An *In Vitro* Determinations of the Inhibition of Free Radicals Activity, Carbohydrate-Hydrolysing and Lipoxygenase Enzymes by the Leaf Extracts of *Moris alba L*

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Abstract: This study determined the effect of the some extracts of *moris alba L* leaf against diabetes and selected free radicals using different standard procedures. Free radical scavenging and antidiabetic activities of these extracts (aqueous and ethanol) were tested in six assays, which include metal chelating, 1, 1-diphenyl-2-picryl hydrazyl (DPPH), nitric oxide, 2, 2-azinobis (3-ethylbenzothiazoline-6) sulphonic acid (ABTS), hydroxyl radical and reducing power in five different concentrations (0.065-1.000 mg/mL), the antidiabetic activity was assessed via the inhibition and kinetics of carbohydrate-hydrolysing enzymes including alpha amylase and alpha glucosidase. The aqueous extract showed the best activities in most of the radical scavenging assays including DPPH, ABTS, nitric oxide, hydroxyl radicals and metal chelating (with IC_{50} values of 0.625, 3.923, 2.719, 0.968 and 2.182 mg/mL) respective values. Moreover, aqueous extract also possessed best reducing ability with 0.41 at the lowest concentration (0.065mg/mL) and 0.78 at the highest concentration (1.0 mg/mL). Similarly, the aqueous extract exhibited the strongest inhibition of alpha amylase activity (0.798 mg/mL) and the strongest alpha glucosidase inhibitory potentials as well (1.591 mg/mL). The anti-inflammatory results depicted the aqueous extract (40.66 mg/mL) revealing the best inhibition of LOX among the two extracts although the activity of quercetin on this enzyme was superior (39.01 mg/mL). In conclusion, it can be suggested that *Moris alba L* possesses antioxidative and antiinflammatory activities and scientifically indicative of the use of the plant as a potential antidiabetic agent.

Key words: *Moris alba L* · Antidiabetic · Antioxidant · Phytochemicals · α -Amylase · α-Glucosidase

INTRODUCTION

Oxidative stress can be defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of the later due to different factors such as aging, drug actions and toxicity, inflammation and/or addiction [1]. Oxygen is highly reactive specie that has the ability to become part of potentially harmful and damaging molecules (Free Radicals) Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT—enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidise (GSH–Px) antioxidants (1). The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications [2]. It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes [3]. There are almost 150 million people worldwide suffering from diabetes [4]. However, type 2 diabetes (T2DM) is the most encountered form of diabetes, which accounts for more than 80% of total cases [5].

So, the use of antioxidant substances that scavenge and eradicate ROS may prevent or minimize these oxidation-related diseases. Selkoe [6] suggested the use of antioxidants and free radical scavengers as possible treatment options for certain features of Alzheimer's disease. Moreover, targeting inhibitors of LOX and acetyl

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choline esterase (AChE) may be an indication of potential therapeutic use in treatment of cognitive dysfunction. The efficacy of natural antioxidants and anti-inflammatory drugs in treating inflammatory is known [7].

The medicinal plants may provide a useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapy [8]. More so, about 400 plants species as well as 700 recipes and compounds have been scientifically evaluated for treatment of type 2 diabetes [8]. Of these medicinal plants widely used in the field of herbal medicine, *Morus alba* have been reported to possess several medicinal properties including hypoglycemic effect [9, 10]. *Morus alba*, known as mulberry, has long been used as an antioxidant, antidiabetic and anti-inflammatory remedy in traditional medicine [11]. A toxicity study of *Morus alba* leaves revealed no remarkable acute or subacute toxicities [12]. Mulberry water extracts contain polyphenols, including gallic acid, chlorogenic acid, rutin and anthocyanins [13].

Thus, the current researches evaluated the antioxidant, antidiabetic and anti-inflammatory activity and of mulberry leaf in an *in vitro* studies.

MATERIALS AND METHODS

Plant collection, identification and authentication: The *Moris alba L* was harvested and collected freshly from the native farms in Akure and authenticated by Rufus Jose in the Environmental Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

Chemicals and Reagents: Porcine pancreatic α-amylase, rat intestinal α-glucosidase, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, acarbose and para-nitrophenyl-glucopyranoside (pNPG) were obtained from Sigma–Aldrich Co. St Louis, USA. Starch soluble (Extra pure) was a product of J.T. Baker Inc., Phillipsburg, USA, while other chemicals and reagents used were of analytical grade. The water used was glass-distilled.

