenol (3.69% peak area) and γ-sitosterol (3.54% peak area) highlight the enhanced bioactive profile when *M. indica* was combined with *V. amygdalina* leaf.

The bioactive fingerprints of the combined extracts of *M. indica* (mango) and *V. amygdalina* leaf (Figure 7) showed a broader spectrum of phytochemicals compared to the individual extracts, indicating a synergistic effect that enhances their antioxidant and therapeutic potential. The combined extracts contain compounds such as dihydroxyacetone, eugenol, and γ-sitosterol. Dihydroxyacetone, commonly used in cosmetics, has shown promising antioxidant properties

that contribute to the overall antioxidant activity of the combined extracts, helping to reduce oxidative damage at the cellular level. Eugenol, a well-known phenolic compound found in cloves, is renowned for its antioxidant, anti-inflammatory, and antimicrobial properties, adding significant therapeutic value to the combined extracts. γ-Sitosterol, a plant sterol with a structure similar to cholesterol, has been studied for its cholesterol-lowering effects and its ability to enhance the antioxidant defences of cells, as well as exhibiting anti-inflammatory and anticancer properties.

Figures 1a and b present the GC-MS chromatograms of the two extracts and *V. amygdalina* alone respectively. These Figures illustrate the phytochemical profiles of the samples, highlighting the distinct peaks corresponding to the various compounds present in each extract. The GCMS analysis provides a detailed understanding of the chemical composition and the relative abundance of bioactive compounds in the *M. indica* + *V. amygdalina* leaf combination and *V. amygdalina* leaf alone.

Figures 2a and b demonstrate the free radical scavenging ability against DPPH a mixture of the extracts *and V. amygdalina* only respectively. There was no activity at the initial concentration (0 mg/ml), but at 0.43 mg/ml, the %DPPH left was 100, indicating no scavenging activity. As the concentration of the extract increases, the %DPPH radical remaining decreased significantly, showing 52.4% at 0.86 mg/ml, 36.9% at 2.14 mg/ml, and 19.3% at 3.43 mg/ml of the extract. In recent years, exploring natural sources for bioactive compounds with potential health benefits has gained significant attention. *M. indica* (mango) and *V. amygdalina* (bitter leaf), both widely distributed in Africa and particularly in Nigeria, have been studied extensively for their bioactive constituents, antioxidant properties, and potential anti-diabetic effects.

Free radical scavenging activity of *M. indica* and *V. amygdalina* mixture against DPPH (Figure 2b), showed a concentration-dependent scavenging potential. This result corroborates the work of Ijeh *et al*. (2022) and Ajibola *et al*. (2020) who reported similar DPPH scavenging activities in *V. amygdalina* leaf extracts, albeit at different concentrations, reinforcing the antioxidant capabilities observed in this study., who reported. Comparatively, the DPPH scavenging activity of *V. amygdalina* leaf alone (Figure 2a) was lower than the mixture, indicating lower radical scavenging efficiency caused by less bioactive constituents. At 0 mg/ml, there was no activity, while at 0.86 mg/ml, the %DPPH remaining was 73.6%. This decreased to 52.3% at 1.29 mg/ml, 36.9% at 2.14 mg/ml, and 26.1% at 3.43 mg/ml. The *V. amygdalina* leaf showed a lower scavenging activity when compared to a mixture of *M. indica* and *V. amygdalina* extract. This observation is consistent with the findings of previous studies (Udeh *et al*., 2020), which suggested that the combination of *M. indica* and *V. amygdalina* leaf extracts enhances antioxidant potential due to synergistic interactions among their bioactive constituents.

Figure 3a illustrates the ferric-reducing antioxidant power (FRAP) of *V. amygdalina* leaf. At 0 mg/ml, there was no activity, but at 0.85 mg/ml, the percentage of iron (III) reduced was 1729.36%. This percentage iron (III) reduced increased progressively with concentration, reaching 2019.27% at 4.23 mg/ml. Figure 3b shows the FRAP of *M. indica* + *V. amygdalina* leaf. At 0 mg/ml, there was no activity, while at 0.65 mg/ml, the percentage of iron (III) reduced was 1723.85%. This value increased with concentration, reaching 2246.79% at 3.23 mg/ml, demonstrating a higher reducing power compared to *V. amygdalina* leaf alone. Ferric-reducing antioxidant power is usually employed for measuring the ability of extracts to reduce Fe^{3+} to Fe^{2+}. The mixture of the extracts demonstrated a stronger reductive capacity than when *V. amygdalina* extract was used alone. The higher FRAP values observed in the mixture of the two extracts, compared to *V. amygdalina* extract alone suggests enhanced ferric-reducing antioxidant potential (Figures 3a

and b). This finding aligns with the study of Ogunrinola *et al*. (2021), who highlighted the superior antioxidant activity of combined plant extracts compared to individual extracts. A mixture of the two extracts exhibited a more potent hydrogen peroxide scavenging effect than *V. amygdalina* extract alone. This perhaps suggests that more phytoconstituents were present in the mixture, which acted additively or synergistically to produce stronger effects (Figures 3a and b). This result supports the potential use of these extracts in mitigating oxidative stress-related damage, as proposed by a recent study (Oboh *et al*., 2023).

