*Journal of Pharmaceutical Research International*

***22(6): 1-12, 2018; Article no.JPRI.42218***
***ISSN: 2456-9119***
*(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)*

# An *In-vitro* Antioxidant and Antidiabetic Evaluation of Traditional Medicinal Plants of Botswana

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MNK performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Author GS designed the study, managed the analyses of the study and supervised the experiments. Author CK wrote the protocols, was conducting the lab work and assisting in preparing the results. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JPRI/2018/42218
Editor(s):
(1) Dr. Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
(2) Dr. Ali Nokhodchi, Professor of Pharmaceutics and Drug Delivery, School of Life Sciences, University of Sussex, UK.
Reviewers:
(1) M. V. N. L. Chaitanya, Guru Nanak Institutions Technical Campus, India.
(2) Muruganandan Shanmugam, Wayne State University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/25270

***Original Research Article***

***Received 12th April 2018***
***Accepted 18th June 2018***
***Published 26th June 2018***

## ABSTRACT

The present study was aimed at the *in-vitro* analysis of the antioxidant and antidiabetic potential of the methanol extracts of four perennial plants which are indigenous to Botswana and further to investigate whether their antihyperglycemic effects are working through the antioxidant system. The plants used in this study were the aerial parts of *Ocimum gratissimum,* corms of *Hypoxis hemerocallidea*, aerial parts of *Momordica balsamina* and the leaves and stems of *Lippiascaberrima. T*he extracts were prepared in 70% methanol and they were labeled as MEOG, MEHHC, and MEMB, MELS (l) and MELS (s) respectively. All the four plant extracts showed a notable antioxidant and antidiabetic activity, the most potent one being *H. hemerocallidea* and the least potent being *L. scaberrima* stems. The total antioxidant status was evaluated by DPPH, ABTS and TBA assays. Total phenolic content was determined for each extract and converted to mg of

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gallic acid equivalents/g of dry extract (mg GAE/g).The crude methanol extracts showed many components with high radical scavenging activity in the TLC-DPPH bioautogram. The antidiabetic potential was determined by evaluating the inhibition of the extracts on α-amylase activity, and the results indicated that MEHHC showed the highest effect followed by MEMB, MEOG MELS (l) and MELS(s). The results obtained in the present investigations indicated that the extracts used in this study have bioactive compounds with antioxidant and antidiabetic properties.

*Keywords: Antidiabetic potential; antioxidant activity; α-amylase; Reactive Oxygen Species (ROS).*

## 1. INTRODUCTION

Diabetes is a metabolic disorder involving abnormal metabolism of carbohydrates, fats and proteins, ultimately leading to elevated levels of plasma glucose. It is one of the most considerable endocrine disorders and is currently one of the major contributors to human mortality. In diabetes, hyperglycemia generates reactive oxygen species (ROS) which in turn cause lipid peroxidation and membrane damage and thus, plays a vital role in the production of secondary complications in diabetes mellitus such as kidney, eye, blood vessel, and nerve damage. Researchers are interested in search of new drugs from medicinal plants for their biological activities like an antioxidant and antidiabetic property. Plants containing natural antioxidants (tannins, flavonoids, vitamins C and E) can preserve β-cell function and prevent diabetes induced by ROS. The current drugs which are used in controlling diabetes are not only expensive but also have their limitations and are known to produce serious side effects. Therefore, the search for safer, specific and effective hypoglycemic agents has continued to be an essential area of investigation with natural extracts from readily available traditional medicinal plants offering potentials for a discovery of new and safer antidiabetic drugs. The World Health Organization (WHO) has listed 21 000 plants, which are used for medicinal purposes around the world [1]. In Southern Africa, approximately 13.8% of the flora is used in traditional medicine [2].

Four perennial plants indigenous to Botswana which are used in traditional medicine to treat various ailments as described below were used in this study. The plants can be harvested during the rainy season from October to April. *Ocimum gratissimum* is a shrub belonging to Lamiaceae family. It is commonly known as the African basil or wild basil. It can reach heights of 1-2 meters, with a ribbed stem and simple leaves which are oppositely arranged. It may have antimicrobial properties because it is traditionally used to treat conditions like diarrhoea, respiratory infections, eye, and skin disorders [3]. This plant is also commonly used in Ayurvedic medicine [4].

*Hypoxis hemerocallidea* belongs to the star lily family (Hypoxidaceae). It is commonly known as the African potato. It is a tuberous plant with linear or broad lance-shaped leaves which are hairy. The sheets can measure about 400 mm. The flowers usually close at midday and are star-shaped with a bright yellow colour. This plant has recently attracted a lot of research interest because of its possible immune stimulant properties in its tuberous part. The healing properties may be a result of phytochemicals which are antioxidants and phytosterols [5] which may reduce the low-density lipoproteins (LDLs) in the blood. Traditionally it has been used to treat many conditions like hypertension, diabetes mellitus and arthritis.