Extracts Preparation: A slight modification of Onoagbe and Esekheigbe (39) method was used to prepare the extract. The freshly collected stems were rinsed with distilled water to remove foliar contaminants after which the leaves were separated from the twigs and stems. The leaf component were then air-dried at room temperature and subsequently ground into fine powder materials separately. Thereafter, exactly 1000 g each of the powdered samples were extracted in ethanol (AR) (2500ml) and distilled water (2000ml). The flasks were placed on Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h at 110 rpm to allow for proper agitation. All extracts were filtered using Whatman No. 1 filter paper. The ethanol extract was concentrated under reduced pressure at 40 °C using rotary evaporator (Cole-Parmer, SB 1100, Shanghai, China), while hydroethanol water extracts were freeze-dried using lyophilizer (Virtis Bench Top, SP Scientific Series, USA). All extracts were kept air-tight and refrigerated (4 °C) prior to further experimental analysis.

Isolation of the Phytochemicals

Isolation of Flavonoids: Solvent extraction of dried powder (1000g) of *Moris alba L* was carried out [14] using 5L of 80% methanol in a glass container for a successive extraction for 72 hours. The methanol extract obtained was concentrated by evaporation (40-50°C) in a rotary vacuum evaporator. Using a separating funnel, the methanol extract was dissolved in distilled water and soluble fractions were filtered, concentrated in vacuo and the aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr-1), ethyl ether (Fr-II) and ethyl acetate (Fr-III) separately. Each step was repeated thrice for complete extraction, fraction I was discarded because it contained fatty substances, whereas fraction II and III were concentrated and used for determining free and bound flavonoids respectively. Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid (10 mL/g plant material for 2 hr), filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled together separately, neutralized by distilled water with repeated washings and concentrated to obtain flavonoids (565.90 mg). Both fraction II and fraction III were taken up in small volume of ethanol (2-5 mL) before chromatographic examination.

Isolation of Saponins: This was carried out according to the method of Woo *et al.* [15]. About one thousand grams (1000 g) of the powdered leaves of *Moris alba L* was dissolved in a glass container containing 5 L petroleum ether for a successive extraction for 72 hours to defat it. The pet ether extracts was discarded while the marc was further subjected to ethanol extraction (5 L). The marc from the ethanol extraction was discarded while the ethanol extract was diluted with distilled water (3 L) and partitioned with Diethyl ether (1:5). The ether extract was removed and the aqueous extract collected and further partitioned with n-Butanol (1.5 L). The aqueous extract

was removed and n-Butanol fraction partitioned with 1% KOH (500 mL). The n-Butanol collected contained saponins (443 mg) which was dried at room temperature and kept in the refrigerator.

Extraction of Tannins: The method described by Wall *et al*. [16] was followed. Precisely, 1000 g of the powdered plant was dissolved in 5000 mL of methanol (AR) after 72 hours of successive extraction; it was filtered and concentrated with freeze dryer. The methanol extract (110.30 g) was suspended in 90% methanol (1 L) and then partitioned with hexane (15 L) to defat it. The hexane extract was discarded and 90% methanol partition was concentrated and suspended in distilled water and further partitioned with dichloromethane (2 L). The aqueous extract was collected while the crude dichloromethane partition   was washed with 1% NaCl to remove tannins and then evaporated to dryness (170.20 mg). The dichloromethane partition was essentially free of tannins while the 1% NaCl solution became a waste.

***In vitro* antioxidant assays**
1-diphenyl-2-picrylhydrazyl(DPPH)RadicalScavenging Assay: Antioxidant activity of the extracts (crude and isolated phytochemicals) of *Moris alba L* was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH, according to the method described by Turkoglu *et al*. [17]. Briefly, 1 mL of a 0.2 mM DPPH methanol solution was added to 1 mL of various concentrations (0.065-1.000 mg/mL) of the extracts and incubated at 25 °C for 30 min. The absorbance of the resulting mixture was measured against blank at 516 nm using a microplate reader (BIO RAD, Model 680, Japan). The percentage inhibition rate (I%) on the DPPH radical was calculated following the expression below (equation 1) and IC_{50} value was extrapolated from the calibration curve.

$$Percentage\ inhibition\ (I\%) = Abs_{blank} - Abs_{extract} / Abs_{blank}$$
$$x\ 100$$

