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RESEARCH ARTICLE

In vitro antioxidant, α-amylase and α-glucosidase activities of methanol extracts from three Momordica species

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Abstract

Antioxidant based drug preparations are used in the prevention and management of complex diseases which include atherosclerosis, stroke, diabetes, alzheimer's disease and cancer. Diabetes mellitus is a metabolic disorder of glucose metabolism. The management of blood glucose level is the hallmark in the treatment of this ailment, which may be achieved through the use of oral hypoglycemic drugs such as biguanides, insulin secretagogues, and α-amylase and glucosidase inhibitors. Although several biological activities had been reported for *Momordicafoetida* and *Momordicacharantia*; it appears there is limited information on the biological activity of *Momordicacissoides*. The purpose of this study is to compare the antioxidant, α -amylase and α-glucosidase inhibitory activities of *Momordicacharantia* (M1), *Momordicafoetida* (M2), and *Momordicacissoides* (M3) to establish a chemotaxonomic relationship between them. The antioxidant activities measured by DPPH scavenging properties, metal ion chelation, hydrogen peroxide scavenging and ABTS revealed that *M. foetida* had the highest inhibition potential, followed by *M.charantia* and the least being *M.cissoides*. In contrast, the antioxidant activities measured by FRAP, the total phenolic content, flavonoids and tannins revealed that *M. cissoides* had the best antioxidant potential, while *M. foetida* had the least activity. The comparative α-amylase and glucosidase inhibitory studies performed demonstrated that the extracts of *M.cissoides* had the highest inhibitory potentials. Thus, the plant can be used in the management of diabetes.

Keywords: Mcissoides; amylase; glucosidase; antioxidants; diabetes

Introduction

Free radicals, which belong to a group of reactive oxygen species (ROS), are produced through endogenous source, that is, the human body itself, and exogenous sources such as tobacco smoke, burning of fossil fuels, and ozone. The imbalance between the production of ROS and the activity of the antioxidant defences is referred to as oxidative stress [1]. The preventive or protective effects of herbs or spices against the harmful consequences of oxidative stress are due to the existence of naturally obtainable antioxidants in them [2]. Antioxidant based drug preparations are used in the exclusion and controlling of complex diseases which include atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer [2, 3].

Diabetes mellitus (DM) has repeatedly been described as a metabolic disorder characterized by hyperglycemia which develops as an upshot of defects in insulin secretion, action, or both [4]. It is one of the communal metabolic problems with micro-and macrovascular complications that results in significant morbidity and mortality. Diabetes mellitus is also one of the five main causes of death in the world. In contemporary medicine no satisfactory effective therapy is still obtainable to cure diabetes mellitus. There is a growing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic

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agents. Many traditional medicinal plants have been reported to have hypoglycemic activity [5].

Momordica is a genus of about 60 species of annual or perennial climbers that are herbaceous or rarely small shrubs belonging to the family *cucurbitaceae*. They are natives of tropical and subtropical Africa,Asia and Australia. Most species yield floral oils and are visited by specialist pollinators in the apid tribe *ctenoplectrini*. A molecular phylogeny that includes all species is available [6, 7].

Momordica foetida Schumach. et Thonn. from the *cucurbitaceae* family, is a medicinal plant, widely distributed in tropical Africa, South and West tropical Africa. The plant has both male and female flowers. *Momordica foetida* has been used in the treatment of malaria, hypertension, peptic ulcers, diabetes mellitus, and as a purgative. Curcubitane triterpenoids and polyphenolic compounds have been isolated from the leaf extracts while alkaloids and glycosides were obtained from the whole plant extracts [8].

*Momordica Charanti*a (bitter melon or bitter gourd) is a flowering vine also in cucurbitaceae family which is widely cultivated in Asia, India, East Africa and South America. The plant is used as a natural remedy for diabetes, topically for sores, wounds and infections. It is also used to expel worms and parasites; as an emmenagogue ; and as antiviral for measles, hepatitis and feverish conditions. The plant is also being used to induce abortion. Several alkaloids, triterpenoids and glycosides have been isolated from the plant [9, 10].

*Momordica cissoide*s Linn. is a plant used in traditional folk medicine by many African countries for the treatment of several illnesses like; epilepsy, headache, madness and gonorrhoea. The plant has been reported for its anticonvulsant and antioxidant activity [11]. There are so many species of *momordica* but it appears that the information on *Momordica cissoide*s is limited.