*Momordica balsamina,* also known as the African pumpkin, is a short-lived wild climber which belongs to Cucurbitaceae family. It has bright orange fruits with small spikes on the surface. *M. balsamina* has a high protein content as well as a variety of essential micronutrients like potassium, phosphorus, calcium, magnesium, iron and zinc [6]. Its leaves, fruits, seeds and bark offer a wide range of medicinal use including immune stimulant properties. The healing properties of the plant are due to chemicals such as resins, alkaloids, flavonoids, glycosides, steroids and terpenes [6]. The leaves and young fruits can be eaten after cooking, but the ripe fruit can cause diarrhoea and vomiting. Traditionally it is used as an analgesic and to treat conditions like excessive uterine bleeding, cardiovascular disorders, gastrointestinal problems, diabetes mellitus, syphilis and rheumatism.

*Lippia scaberrima* is one of the five indigenous *Lippia* species found in Southern Africa [7]. It is a perennial aromatic shrub which belongs to the family Verbenaceae. It has woody stems and can grow up to a height of about 50- 60cm. It has a high content of essential oils (phenols) which

give fragrance to the plants. The main components of the oils are carvone, 1,8- cineole and limonene [7]. The leaves have the highest phenolic content, and the twigs have a lower phenol content. These phenols may be potentially powerful antioxidants [8]. There is no oil from the roots and stems of fresh or air-dried plant material [9]. *L. Scaberrima* is a favorite local traditional tea which can be drunk alone or mixed with other commercial beverages to add flavour and aroma. The stems and leaves together with the flowers are used to make tea. In traditional medicine, it is used to treat gastrointestinal and respiratory problems [10].

The present study was aimed at the *in-vitro* analysis of the methanolic extracts of the above plants to estimate their antihyperglycemic effects and to check whether they are working through the antioxidant system. This kind of analysis in these four plants has not been evaluated before to the best of the authors' knowledge.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plants

All the four seasonal plants were collected locally from Botswana. For *O. gratissimum* and *M. balsamina* the aerial parts were used, for *H. hemerocallidea* the underground corms were used, and for *L. scaberrima*the leaves and the stems were used. The identification of the plants were done by Dr. M. Muzila at the University of Botswana Herbarium. The voucher specimens were submitted in the herbarium and voucher numbers were (2006/G, A01), (G2016/, A02), (G2016/, A01) and (KNMW1/ 2016) respectively.

### 2.2 Preparation of the Extract: Methanol Extract

For *H. hemerocallidea* (African potato) the corms were cut into small pieces, dried in the shade, coarsely powdered and soaked in 70% methanol for three days at room temperature. For *O. gratissimum* and *M. balsamina* the aerial parts of the plant were cut into small pieces and dried in the shade, then coarsely powdered and soaked in 70% +30% mixture of methanol and distilled water for three days at room temperature. For *L. scaberrima* the complete stems and leaves were dried at room temperature, then separated into stems and leaves and ground into a powder separately. The stems and leaves were soaked in 70% +30% mixture of methanol and distilled water for three days at room temperature. All the extracts were filtered and made solvent-free by using a Buchi type rotary evaporator ($65^{oC}$) and dried completely in the vacuum. The yields were 7.8%, 7.3%, 7.6%, 7.9% and 6.9% (W/W) for *H. hemerocallidea, O. gratissimum, M. balsamina, L. scaberrima* leaves and stems respectively. The extracts obtained were used to carry out the experiments as MEOG (Methanol extract of *O. gratissimum),* MEMB (Methanol extract of *M. balsamina),* MEHHC (Methanol extract of *H. hemerocallidea* corm), MELS(l) (Methanol extract of *L. scaberrima* leaves) and MELS(s) (Methanol extract of *L. scaberrima* stems) respectively.

### 2.3 Chemicals

All the chemicals used were analytical grade and bought from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA. Reagents DPPH (2,2-diphenyl-l-picrylhydrazyl) reagent (molecular formula $C_{18}H_{12}N_5O_6$ molecular weight 394g/ mol) was purchased from Fluka Chemicals (Steinheim, Germany), Ascorbic acid and anhydrous sodium carbonate were all analytically pure and were purchased from Unilab (South Africa). Gallic acid (AR) was obtained from Sigma Chemicals (Steinheim, Germany). The solvents used for the extraction process were also of analytical grade.

### 2.4 Animals

Male albino rats of Wistar strain (*Rattusnorvegicus*) of body weight ranging 200-250 grams were housed in colony cages at ambient temperatures of 25°C ± 2°C and 50-55% relative humidity with 12 Hours light and dark cycle. They had water and food *ad libitum.* Experiment was conducted as per the internationally accepted principles from the laboratory animal care unit of University of Botswana.

### 2.5 Phytochemical Screening

The phytochemical tests were carried out in duplicates as previously detailed elsewhere [11] and briefly described here.

#### 2.5.1 Flavonoids

The extract (1 mL) was added to a concentrated sulphuric acid (0.2 mL) and 0.5 g of Mg. A pink or red coloration that disappear on standing (3 min.) indicated the presence of flavonoids. Or, Lead

acetate solution (10%) drops were added to the extract (1 mL). Formation of a yellow precipitate showed the presence of flavonoids.

#### 2.5.2 Tannins

The extract (1 mL) was added to 2 mL of water followed by drops of dilute ferric chloride solution (0.1%). A green to blue-green (cathectic tannins) or a blue-black (gallic tannins) coloration were positive indicators.