Figures 4a and b present the hydrogen peroxide scavenging capacity of *M. indica* + *V. amygdalina* leaf. At 0 mg/ml, there was no activity, while at 19.4 mg/ml, the % hydrogen peroxide scavenging was 94.88%. This value slightly decreased with higher concentrations, showing 92.31% at 58.2 mg/ml. Figure 4b illustrates the hydrogen peroxide scavenging capacity of *V. amygdalina* leaf alone. At 0 mg/ml, there was no activity, but at 14.6 mg/ml, the % hydrogen peroxide scavenging was 105.03%. This value remained relatively stable with a slight decrease to 103.57% at 36.5 mg/ml, indicating a strong scavenging capacity.

Figure 5a show the iron-chelating activity of *M. indica* + *V. amygdalina* leaf. At 0 mg/ml, there was no activity, while at 1.29 mg/ml, the percentage of iron chelating was 9.054%. This percentage increased with concentration, reaching 32.73% at 28.4 mg/ml. Figure 5b presents the iron chelating activity of *V. amygdalina* leaf. At 0 mg/ml, there was no activity, but at 1.69 mg/ml, the percentage of iron chelating was 8.58%. This percentage increased to 21.83% at 33.79 mg/ml, showing lower chelating activity when compared to the combination. The iron (II) chelating effect of the mixture of *M. indica* and *V. amygdalina* extracts was stronger than *V. amydalina* extract alone. This observation can be attributed to the higher quantity and quality of bioactive principles present in the mixture. This finding is in agreement with the earlier report of the work of Olaniyan *et al*. (2021) highlighted comparable iron-chelating properties in *M. indica* and *V. amygdalina* leaf extracts.

The percentage lipid peroxidation was 100% at 0 mg/ml of *M. indica* + *V. amygdalina* leaf extract on the liver (Figure 6a). This remained unchanged even at 2.69 mg/ml, then decreases significantly to 56.7% at 5.39 mg/ml. For *M. indica* + *V. amygdalina* leaf extract on the pancreas (Figure 6b), at 0 mg/ml, the % lipid peroxidation was 100%. This slightly decreased to 98.2% at 1.61 mg/ml and further to 77.1% at 2.69 mg/ml. For *V. amygdalina* leaf extract on the pancreas (Figure 6c), at 0 mg/ml, the % lipid peroxidation is 100%. This remained at 100% at 1.22 mg/ml, then decreased to 88.3% at 3.24 mg/ml. For *V. amygdalina* leaf extract on the kidney (Figure 6d), at 0 mg/ml, the % lipid peroxidation is 100%. This remained at 100% at 1.22 mg/ml, then decreased to 88.2% at 3.24 mg/ml.

Figure 7a show the nitric oxide radical scavenging ability of the mixture of both extracts and *V. amygdalina* alone respectively. At 0 mg/ml, there was no activity, while at 2.08 mg/ml, the % nitric oxide scavenged was 52.23%. This increased with concentration, reaching 58.54% at 10.4 mg/ml. Figures 7b present the nitric oxide radical scavenging ability of *V. amygdalina* leaf alone. At 0 mg/ml, there was no activity, while at 3.44 mg/ml, the % nitric oxide scavenging was 46.23%. This increased with concentration, reaching 61.85% at 17.2 mg/ml, suggesting a higher scavenging activity when compared to the combination. The strength of antioxidant agents has also been routinely assessed by measuring their ability to scavenge nitric oxide (NO) radicals. In the present study, a mixture of the two extracts produced a significantly higher NO radical scavenging effect than *V. amygdalina* extract alone. This stronger effect could be attributed to the polyphenolic presence in the mixture than the *V. amygdalina*.

Comparative analysis with existing literature supports the findings regarding the antioxidant and antidiabetic potentials of *M. indica* and *V. amygdalina* leaf extracts. Additionally, the. However, discrepancies in potency and efficacy among different studies highlight the critical need for standardized methods and conditions in evaluating bioactive properties (Oyedemi *et al*., 2019). Variations in geographical origin, plant genotype, extraction methods, and assay protocols can contribute to inconsistencies in reported results.