Equation 1

where Abs is the absorbance

Nitric Oxide Scavenging Ability: The potential of *Moris alba L* extracts to scavenge nitric oxide radical was determined according to the procedure reported by Garrat [18]. Exactly 2 mL of 10 M sodium nitroprusside was prepared in 0.5 mL phosphate buffer saline (pH 7.4) and mixed with 0.5 mL of different concentrations of the extracts (0.065-1.000 mg/mL) in a 96-wells plate. The resulting mixture was incubated at 25 °C for 2 h, after which 0.5 mL was taken from the incubated mixtures and added to 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid). The mixture was also incubated at 25 °C for 5 min. Thereafter, 1 mL naphthyl ethylenediamine dihydrochloride (0.1% w/v) was added to the mixtures and the resulting solution was later incubated at 25 °C for 30 min. The absorbance was read at 540 nm and the IC_{50} was then estimated from calibration curve following the determination of percentage nitric oxide radical inhibition (Equation 1) by *Moris alba L* extracts.

Metal Chelating Ability of the Extracts: Metal chelation ability of *Moris alba L* extracts was assayed following the procedure of Dinis *et al*. [19]. Notably, 0.1 mL of the extract (0.065-1.000 mg/mL) was added to 0.5 mL of 0.2 mM ferrous chloride solution. The reaction was initiated by adding 0.2 mL of Ferrozine (5 mM) and incubating at 25 °C for 10 min. The absorbance was read at 562 nm in a microplate reader (BIO RAD, Model 680, Japan). Vitamin C was used as control and the chelating potential of the extracts that competed with Ferrozine for the ferrous ions was revealed from the colour reduction. IC_{50} value was extrapolated from the calibration curve.

Reducing Power Property: The reducing property of the extracts was assessed according to the method of Oyaizu [20]. 2.5 mL different concentrations (0.065-1.000 mg/mL) of *Moris alba L* extracts were added to 1 mL of distilled water and then mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated at 50 °C for 20 min before the addition of 2.5 mL trichloroacetic acid (10%). The resulting mixture was centrifuged at 3 000 rpm for 10 min. After this, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% $FeCl_3$. The colour change of the resulting solution was then taken at 700 nm. The IC_{50} value was extrapolated from the calibration curve

Azinobis   (3-ethylbenzothiazoline-6-)Sulfonic   Acid (ABTS) Radical Scavenging Ability: This test was carried out according to the procedure of Re *et al*. [21]. The $ABTS^+$ was generated by reacting 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 h and adjusting the pH to 7 with ethanol. Exactly 0.2 mL of the various dilutions of extracts (0.065-1.000 mg/mL) was added to 2.0 mL $ABTS^+$ solution and the absorbance was measured at 734 nm after 15 min. The ascorbic acid equivalent antioxidant capacity was later determined.

Hydroxyl Radical Scavenging Potential: The ability of the various extracts of *Moris alba L* to stop Fe^{2+}/ H_2O_2 induced decomposition of deoxyribose was assayed using the modified method of Oboh *et al.* [22]. In brief, 40 µL of the freshly prepared extracts (0.065-1.000 mg/mL) was added to a reaction mixture containing 20 µL 20 mM deoxyribose, 80 µL of 0.1 M phosphate buffer, 10 µL of 500 mM $FeSO_4$ and the volume was made up to 200 µL with distilled water. The reaction mixture was initiated at 37 °C for 30 min and stopped by adding 50 µL of 2.8% TCA (Trichloroacetic acid). Later, 50 µL of 0.6% thiobarbituric acid solution was added. The reaction mixture was then incubated in boiling water for 20 min and absorbance was read at 532 nm in a microplate reader (BIO RAD, Model 680, Japan). This procedure was repeated for a standard antioxidant by replacing the extracts with ascorbic acid (0.065-1.000 mg/mL) and IC_{50} value was extrapolated from the calibration curve.

In Vitro Antidiabetic Assays

α- Amylase Inhibitory Assay: The α-amylase inhibitory activity and kinetics of inhibition were determined using the methods described by Elsnoussi *et al.* [23] and Kazeem *et al.* [24] respectively. Briefly, 500 µL of each of the varying extracts' dilutions (0.065-1.000 mg/mL) was mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 0.5 mg/mL of α-amylase solution. The resulting mixture was pre-incubated in test tubes at 25 °C for 10 min. Thereafter, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each test tube at timed intervals. The reaction mixtures were incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid colour reagent. The tubes were incubated in a boiling water bath for 5 min and left to cool at 25 °C. Then 15 mL of distilled water was used to dilute the reaction mixtures and the absorbance was measured at 504 nm using a spectrophotometer (Biochrom WPA Biowave II, Cambridge, England).