#### 2.5.3 Saponins

The extract (1 mL) was shaken vigorously with distilled water. A stable persistent froth for 20 minutes was a positive indicator.

#### 2.5.4 Coumarins

NaOH (2 mL, 10%) was added to 1 mL of extract and formation of yellow color indicates the presence of coumarins.

#### 2.5.5 Terpenoids

The extract (2 mL) was added to acetic anhydride (2 mL) and concentrated $H_2SO_4$ drops. Formation of blue, green rings indicated the presence of terpenoids.

#### 2.5.6 Fatty acids

The extract (0.5 mL) was mixed with 5 mL of ether. The solution was allowed to evaporate on filter paper. The appearance of transparency on dried filter paper indicated the presence of fatty acids.

#### 2.5.7 Phenols

Ferric chloride test, an extract (1 mL) was treated with drops of ferric chloride (5%) and observed for the formation of deep blue or black colour.

#### 2.5.8 Amino acids and proteins

The extract (1 ml) was treated with drops of ninhydrin solution (1% ninhydrin solution) and placed in a boiling water bath for 2 minutes. The formation of purple colour was a positive test.

#### 2.5.9 Quinones

An extract (1 mL) was treated with conc. HCl drops and observed for the formation of yellow precipitate or coloration.

#### 2.5.10 Oxalate

The extracts (2 mL) were treated with a few drops of glacial acetic acid. A greenish black coloration indicates presence of oxalates.

### 2.6 Antioxidant Capacity

#### 2.6.1 2,2`-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

ABTS radical scavenging activity was determined by the method described by Pellegrini et al. [12]. The ABTS radical cations are produced when ABTS (7 mM) reacts with potassium persulfate (2.45 mM) when incubated at room temperature in the dark for 16 hrs. The solution thus obtained was further diluted with phosphate buffer saline (PBS) to give an absorbance of 1.000. Different concentrations of the test sample in 50 µl were added to 950 µl of ABTS working solution to give a final volume of 1 ml. The absorbance was recorded immediately at 734 nm. Gallic acid was used as reference standard. Inhibiting concentrations of extracts were tested at 5, 10, 15, 20 and 25 mg/ml. Reference standard (gallic acid) was tested at the same concentrations. The percent inhibition was calculated from the following equation:

% Inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance Control] x100

#### 2.6.2 Thin layer chromatography (TLC) - semi-quantitative DPPH assay

0.2% DPPH solution in methanol was prepared and kept in the fridge for further use. The grid space was marked with 1.0 $cm^2$ space on an aluminum-based TLC sheet (Merck silica gel 60F254) and a stock solution of all the extracts together with the standard were prepared in methanol. A series of dilutions of the stock together with the standard were prepared ranging from 400 µL to 0.01 µL for the last dilution. The grid on the TLC sheet was labeled with extract on the horizontal axis and concentration of extract on the vertical axis. The extracts of different concentrations and the standards were plotted on the TLC sheets and the spots allowed to dry for at least 2 hours. Care was taken to keep the volume of the extracts spotted the same so that all the spots were of the same size for a fair comparison. The sheet was sprayed with 0.2% DPPH solution and the

appearance of yellow spots against the white background showed the antioxidant activity. Photographs of the TLC were taken after 2 hours, 6 hours, and 24 hours and this could be used for further references because the DPPH gets faded with time. This procedure was adapted and revised from the methods which were previously used by Juma and Majinda [13].

#### 2.6.3 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay - spectrophotometric method

The free radical scavenging activity was measured using DPPH method modified by Yeboah and Majinda [14]. Solutions of 500 µM DPPH (i.e. 0.02% or 0.2 mg/mL) in methanol (AR) was prepared. Also different concentrations of each of the plant extracts and standards were prepared (ascorbic acid and gallic acid) ranging from 0.001-0.05 mg/mL in methanol. Each extract or standard solution (2 mL) was added to an equal volume of the DPPH solution, making a total reaction volume of 4 mL. A control reaction mixture was prepared consisting of 2 mL methanol without extract and an equal volume of DPPH solution. The test tubes were tightly closed, vigorously shaken and placed in a dark cupboard for 30 minutes. The absorbance of each solution was measured at 517 nm, and methanol was used as the blank for baseline correction, after 2 h and finally after 24 h. The percentage inhibition of DPPH, I % was calculated using the following formula:

> % Inhibition (I %) = [(Absorbance of control – Absorbance of test sample)/ Absorbance Control] x 100

From the inhibition curves (I % versus sample concentration in µg/mL) the concentration of extract or standard required to inhibit DPPH radical activity by 50% ($IC_{50}$) was determined from non-linear regression equations that best fitted the curves. The experiment was carried out in triplicate and the $IC_{50}$ values reported as the average of three trails in µg/ mL + the standard deviation.

#### 2.6.4 Thiobarbituricacid (TBA) assay

The method followed was described by Rezaeizadeh et al. [15]. Briefly, extracts (1 mg/mL, 2 mL) were added to aqueous trichloroacetic acid (20%, 1 mL) and thiobarbituric acid (0.67%, 2 mL). After boiling for 10 min. the mixture was cooled and centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was recorded at 532 nm. The antioxidant activity was calculated by percentage of inhibition as follows: % Inhibition = 100-[(A1-A0) ×100]. Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts. Measurements were done in triplicates.