Several studies have shown the antidiabetic activity of *M.foetida* and *M.Charantia* [8, 10, 12], no erstwhile report has been given on the antidiabetic potential of *M.cissoides.*The earlier report of the comparative phytochemical screening of the three medicinal plants which indicated that *M.cissoides* is rich in saponins, phenols and tannins was documented by Akinwumi [13], p rompted this research. This might also be useful to ascertain their chemotaxonomic relationship. Thus, this research is a follow up to the previous one. It is aimed at evaluating the effect of the *M.foetida*, *M.Charantia* and *M. cissoides* for antioxidant, α-amylase and α-glucosidase activities.

Material and methods

Sample preparation

The Fresh leaves of *M. foetida, M. charantia* and *M.cissoides* were collected from the Sawmill area, Ikere road, Ado- Ekiti, Ekiti State, Nigeria. The plants were identified by Mr. Omotayo at the Herbarium section of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti. The leave samples were air dried for three weeks at room temperature and ground into powder using an electric blender. The resultant powder were soaked in methanol for 72 hrs, filtered and concentrated at 40^{o}C using rotary evaporator. The methanol extracts were then stored at - 4^{o}C pending analysis.

DPPH Radical-Scavenging Activity Assay

Free radical scavenging activity of the leaves of Momordica plant extracts were measured according to the method of Wickramaratne et al [14], with slight modifications. This assay was based on the fact that DPPH would be reduced to hydrazine. 0.2% solution of DPPH was added to various concentrations of extracts ranging from 10 to 50 μg /ml in a 96-well plate. The solution was incubated at room temperature in the dark for 30 minutes, thereafter; the absorbance was measured at 490 nm using a microplate reader. The diluting solvent was used as a blank and the scavenging activity was calculated as follows:

$$\%\ inhibition\ =\ \frac{[A_{490}\ of\ blank - A_{490}\ of\ sample]}{A_{490}\ of\ blank} \times 100$$

Hydrogen Peroxide Scavenging

40mM solution of $H_2\,O_2$ was prepared in 0.1M phosphate buffer (pH 7.4). Various concentrations (10 -50 μg /ml) of the extracts were added to 0.6ml $H_2\,O_2$ solution and the total volume was made up to 3ml. The absorbance of the reaction mixture was then measured at 230nm using a spectrophotometer. Phosphate buffer was used as a blank and the extent of $H_2\,O_2$ scavenging of the plant extracts was calculated as:

$$\%\ inhibition\ =\ \frac{[A\ of\ blank - A\ of\ sample\ ]}{A\ of\ blank} \times 100\ [15]$$

Metal chelating activity assay

0.16 ml of deionized water and 0.005 ml of 2 mM $FeCl_2$ was added to 0.05 ml of extract at different concentrations (10-50 μg /ml). 0.01 ml of 5 mM ferrozine was added after 30 s. The absorbance of the resultant Fe^{2+} –Ferrozine complex formed from the reaction above was measured at 562 nm after 10 minutes at the room temperature. The chelating activity of the extract for Fe^{2+} was calculated as

$$Chelating\ rate\ (\%)\ =\ \frac{(A0 - A1)}{A0} \times 100$$

Where A0 represents the absorbance of the control blank without extract and A1 was the absorbance of the extract [16].

Total phenolic content determination

The total phenolic content of the plant samples were determined according to the method described by Nagulendran et al [17] with slight modifications. 0.05 ml of the plant extract with concentration of 1 mg/ml was added to 0.05 ml of Folin-Ciocalteu reagent, after 3 minutes, 0.1 ml of 2% $Na_2 CO_3$ was added. Afterwards, the mixture was shaken for 2 h at 28 ^{0} C, and then absorbance was measured at 760 nm. Quercetin was used as the standard.

Tannin determination

50 ml of distilled water was added to about 500 mg of the sample and the mixture shaken for 1 hour using a mechanical shaker. This was filtered into a 50 ml standard flask and made up to the mark with distilled water. 0.1 ml of the filtrate was mixed with 0.06 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M $KFe(CN)_6$. The absorbance was afterwards measured using a spectrophotometer at 605 nm within 10 minutes. The absorbance of the blank was also measured at the same wavelength. Gallic acid was used as a standard [18].