Same procedure was repeated for acarbose which serves as the positive control by preparing it in distilled water at same concentrations (0.065-1.000 mg/mL) as extracts. The values were compared with those of acarbose used as control. The result of the triplicate determinations of α-amylase inhibitory activity was expressed as % inhibition (Equation 1). The concentration of the extracts causing 50% inhibition (IC_{50}) of α- amylase activity was calculated from its standard calibration curve.

α- Glucosidase Inhibitory Assay: The alpha-glucosidase inhibitory activity was according to the method of Elsnoussi *et al.* [23]. Briefly, 50 µL of varying concentrations (0.065-1.000 mg/mL) of *Moris alba L* extracts were mixed with 100 µL of 0.1 M phosphate buffer (pH 6.9) containing 1.0 M of the α- glucosidase solution. The mixtures were incubated in 96-well plate at 25 °C for 10 min. Thereafter, 50 µL of 5 mM p-NPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min and stopped with 50 µL of 0.1 M Na_2CO_3. The absorbance was read at 405 nm using a microplate reader (BIO RAD, Model 680, Japan) and the values were compared with acarbose were used as control.

LOX Inhibitory Assay: A modified protocol of the LOX/Ferrous oxidation (FOX) [25] was used to investigate in 15-LOX inhibitory activity of the extracts. The ferrous ion (Fe^{2+}) oxidation xylenol orange (FOX) methods is based upon oxidation of Fe^{2+} (Reagent) to ferric ions (Fe^{3+}) by oxidizing agents (Extract or control) which then binds with xylenol orange (Reagent) to produce a complex colour which absorbs maximally at 560 nm. The intensity of the resultant colour is proportional to the amounts of the oxidizing agents present by using linoleic acid as the substrate for LOX.

In brief, 20 µL of plant extracts or control was added to 50 µL of 15-LOX (100 µg/mL, Sigma-Aldrich) in a well of a 96-well microtitre plate and incubated for 5 min at 25°C. After incubation, 50 µL of substrate (10 µL linoleic acid, with 30 µL absolute ethanol added and made up to 120 mL with borate buffer) was added to the mixture and the microtitre plate was further incubated for 20 min at 25°C. After the second incubation period, 100 µL of FOX reagent (Made of 90 mL absolute methanol and 10 mL distilled water, add 166.66 µL concentrated sulphuric acid, 7.6 mg xylenol and 2.8 mg ferrous sulphate) was added to each well and the microtitre plate was incubated again at 25°C for 25 min in the dark. After incubation, the microtitre plate was placed in a microtitre plate reader and the absorbance was read at 560 nm. Quercetin (1 mg/mL) and indomethacin (1 mg/mL) were used as positive controls, representing known LOX and cyclo-oxygenase (COX) inhibitors respectively. The experiments were done in triplicate at three different times. The enzyme activity (EA) of the negative control was taken as 100%, hence the EA of extracts/positive controls were calculated thus;

$$EA = EA = abs \; X \; 100\% \qquad \text{Equation 2}$$

where abs= absorbance and IC50 was calculated as the concentrations of the extracts that resulted in 50% inhibition of 15-LOX.

Calculation of 50% Inhibitory Concentration (IC$_{50}$): The concentration of the plant extracts required to scavenge 50% of the radicals (IC$_{50}$) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by:

I % = (Ac-As)/Ac X 100%   Equation 3 [26]

where Ac is the absorbance of the control and As is the absorbance of the sample. The concentration of *Moris alba L* extracts causing 50% inhibition (IC$_{50}$) of DPPH radical was calculated from the standard calibration curve.

Statistical Analysis: Statistical analysis was carried out using GraphPad Prism 5 statistical package (GraphPad Software, USA). One-way analysis of variance (ANOVA) was used to analyse the data followed by Bonferroni test. Results were expressed as mean±standard error of mean (SEM) of triplicate determinations. Statistical significance of the mean values was considered at P < 0.05. The data was analyzed statistically using Tukey-Kramer test.

RESULTS

Percentage Yield of the Extracts: The aqueous extract (310.81 g) of *Moris alba L* revealed the highest amount of the yield following extraction with same quantity of the starting materials followed by ethanol (297.02g).