#### 2.6.5 Total Phenolic Content (TPC)

The total phenolic content (TPC) of the extracts were determined using the Folin-Ciocalteu reagent method as described by Yeboah and Majinda [14]. Five different concentrations of the standard, gallic acid, in methanol were prepared ranging from 0.01 to 0.05 mg/mL. 5 mL of 90% aqueous methanol and 0.5 mL Folin-Ciocalteu reagent were added to 0.5 mL of each of the standard solutions and to 0.5 mL of each extract solution (1 mg/mL) in screw cap test tubes. After 3 min, 1 mL of 2% $Na_2CO_3$ was then added to each test-tube and the mixture was vigorously shaken for 2 minutes and left to stand for 2 hours at room temperature. The absorbance of the supernatant solution was determined at 725 nm using 90% aqueous methanol as a solvent blank. A gallic acid standard curve was prepared and the equation derived by linear regression ($y = 36.84 x + 0.1069$) was used to determine the TPC of each extract in mg of gallic acid equivalents/g of extract (mg GAE/g). The experiment was performed in triplicate and TPC was reported as the average value of 3 trials ± the standard deviation

### 2.7 Antidiabetic Potential

#### 2.7.1 α-Amylase activity

The assay was carried out following the standard protocol by Hansawasdi et al. [16] with slight modifications. Starch azure (2 mg) was suspended in 0.2 mL of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing substrate solution were boiled for 5 min and then pre-incubated at 37°C for 5 min. Ethanol extract of *L. scaberrima* was dissolved in DMSO in order to obtain concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. Then, 0.2 mL of plant extract of particular concentration was added to the tube containing the substrate solution. In addition, 0.1 mL of porcine pancreatic amylase in Tris–HCl buffer (2units/mL) was added to the tube containing the plant extract and substrate solution. The reaction

was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer (Perkin Elmer Lambda 25 UV–VIS spectrophotometer). Same procedure was followed for other plants extracts to test their α-amylase inhibitory effects. Acarbose, a known α-amylase inhibitor was used as a positive control. The experiments were repeated thrice. The α-amylase inhibitory activity was calculated by using following formula:

$$\% \text{ α-amylase inhibitory activity} = [(Abs100\%_{control} - Abs\ sample)/ Abs100\%_{control}] \times 100$$

The α-amylase inhibition was plotted against the extract concentration and the $IC_{50}$ values were determined from the graph.

## 2.8 Statistical Analysis

All data were expressed as the mean ±S.E. mean of n= 5. Analysis of variance was performed by one way ANOVA and the significant difference between the means were determined by the Holm-Sidak method. The *p*-value ≤0.05 was regarded as significant. In all these cases, Statistical Software Stata 13.1 was used to analyze the data.

# 3. RESULTS AND DISCUSSION

## 3.1 Phytochemical Screening

Phytochemicals are non-nutrient plant chemicals that produce antioxidant benefits such as anticarcinogenic, antimutagenic and anti-inflammatory effects. A number of biochemical reactions produce reactive oxygen species (ROS) or free radicals. Under normal circumstances the production of ROS are controlled by the antioxidant defense system, but under certain pathological conditions the ROS are not efficiently removed by this defense system. The excessive ROS may alter cell integrity through attacking the lipids, proteins and DNA leading to debilitating diseases and disorders such as coronary heart diseases, rheumatoid arthritis, cancers and Alzheimer's disease. Therefore antioxidant phytochemicals may be beneficial for the prevention and treatment of such chronic diseases.

Oxidative stress suppresses normal physiological health hence improving oxidative capacity especially through natural nutrition. This is important for normal physiological processes. The results in Table 1 show that all the five extracts contain important phytochemicals like flavonoids and tannins, and differing presence of other phytochemicals. This suggests that these plants have cell protective properties due to these antioxidants.

## 3.2 ABTS Radical Scavenging Activity

Several methods for measuring antioxidant capacity have been developed. These assays generate different radicals and/ or target molecules. Therefore using a few of these assays at a time ensures a wider coverage of antioxidants that may be there in the study sample. ABTS decolourisation assay method is commonly used to screen for antioxidant activity in both lipophilic and hydrophilic antioxidants. ABTS is a blue/ green stable radical that reacts quickly with molecules that are able to donate hydrogen atom or electrons, such as antioxidants, thus converting the ABTS radical cation to its neutral colourless form. The concentration of the antioxidant and the duration of the reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. The phenolic antioxidant activity of the plants was assessed by looking at the ability of different phenolic compounds in the plant extracts to scavenge the ABTS radical cation.

## 3.3 Thin Layer Chromatography (TLC) Assay

TLC assay was used to determine the antioxidant activity of the extracts compared to gallic acid standard. This procedure was used to separate and differentiate between individual components of the extracts. Gallic acid is a natural antioxidant which is also a water-soluble phenolic acid. The antioxidant activity of the samples was determined by DPPH free radical assay as described in the methodology. The strength of the antioxidant potential is shown by the intensity of the decolourisation of DPPH with the plant extracts as shown in Fig. 2. The results show a better presence of antioxidants in MEHHC and MEOC. MEMB and MELS had the least antioxidant content.