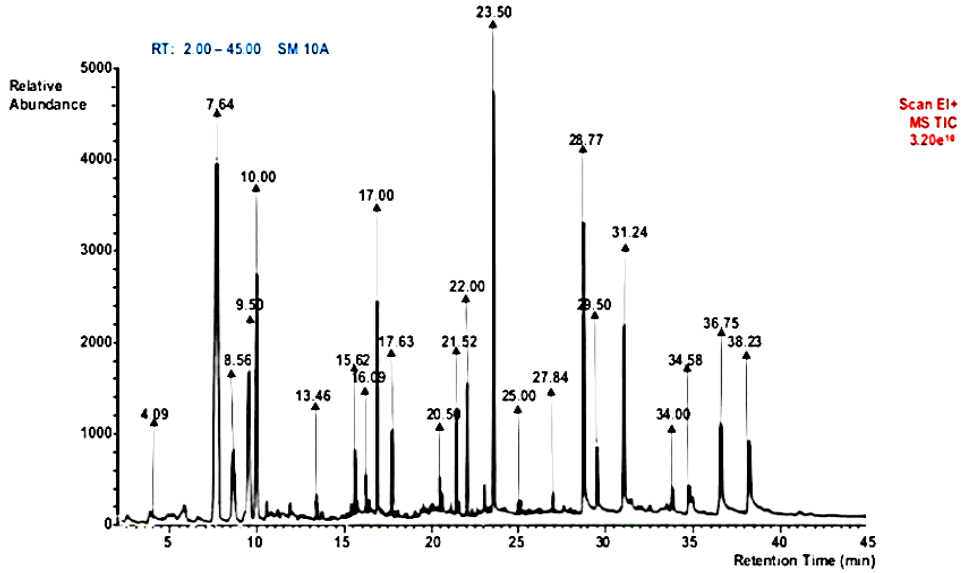

Figure 1a: GC-MS Chromatogram of *M. indica* + *V. amygdalina* leaf.

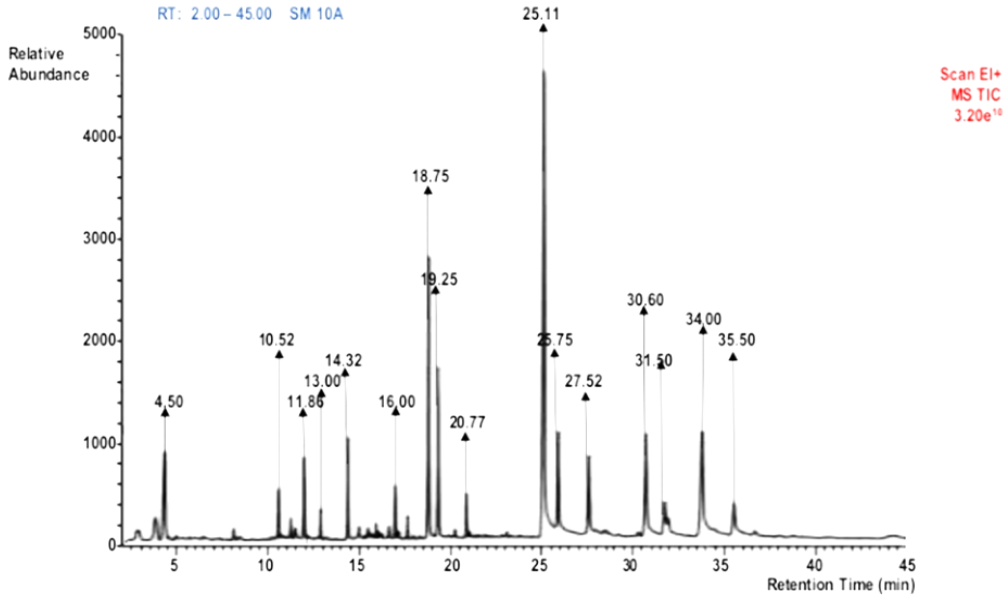

Figure 1b: GCMS Chromatogram of *V. amygdalina* leaf.

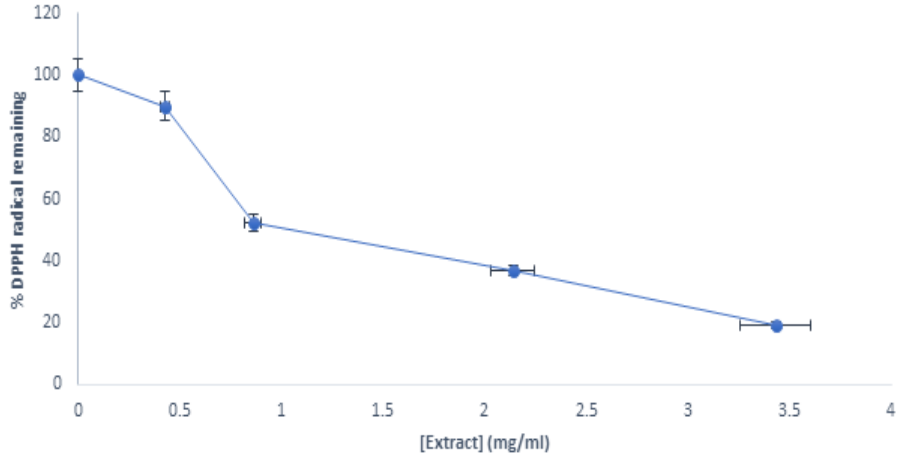

Figure 2a: Free radical scavenging ability of *M. indica* + *V. amygdalina* leaf against DPPH. Data are represented as mean ± S.D (n=3) at P < 0.05.