DISCUSSION

In vitro DPPH radical-scavenging and superoxide anion activities of the extracts (Aqueous and ethanol) compounds are summarized in Table 1. In the DPPH radical-scavenging assay, aqueous extract show significant radical-scavenging activity with lower IC$_{50}$ values (0.625) than ethanol extract (0.914) but a little higher than ascorbic acid (0.602). ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT—enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidise (GSH–Px) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications [2]. According to epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycemia [3]. Using the *in vitro* studies, the compound sodium nitroprusside decomposes in aqueous solution at physiological pH (7.2) producing NO., making it an ideal assay to mimic the body system in scavenging the free radical [27]. This agrees with Moyo *et al.* [28] who states that the active components in the water extract are capable of donating hydrogen to a free radical to remove electron which is responsible for radical's reactivity.

As noted by Kazeem and Ashafa [24], hydroxyl radicals are highly reactive in causing enormous biological damage to any living cell, but this untoward effect may be mitigated by the presence of ethanol extract of *P. Prunelloides*, which is also supported by the results of this study, as it's seen in the IC$_{50}$ value. As shown in table 1, aqueous extract has a significantly lower IC$_{50}$value in the nitric oxide (3.923), ABTS (2.719), hydroxyl radical (0.968) and metal chelating (2.182) respectively when compare with the ethanol extract. It might be inferred that the synergistic relationship among most of the phytochemicals might be responsible for the overall medicinal effects of *Moris alba L*, because the antioxidant and antidiabetic efficacies of medicinal plants has been linked to their high phenolic contents [29].

Aqueous and ethanol extracts of *M. alba L* were investigated for inhibitory activity against α- amylase and α-glucosidase. The inhibitory were summarized in Table 4. For α- amylase, the aqueous extract shows a significantly low IC$_{50}$ (0.798) when compared to the ethanol (1.938) extract and acarbose (0.912). Similarly, in α– glucosidase anti-diabetic assay, the aqueous extract (1.591) shows a significantly lower value when compared

Table 1: IC$_{50}$ (mg/mL) values for the antioxidant properties of *Moris alba L* leaf extracts.

Extracts	DPPH	Nitric oxide	ABTS	Hydroxyl	Metal chelating
	Assays IC$_{50}$ (mg/mL)				
Aqueous	0.625±0.04 [a]	3.923±0.05 [a]	2.719±0.04 [a]	0.968±0.03[a]	2.182±0.14[b]
Ethanolic	0.914±0.08 [b]	4.014±0.10[a]	2.798±0.05 [a]	2.411±0.07[b]	4.591±0.28[a]
Ascorbic acid	0.602±0.03[a]	3.256±0.06 [a]	2.150±0.05 [a]	0.861±0.01[a]	3.972±0.32[b]

The values are presented as mean±standard error of mean (SEM) of triplicate determinations. Means along the same row not sharing a common superscript for each parameter are significantly different (p< 0.05).

where, DPPH: 1, 1-diphenyl-2-picryl hydrazyl; ABTS: 2, 2-azino-bis (3-ethylbenzothiazoline-6)-sulphonic acid

Table 2: Reducing potential of *Moris alba L* leaf extracts.

Conc. (mg/mL)	Absorbance (700 nm)		
	Aqueous	Ethanol	Ascorbic acid
0.065	0.41±0.02[a]	0.61±0.02[a]	0.44±0.02[a]
0.125	0.56±0.04[a]	0.64±0.02[a]	0.50±0.02 [a]
0.25	0.59±0.01[a]	0.81±0.02[a]	0.58±0.31[a]
0.5	0.64±0.01[a]	0.89±0.01[a]	0.69±0.01[a]
1.0	0.78±0.02[a]	1.25±0.03[a]	0.82±0.03[b]

The values are presented as mean±SEM of triplicate determinations. Means along the same row not sharing a common superscript for each parameter are significantly different (p< 0.05).

Table 3: IC_{50} (mg/mL) values for the inhibitory activities of *Moris alba L* leaf extracts on the specific activities of α-amylase and α-glucosidase

Samples	Assays IC_{50} (mg/mL)	
	α– amylase	α– glucosidase
Aqueous	0.798±0.06[a]	1.591±0.02[a]
Ethanol	1.938±0.10[b]	2.009±0.10[b]
Acarbose	0.912±0.09[a]	1.840±0.02[a]

The values are expressed as mean±SEM of triplicate determinations. Means along vertical columns not sharing a common superscript are significantly different (p< 0.05) from each other.