**Table 1. Results of phytochemical screening**

| TEST | MEOG | MEC | MEMB | MELS(l) | MELS(s) |
|---|---|---|---|---|---|
| Flavonoids | ++ | +++ | ++ | + | ++ |
| Tannins | ++ | ++ | + | ++ | ++ |
| Saponins | + | - | - | + | - |
| Connanins | - | + | + | - | + |
| Terpenoids | - | - | - | - | + |
| Fatty acids | - | ++ | - | - | - |
| Phenols | + | +++ | ++ | ++ | + |
| Amino acids | - | - | - | - | - |
| α proteins | - | - | - | - | - |
| Quinones | + | - | - | - | + |
| Oxalate | - | - | - | - | - |

*Key: +++strongest activity,++strong activity, +weak activity, -no activity*
*MEHHC - Methanol extract of H. hemerocallidea corm, MEOG - Methanol extract of O. gratissimum, MEMB - Methanol extract M. balsamina, MELS (l) - Methanol extract of L. scaberrima (leaf) and MELS(s) - Methanol extract of L. scaberrima (stem)*

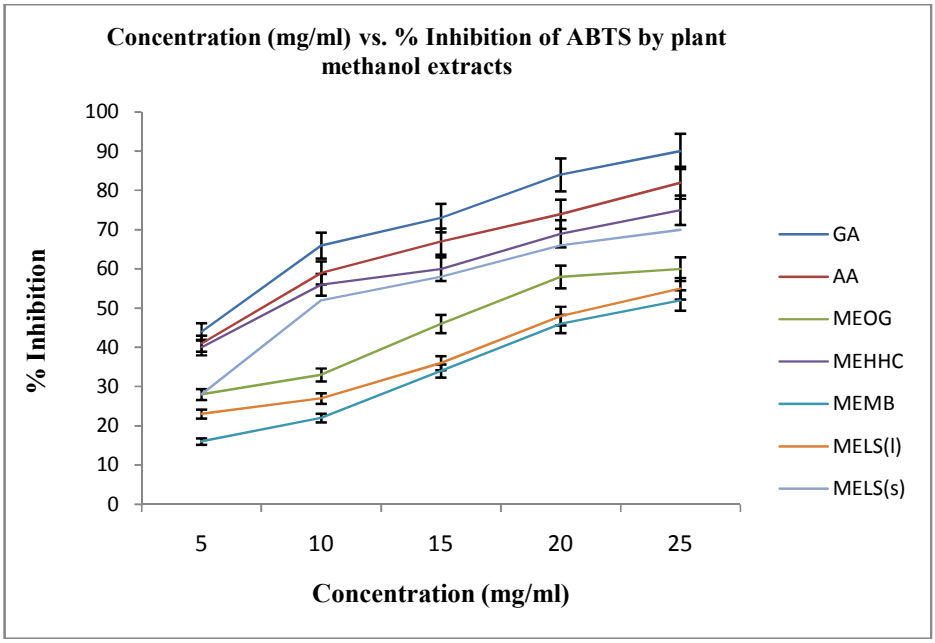


**Fig. 1. % inhibition of ABTS for different plant extracts against GA and AA**
*GA - Gallic acid, AA - Ascorbic acid, MEHHC - Methanol extract of H. hemerocallidea corm, MEOG - Methanol extract of O. gratissimum, MEMB - Methanol extract M. balsamina, MELS(l) - Methanol extract of L. scaberrima (leaf) and MELS(s) - Methanol extract of L. scaberrima (stem)*

## 3.4 DPPH Assay

This method has been commonly used to test for the antioxidant activities of materials due to its relatively short time required for analysis. In the presence of an antioxidant, the DPPH will become discoloured, and the colour change is measured spectrophotometrically. The evolution of colour after addition of the test material is used as an indicator of the antioxidant strength of the article. Compared against the concentration of the naturally occurring antioxidants Gallic acid and Ascorbic acid, MEHHC showed the highest and maximum antioxidant power at 0.34 mg/ ml, closely followed by the remaining four extracts. MELS leaves showed the least antioxidant strength compared to the other plants and had maximum DPPH inhibition at a concentration of 0.5 mg/ ml. The radical scavenging activity of the different plant extract increased with the intensity of the extracts.