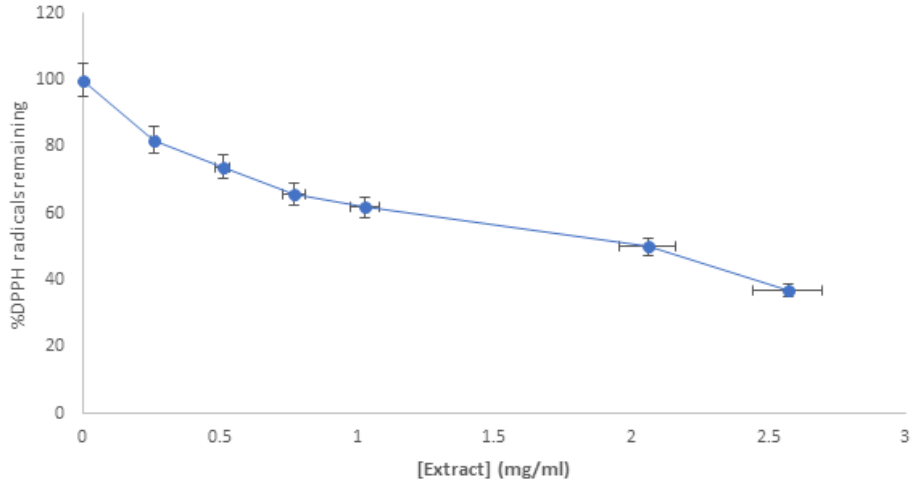

Figure 2b: Free radical scavenging ability of *V. amygdalina* leaf against DPPH. Data are represented as mean ± S.D (n=3) at P < 0.05.

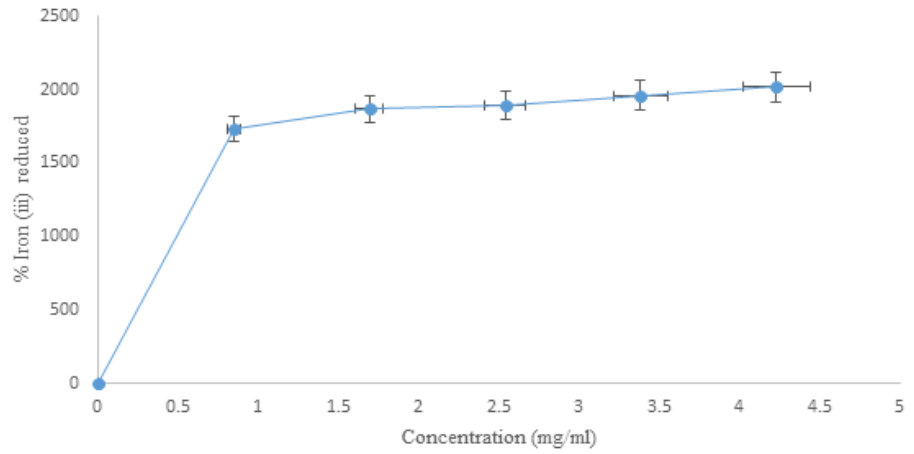

Figure 3a: Ferric reducing antioxidant power (FRAP) of *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

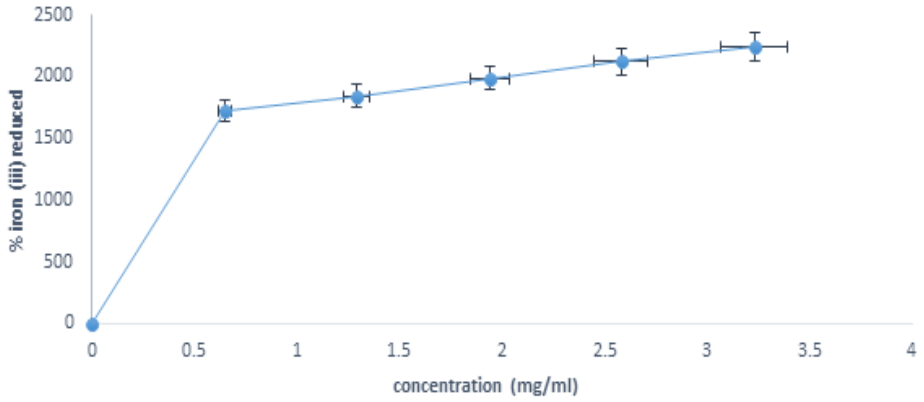

Figure 3b: Ferric reducing antioxidant power (FRAP) of *M. indica* + *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

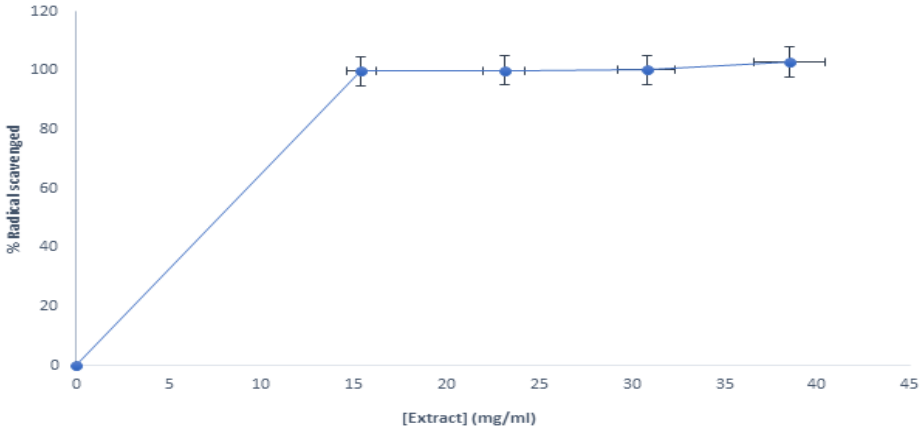

Figure 4a: Hydrogen peroxide scavenging capacity of *M. indica* + *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