Table 4: IC_{50} (mg/mL) values for the inhibitory anti-inflammatory activities of *Moris alba L* leaf extracts on the specific activity of 15-Lipoxygenase enzyme

Samples	IC_{50} (mg/mL)
	15-Lipoxygenase
Aqueous	40.66±9.15[a]
Ethanol	102.91±23.11[b]
Quercetin	39.01±11.30[a]
Indomethacin	129.41±26.11[c]

The values are expressed as mean±SEM of triplicate determinations. Means along vertical columns not sharing a common superscript are significantly different (P < 0.05) from each other.

to the ethanol extract (2.009) and acarbose (1.840). This result compliment the report of Nair *et al*. [30]. Who showed that the inhibition of α-glucosidase and α-amylase enzymes by the extracts of *M. alba L* in the present study provides a strong biochemical basis for the management of diabetes via the control of glucose absorption. α-amylase inhibitors bind to α-bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide [30]. These findings corroborate previous studies that reported extracts with strong inhibitory potential against α- glucosidase as an ideal antidiabetic agent both *in vitro* and *in vivo* [31, 32]. This agrees with Alimi and Ashafa

[33], ethanol extract (IC_{50} 5.52 mg/mL) was chosen as a mild inhibitor of α-amylase activity, as it exhibited a remarkably higher IC_{50} than that of acarbose. Conversely, decoction extract demonstrated the strongest inhibition against the specific activity of α-glucosidase (IC_{50}: 0.05 mg/mL) when compared with other extracts and acarbose.

Aqueous and ethanol extracts of *M. alba L* were investigated for inhibitory activity against α- amylase and α-glucosidase. The inhibitory were summarized in Table 4. This activity of *M. alba L* as reported in table 4 showed that aqueous extract of *M. alba L* showed a significant low IC_{50} value of the 15-Lipoxygenase. This is supported by the work of Viji and Helen [34] who stated that *Bacopa monnieri* also significantly inhibited 5-lipoxygenase (5-LOX), 15 (LOX) and cyclooxygenase- 2 (COX-2) activities. *Bacopa monnieri* possesses significant anti-inflammatory activity that may well be relevant to its effectiveness in the leading of various inflammatory conditions in traditional medicine [35]. The anti-inflammatory activity of *Bacopa monnieri* is due to the triterpenoid and bacoside present in the plant. The ability of the fractions containing triterpenoids and bacosides inhibited the production of proinflammatory cytokines such as tumour necrosis factor −alpha and interleukin-6 [34]. This was tested using lipopolysaccharide activated peripheral blood mononuclear cells and peritoneal exudates cells *in vitro*. So, *Bacopa monnieri* has the ability to inhibit inflammation through modulation of proinflammatory mediator release [34].

The development and use of acetyl salicylic (Aspirin) and other non-steroidal anti-inflammatory drugs (NSAIDs) have in the past been embraced in the management of pain and inflammatory ailments. However, in recent times due to side effects (Gastrointestinal bleeding etc.) originating from these NSAIDs, interest in medicinal plants effective against pain and inflammation [1, 26] has continued to gain wider publicity.

From the study done so far, it has been elucidated that flavonoids are major anti-inflammatory agents. Some of them act as phospholipase inhibitors and some have been reported as TNF-α inhibitors in different inflammatory conditions. Biochemical investigations have also shown that flavonoids can inhibit both cyclooxygenase and lioxygenase pathways of arachidonic metabolism depending upon their chemical structures [36, 37]. Leukotrienes are the potent mediators of inflammatory and allergic reactions and regarding their pro-inflammatory properties the inhibition of 15-lipoxygenase pathway is considered to be interesting in the treatment of a variety of inflammatory diseases [38].

Anti-inflammatory and free radical scavenging activities of the methanolic extract of *Ricinus communis* root was studied by llavarasan *et al.* [39] in Wistar albino rats. The methanolic extract exhibited significant anti-inflammatory activity in carrageenan-induced hind paw edema model. The observed pharmacological activity may be due to the presence of phytochemicals like flavonoids, alkaloids and tannins in the plant extract [39].

CONCLUSION

In this study, results obtained so far indicate that aqueous and ethanol extracts *M. alba L* investigated have a potential to be used as an antioxidant and anti-diabetic and anti-inflammatory agent. However, weak activity obtained from the other extract is an indication that active compound(s) may be present in low amounts or there is an alternative pathway to which the biological activity is prolific and consistent with those found in the literature, since plant phenolics are known for their potent antioxidant activities.

ACKNOWLEDGEMENT

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