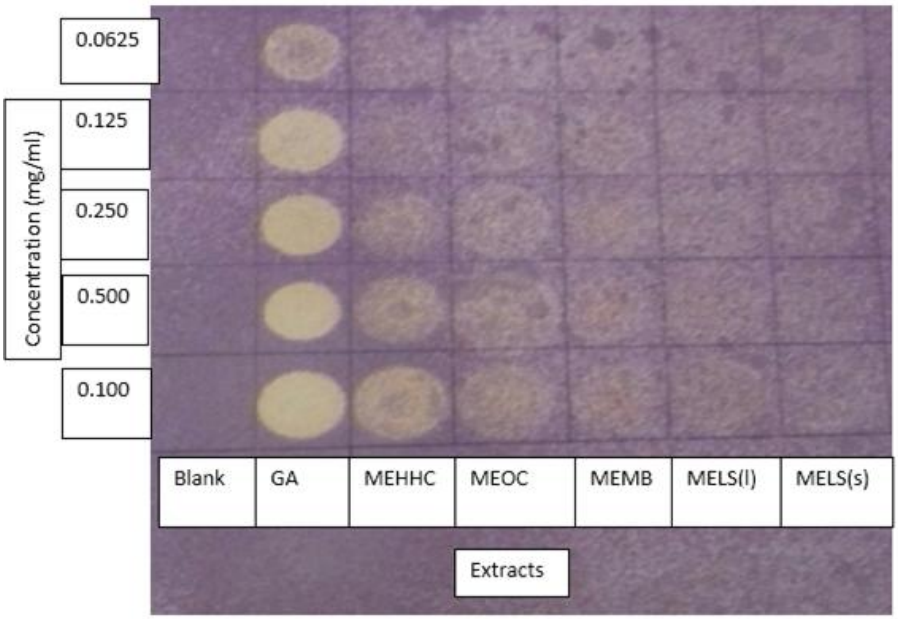


**Fig. 2.Semiquantitative TLC-DPPH radical scavenging activity of different extracts**
*MEHHC Methanol extract of H. hemerocallidea corm, MEOG Methanol extract of O. gratissimum, MEMB Methanol extract M. balsamina, MELS(l) Methanol extract of L. scaberrima (leaf), and MELS(s) Methanol extract of L. scaberrima (stem)*

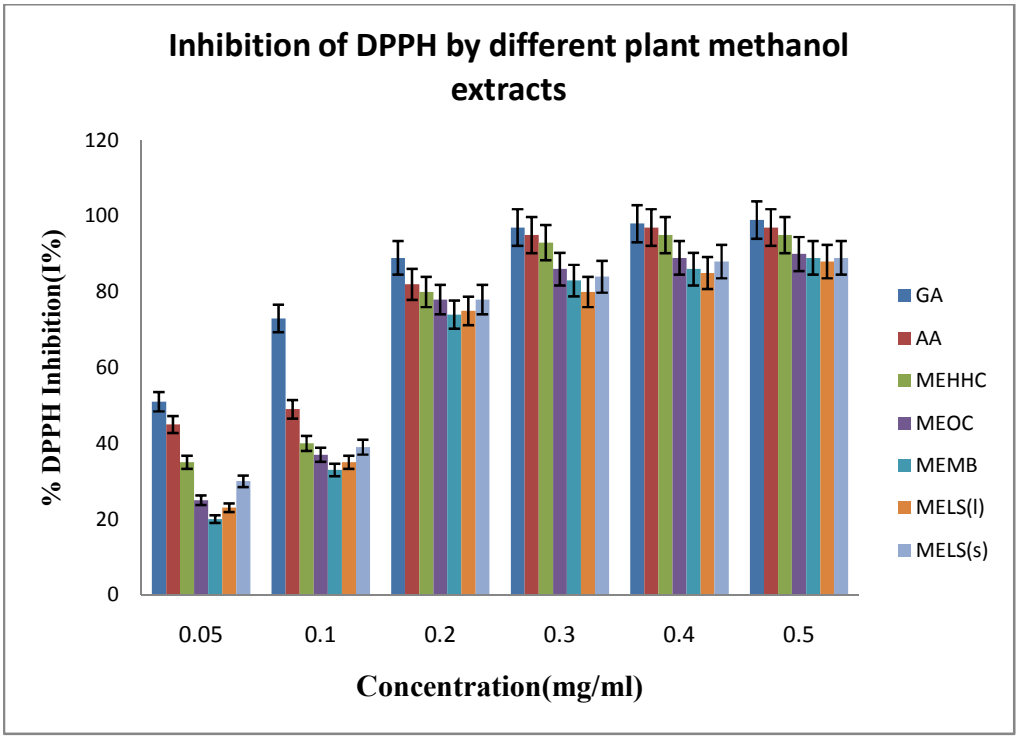


**Fig. 3. % Inhibition of the different plant extracts in DPPH assay**
*GA - Gallic acid, AA - Ascorbic acid, MEHHC - Methanol extract of H. hemerocallidea corm, MEOG - Methanol extract of O. gratissimum, MEMB - Methanol extract M. balsamina, MELS(l) - Methanol extract of L. scaberrima (leaf)and MELS(s) - Methanol extract of L. scaberrima (stem)*

### 3.5 TBA Assay

The thiobarbituric acid assay is the most generally used test in the determination of lipid peroxidation levels of malondialdehyde (MDA) in biological samples [17]. Malondialdehyde is a naturally occurring product formed during the radical induced decomposition of polyunsaturated fatty acids, and it is considered as an essential marker of oxidative stress caused by fatty acids peroxidation. The MDA inhibitory activities of all the methanol extracts are stronger than that of ascorbic acid (AA) but lower than that of Butylatehydroxyanisole (BHT) which is a synthetic antioxidant. The comparison of the five extracts as presented in Table 2 indicate that these extracts possess some antioxidant potential to react against free radicals. At 30 minutes, MEHHC showed the maximum possible (70.5% inhibition) followed by MEMB, MEOG, MELS(l) and finally MELS(s) at 45.0% inhibition. The inhibition of the extracts at one hour increased only minimally from that observed at 30 minutes.