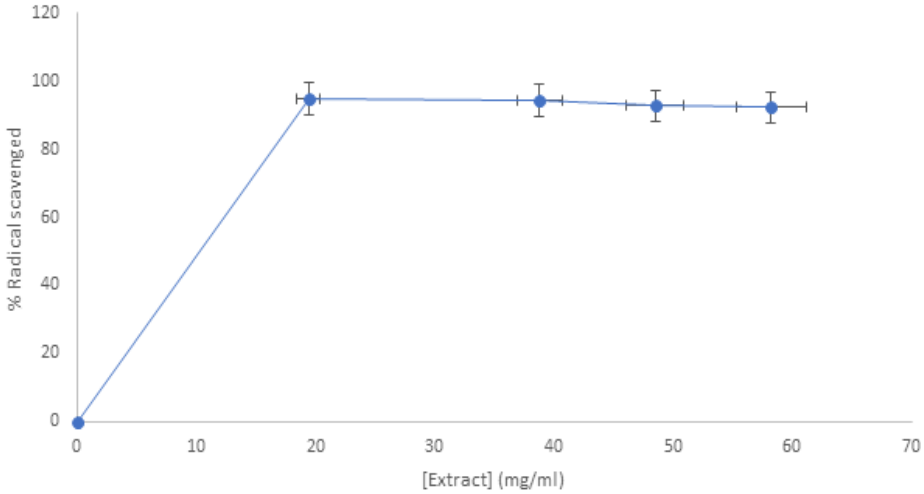

Figure 4b: Hydrogen peroxide scavenging capacity of *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

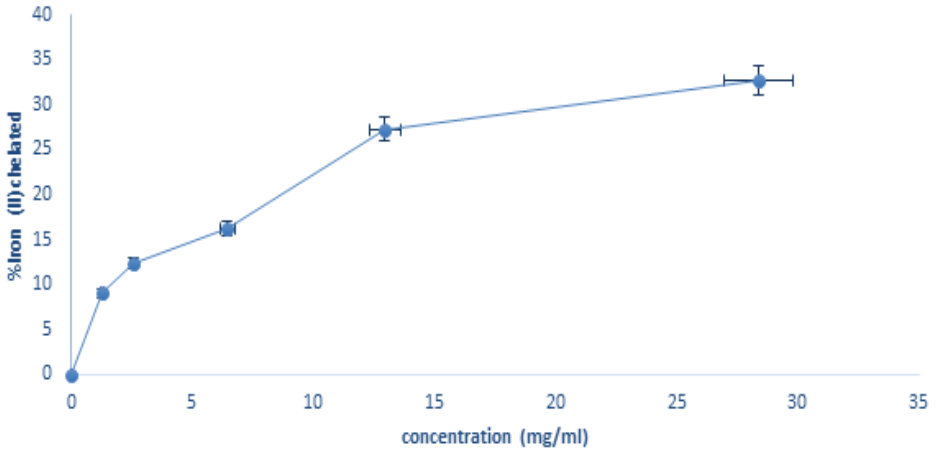

Figure 5a: Iron cheating activity of *M. indica* + *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

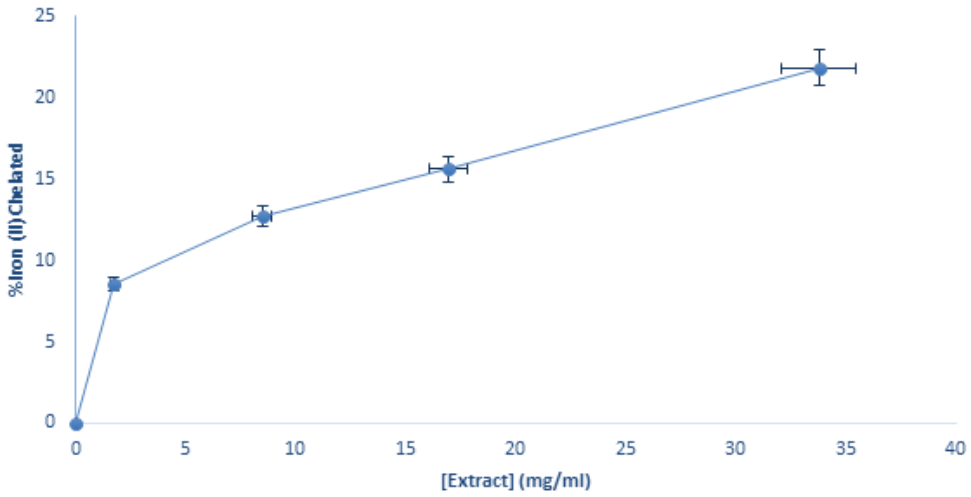

Figure 5b: Iron cheating activity of *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