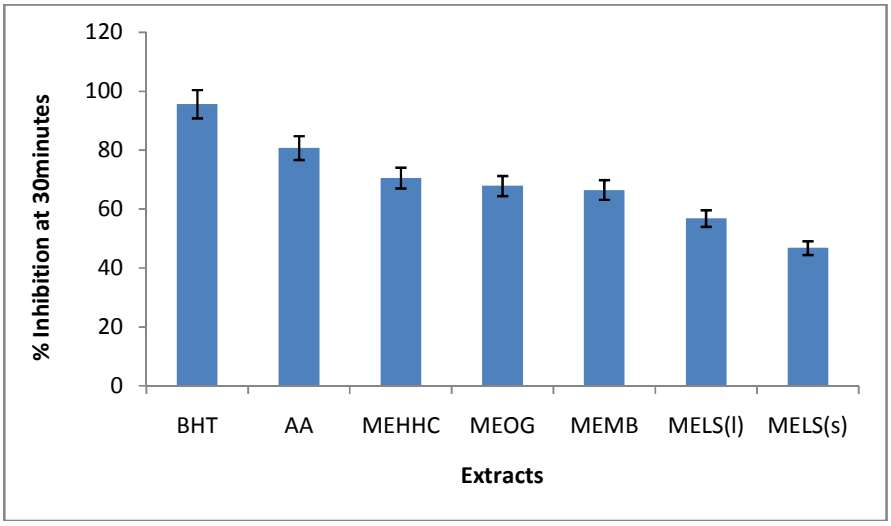


**Fig. 4a. % Inhibition of TBA assay at 30 minutes**

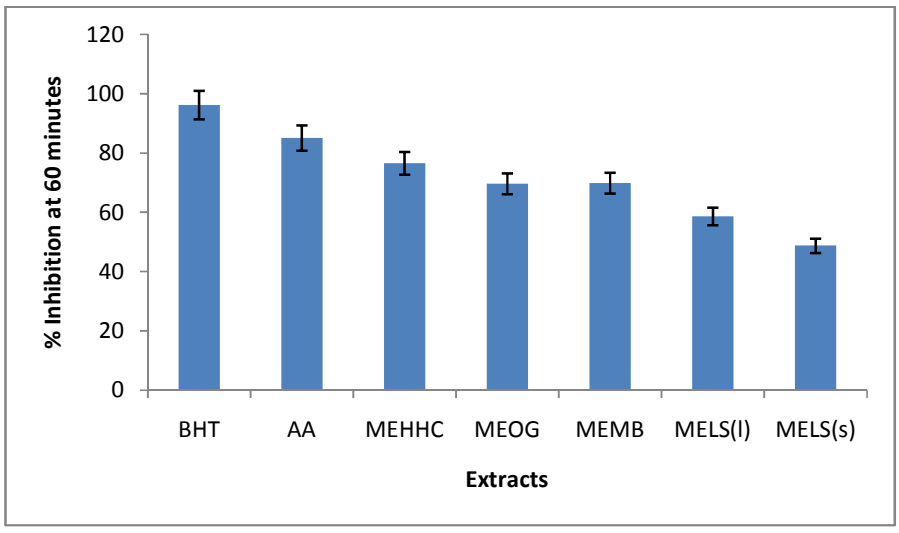


**Fig. 4b. %Inhibition of TBA assay at 60 minutes**

*BHT Butylatehydroxyanisole, AA Ascorbic acid, MEHHC Methanol extract of H. hemerocallidea corm, MEOG Methanol extract of O, gratissimum, MEMB Methanol extract M. balsamina, MELS(l) Methanol extract of L. scaberrima (leaf), and MELS(s) Methanol extract of L. scaberrima (stem)*

**Table 2.Total phenolic content (mg GAE/ 100g) of the different plant extracts**

| Concentration (mg/ml) | MEOG | MEHHC | MEMB | MELS(leaves) | MELS(stems) |
|---|---|---|---|---|---|
| 0.1 | 0.71 ± 1.6 | 27.66 ± 2.3 | 2.84 ± 1.4 | 1.32 ± 3.4 | 5.62 ± 1.8 |
| 0.2 | 10.64 ± 2.7 | 54.96 ± 1.6 | 17.02 ± 1.9 | 11.54 ± 2.6 | 24.61 ± 2.0 |
| 0.3 | 18.09 ± 1.3 | 60.28 ± 3.1 | 26.24 ± 1.5 | 19.24 ± 2.1 | 33.9 ± 1.6 |
| 0.4 | 31.91 ± 1.2 | 76.6 ± 1.8 | 38.3 ± 2.7 | 33.98 ± 3.5 | 45.22 ± 4.1 |
| 0.5 | 54.96 ± 1.9 | 79.79 ± 2.0 | 49.29 ± 3.1 | 57.03 ± 2.0 | 59.7 ± 3.4 |

*MEHHC Methanol extract of H. hemerocallidea corm, MEOG Methanol extract of O. gratissimum, MEMB Methanol extract M. balsamina, MELS(l) Methanol extract of L. scaberrima (leaf), and MELS(s) Methanol extract of L. scaberrima (stem)*

### 3.6 Total Phenolic Content (TPC) Assay

Phenols are very important plant constituents because of their free radical scavenging ability that is due to their hydroxyl groups. Many natural antioxidants are phenolic compounds so the determination of total phenolic content of a plant extract could give useful information that could be correlated with antioxidant capacity of the plant. Plant phenols are potent antioxidants which play a role in preventing damage to cell components such as DNA, lipids and proteins. Hence phenols may be necessary in the prevention or risk reduction of chronic diseases like cancers and heart diseases. In the extracts used, TPC was calculated from the linear regression equation of the standard curve y =36.84 x+0.1069. From this equation, the equivalent concentration of gallic acid 254.21± 0.43 mg/mL was determined for each extract and converted to mg of gallic acid equivalents/g of dry extract (mg GAE/g). MEHHC showed the highest phenolic content and MEOB had the least content. Phenolic content for the remaining three extracts was similar as shown in Table 2.

### 3.7 α-Amylase Activity

Antidiabetic activity was evaluated by assessing the inhibitory potential of the plant extracts against α-amylase using spectrophotometric assays. A gradual increase in the movement was shown in all the extracts together with acarbose which was used as a standard. A dose-dependent gradual increase was noticed during the experiment with the time. MEHHC showed the maximum effect while MELS(s) showed the least effect (Fig. 5)

Alpha-amylase inhibitors and antioxidant compounds can control diabetes [18], and therefore research is looking into the use of plants antioxidants as an option for safe and more cost-effective antidiabetic drugs.

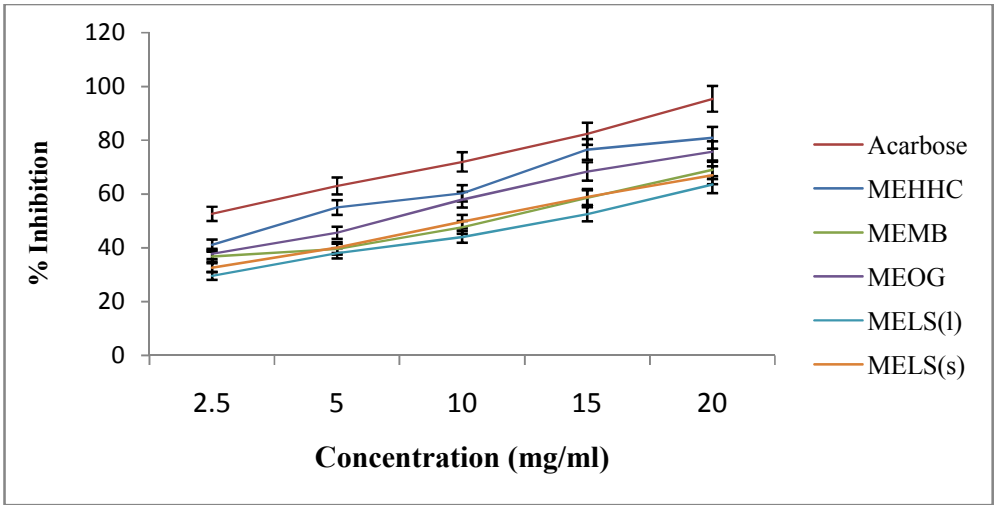


**Fig. 5. The α-amylase inhibitory activity of different plant extracts**

*MEC Methanol extract of H. hemerocallidea corm, MEOG Methanol extract of O, gratissimum, MEMB Methanol extract M. balsamina, MELS (l) Methanol extract of L. scaberrima (leaf), and MELS(s) Methanol extract of L. scaberrima (stem)*

Inhibition of pancreatic α-amylase is one strategy that could be used in the management of diabetes. This inhibition slows down the digestion of carbohydrates thereby lowering blood glucose levels. The antidiabetic activity was evaluated by the inhibitory potential of the plant extracts against α-amylase by spectrophotometric assay. The α-amylase inhibitory potency of the five extracts as compared against acarbose standard was found to be strong as shown in Fig. 5. MEHHC showed the greatest potency with maximum inhibition of 94% at a concentration of 20 mg/ml, while MELS leaves had inhibition of 61.5% at the same intensity. Therefore, the antidiabetic properties of the plants could be through the inhibition of the α-amylase mechanism in the digestive system.

Alternative medicines for type 2 diabetes are based on phytochemicals that can delay or prevent carbohydrate metabolism or enhance glucose absorption [19]. Research evidence suggests that the presence of antioxidants like phenols and other phytochemicals in medicinal plants is responsible for their pharmacological properties such as antimicrobial, antidiabetic, protection against various types of cancers, and protection against cardiovascular disorders [3,6,8,10,20]. The redox properties found in antioxidants enable them to scavenge free radicals and to act as lipid peroxidation inhibitors [20].

## 4. CONCLUSION

All the plant's studies here show unusual antioxidant activity in different systems including TBA, DPPH and ABTS. Furthermore, there is a significant antidiabetic activity in all the five plant extracts, the most potent one being MEHHC and the least potent being MELS stems. MEHHC show more potency as an antidiabetic agent probably because of its higher concentrations of medicinal phytochemicals. This study validates the traditional use of these plants as medicinal plants to treat various ailments. Therefore in conclusion, these plants could potentially serve as an alternative cost-effective and safer option in the management of diabetes mellitus.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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