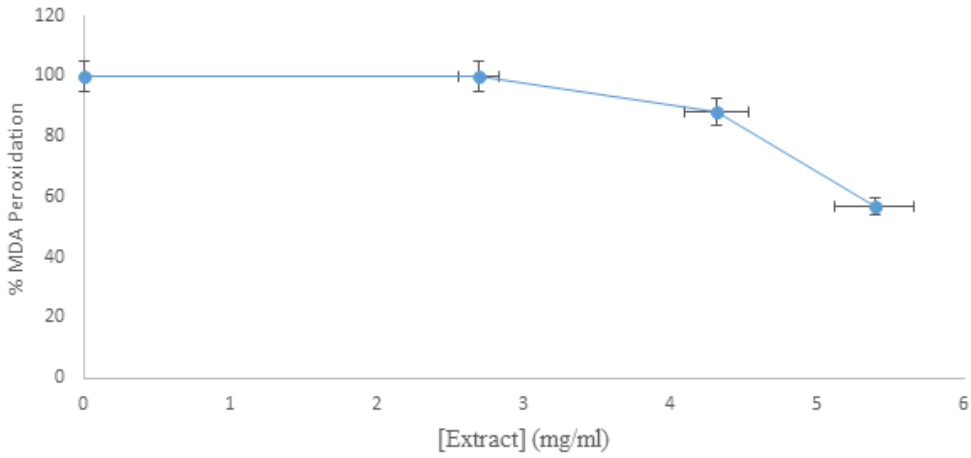

Figure 6a: Inhibitory effect of *M. indica* + *V. amygdalina* leaf extract on hepatic lipid peroxidation. Data are represented as mean ± S.D (n=3) at P < 0.05.

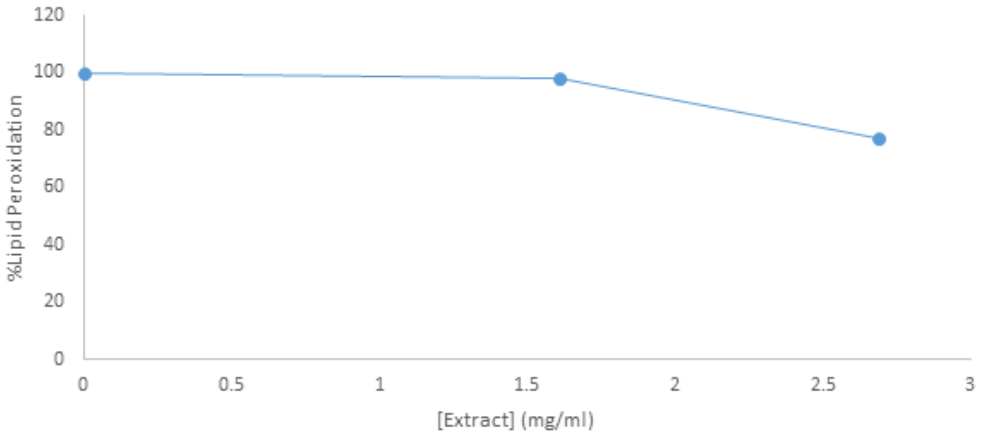

Figure 6b: Inhibitory effect of *M. indica* + *V. amygdalina* leaf extract on pancreatic lipid peroxidation. Data are represented as mean ± S.D (n=3) at P < 0.05.

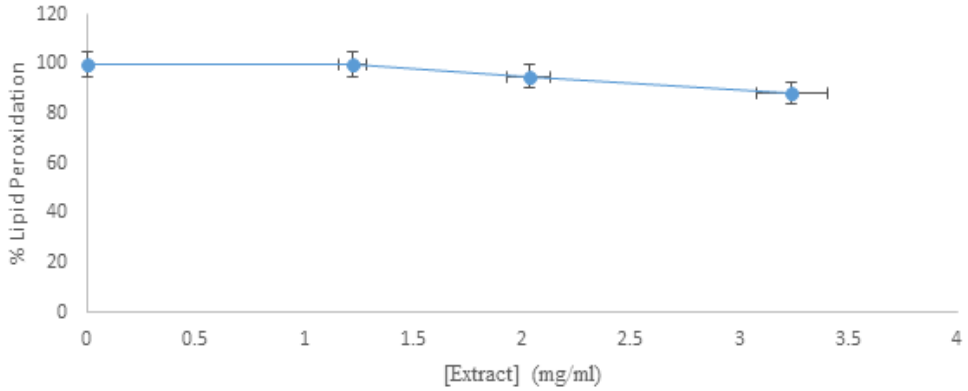

Figure 6c: Inhibitory effect *V. amygdalina* leaf extract on pancreatic lipid peroxidation. Data are represented as mean ± S.D (n=3) at P < 0.05.

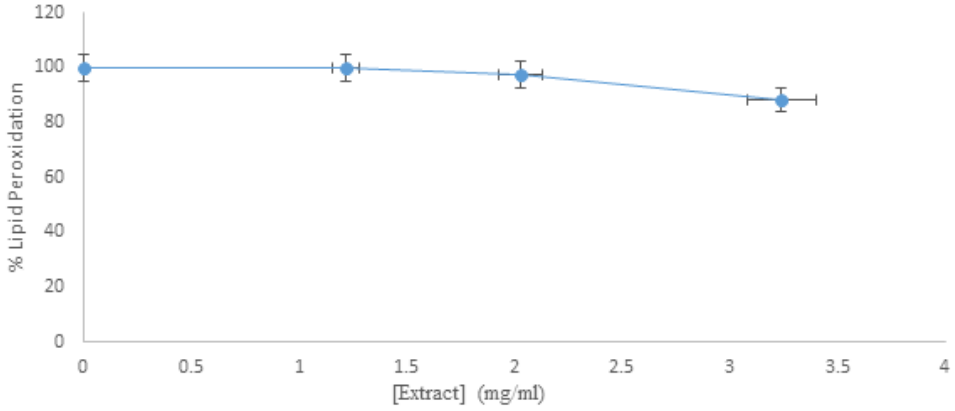

Figure 6d: Inhibitory effect *V. amygdalina* leaf extract on renal lipid peroxidation. Data are represented as mean ± S.D (n=3) at P < 0.05.

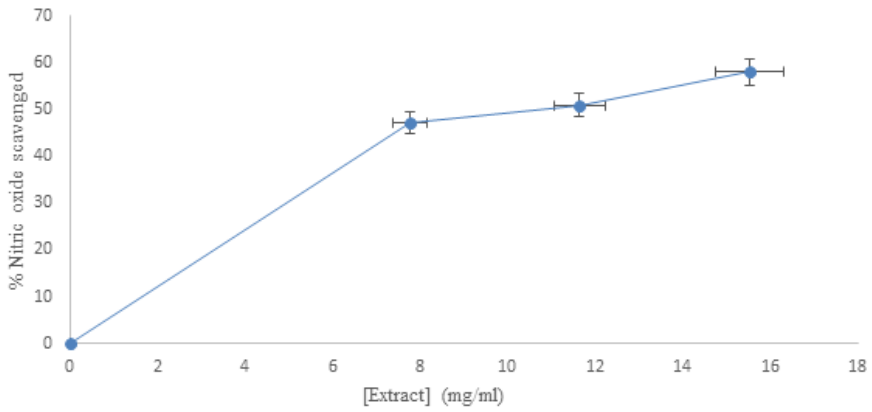

Figure 7a: Nitric oxide radical scavenging ability of *M. indica* + *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

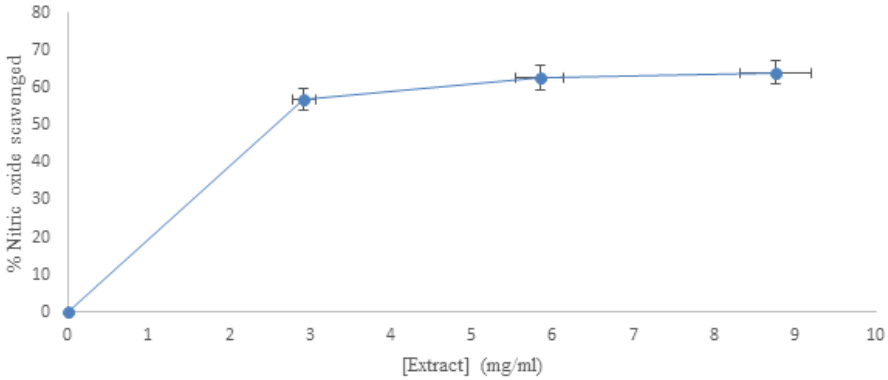

Figure 7b: Nitric oxide radical scavenging ability of *V. amygdalina* leaf. Data are represented as mean ± S.D (n=3) at P < 0.05.

Table 1: Inhibitory Potential of *V. amygdalina* leaf extract on α-amylase activity.

Sample	Volume (µl)	% Inhibition	IC_{50}
Acarbose	10.00	15.20	1.39
	50.00	29.45	
	100.00	38.71	
***V. amygdalina* leaf**	10.00	17.65	9.74
	50.00	46.08	
	100.00	60.24	

Data represent two independent experiments performed in triplicates.

Table 2: Inhibitory Potential of *V. amygdalina* leaf extract on α-glucosidase activity.

Sample	Concentration	% Inhibition	IC_{50}
Acarbose	10.00	16.75	3.08
	50.00	43.90	
	100.00	56.21	
***Vernonia amygdalina* leaf**	10.00	9.80	5.96
	50.00	21.75	
	100.00	36.15	

Data represent two independent experiments performed in triplicates.

Table 3: Glycemic Index of *V. amygdalina* and mixture of *V. amygdalina* and *M. indica*

Sample	Glycemic Index
V. amygdalina	23.74
V. amygdalina* + *M. indica	30.46

Table 4: Inhibitory Potential of a mixture of *V. amygdalina* and *M. indica* leaves extract on α-amylase activity.

Sample	Concentration	% Inhibition	IC_{50}
Acarbose	10.00	41.24	2.47
	50.00	53.20	
	100.00	76.59	
***M. indica* and *V. amygdalina* leaf**	10.00	23.06	8.51
	50.00	57.85	
	100.00	79.21	

Data represent two independent experiments performed in triplicates.

Table 5: Inhibitory Potential of a mixture of *V. amygdalina* and *M. indica* leaves extract on α-glucosidase activity

Sample	Concentration	% Inhibition	IC_{50}
Acarbose	10.00	25.86	2.71
	50.00	45.70	
	100.00	61.05	
***M. indica* and *V. amygdalina* leaf**	10.00	20.13	3.15
	50.00	49.37	
	100.00	63.52	

Data represent two independent experiments performed in triplicates.

Table 6: Bioactive compounds identified in *V. amygdalina* leaf extract.

Peak	RT (min)	Compound Detected	M. Formular	MW (amu)	Peak Area (%)	Comp. (%)	m/z
1	4.50	2,3-Butanediol	$C_4H_{10}O_2$	90	4.79	4.40	43, 45, 90
2	10.52	Benzaldehyde, 4-ethyl-	$C_9H_{10}O$	134	2.66	3.27	51, 105, 134
3	11.86	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	5.05	4.17	41, 68, 108
4	13.00	Phenol,2-methoxy-4-(methoxymethyl)	$C_9H_{12}O_3$	168	2.13	2.00	51, 137, 168
5	14.42	p-Menth-3-en-9-ol	$C_{10}H_{18}O$	154	6.38	5.25	41, 123, 154
6	16.00	Phenol, 2-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O_2$	164	3.19	3.00	55, 77, 164
7	18.75	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.89	15.03	43, 73, 256
8	19.25	Caryophyllene	$C_{15}H_{24}$	204	8.51	7.59	41, 93, 204
9	20.77	trans-10-octadecenoic acid	$C_{18}H_{34}O_2$	282	2.93	2.17	41, 181, 282
10	25.11	Oleic acid	$C_{18}H_{34}O_2$	282	24.47	26.70	41, 55, 282
11	25.75	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	5.85	4.51	43, 74, 270
12	27.52	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	3.72	4.16	69, 165, 208
13	30.60	Methyl stearate	$C_{19}H_{38}O_2$	294	5.32	6.17	41, 67, 294
14	31.50	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.86	2.05	41, 79, 296
15	34.00	Benzenesulfonamide 5-amino 2-methyl N-phenyl-	$C_{13}H_{14}N_2O_2S$	262	6.65	8.21	65, 105, 262
16	35.50	Hexadecyl pentyl ether	$C_{21}H_{44}O$	312	1.60	1.32	41, 81, 312

Table 7: Bioactive compounds identified in a mixture of *M. indica* and *V. amygdalina* leaf extracts

Peak	RT (min)	Compound Detected	M. Formular	MW (amu)	Peak Area (%)	Comp. (%)	m/z
1	4.09	2,3-Butanediol	$C_4H_{10}O_2$	90	0.31	1.21	43, 45, 90
2	7.64	Dihydroxyacetone	$C_3H_6O_3$	90	12.31	7.27	43, 72, 90
3	8.56	Phenol	C_6H_6O	94	2.46	3.12	40, 66, 94
4	9.50	3-Furaldehyde	$C_5H_4O_2$	96	5.23	5.21	40, 95, 96
5	10.00	1,2-Benzenediol	$C_6H_6O_2$	110	8.31	5.25	40, 64, 110
6	13.46	Hexanoic acid	$C_6H_{12}O_2$	116	0.92	1.24	41, 60, 119
7	15.62	Benzaldehyde, 4-ethyl-	$C_9H_{10}O$	134	2.46	3.18	51, 105, 134
8	16.09	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.69	2.00	41, 68, 144
9	17.00	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	$C_9H_{10}O_2$	150	7.69	5.09	43, 135, 150
10	17.63	Methyl 4-(hydroxymethyl) benzoate	$C_9H_{10}O_3$	151	3.38	4.17	77, 107, 151
11	20.50	p-Menth-3-en-9-ol	$C_{10}H_{18}O$	154	1.23	1.71	41, 123, 154
12	21.52	Eugenol	$C_{10}H_{12}O_2$	164	3.69	4.11	77. 103, 164
13	22.00	Caryophyllene	$C_{15}H_{24}$	204	4.92	5.06	41, 93, 204
14	23.50	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.46	15.28	43, 73, 256
15	25.00	Heptadecane	$C_{17}H_{36}$	240	0.77	1.56	43, 57, 240
16	27.84	9, Octadecenamide	$C_{18}H_{35}NO$	281	1.08	1.69	41, 59. 281
17	28.77	Oleic acid	$C_{18}H_{34}O_2$	282	10.15	11.29	41, 55, 282
18	29.50	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	2.77	2.66	43, 74, 270
19	31.24	9.12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	6.77	7.93	41, 81, 280
20	34.00	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.39	1.76	43, 73, 284
21	34.58	Hexadecyl pentyl ether	$C_{21}H_{44}O$	312	1.54	2.92	41, 81, 312
22	36.75	γ-Sitosterol	$C_{29}H_{50}O$		3.54	3.13	43, 55, 414
23	38.23	β-Sitosterol	$C_{29}H_{50}O$	414	2.92	3.15	43, 107, 414

4. CONCLUSION

A mixture of *M. indica* and *V. amygdalina* leaf extract is a stronger antioxidant and antihyperglycemic regimen than either of the leaves used alone. It is therefore concluded that a combination of the leaves of both plants should be employed in the traditional management of diabetes for greater efficacy.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the “Ethics Committee” was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

None.

Credit authorship contribution statement

Author 1: Conceptualization, Investigation, Data curation, editing of the original draft, Supervision.

Author 2: Resources, Formal analysis, Data curation, Investigation, Writing - original draft , Methodology.

Author 3: Data curation, Resources, Investigation, methodology

ORCID Numbers of the Authors

Author 1 (T. Ogunmoyole.): 0000-0002-6185-0602

Author 2 (R.K. Oladele): 0009-0005-4007-0673

Author 3 (O.D. Johnson): 0000-0002-3492-0986

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