Determination of 2,2-Azinobis (3-ethylbenzo-thiazoline-6-sulfonate (ABTS)radical scavenging ability

The ABTS* scavenging ability of the extracts was quantified according to the method of Re et al [19]. The ABTS* radical was generated by reacting a 7 mM l^{-1} ABTS aqueous solution with 2.45 mM l^{-1} $K_2 S_2 O_8$ in the dark for 16 h. The absorbance was adjusted to 0.700 with ethanol at 734 nm. 0.05 ml of appropriate dilution of the extract was added to 0.15 ml ABTS* solution and the absorbance were measured at 734 nm after 15 minutes.

Reducing Power Assay

The reducing power assay was carried out by the method of Nagulendran et al [17]. 2.5ml of the extract solution was mixed with 2.5 ml of 0.2M sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The resultant mixture was then incubated at 50 °C for 20 minutes, after which a 2.5 ml of 100 mg/l trichloroacetic acid solution was added. The mixture was centrifuged at 650 rpm for 10 minutes, 5 ml of the supernatant was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride solution. The absorbance was determined at 700 nm.

Total Flavonoid

The total flavonoid content was measured by the aluminium chloride colorimetric assay. 0.01 ml of extracts or standard solution containing 500μg/ml of quercetin was added to 10 ml standard flask containing 0.04 ml of distilled water. To the above mixture, 0.03 ml of 5% $NaNO_2$ was added, after 5 minutes, 0.03 ml of 10% $AlCl_3$ was then added. At the 6th min, 0.02 ml of 1 M NaOH was added and the total volume made up to 0.2 ml with distilled water. The solution was properly mixed and the absorbance measured against the blank at 510 nm. Total flavonoid content of the extract was expressed as percentage of quercetin equivalent per 100 g sample [17].

α- amylase inhibition activity

The inhibition of alpha amylase activity was carried out using an amended procedure described by Kazeem et al [20]. 20 μl of sample at concentrations 10-50 μg/ml was reacted with 140 μl 0.1M sodium phosphate (pH 6.8) and 10 μl 0.02U/ml amylase prepared in enzyme. The mixture was incubated at room temperature for 15 minutes. 10 μl of 1%w/v starch solution was added and incubated at room temperature for 15 minutes. 10 μl of 96mM dinitrosalicyclic (DNS) acid was added to the resultant mixture above and the plate incubated in boiling water for 5 minutes. The absorbance was recorded at 540 nm using microplate reader, while the % inhibition of α-amylase enzyme was calculated using the formula: $\%\ Inhibition = \frac{A\ control - A\ sample}{Acontrol} \times 100$

α- glucosidase inhibition activity

The inhibitory effect of the *M. species* extracts on α-glucosidase activity was determined according to the chromogenic method described by Kazeem et al [20], with slight modifications. Concisely, 5 units of α-glucosidase were pre-incubated with 10-50 μg/ml of the *M. species* extracts for 15 minutes. 3 mM Paranitrophenylglucopyranoside (PNPG) dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was further incubated at 37°C for 20 minutes and then stopped via the addition of 2 ml of 0.1 M $Na_2 CO_3$. The α-glucosidase activity was determined by measuring the yellow colored p -nitrophenol released from PNPG at 400 nm. Each test was performed in triplicates and the mean absorbance was used to calculate % α-glucosidase inhibition using the formula;

$$\%\ \alpha - glu\cos idase\ inhibition : \frac{Ao-A1}{Ao} \times 100$$

where A_0 was the absorbance of the blank control without extract and A_1 was the absorbance in the presence of the extract.

Statistical Analysis

Data were represented as mean ± SEM of at least three independent experiments. The data were also analysed using Prism Graph Pad software (San Diego, USA).

Results

DPPH radical scavenging activity

The DPPH radical scavenging activities of M1-M3 extracts showed IC $_{50}$ value > 50 μg/ml (Fig. 1A, Table 1). The highest activity was observed in M2 extract at 50 μg/ml with 22.77±0.22 % inhibition and the lowest in M3 (17.94±0.12). At the same concentration, ascorbic acid, the positive control had the % inhibition (96.7±1.03) with IC_{50} 19.2 μg/ml.

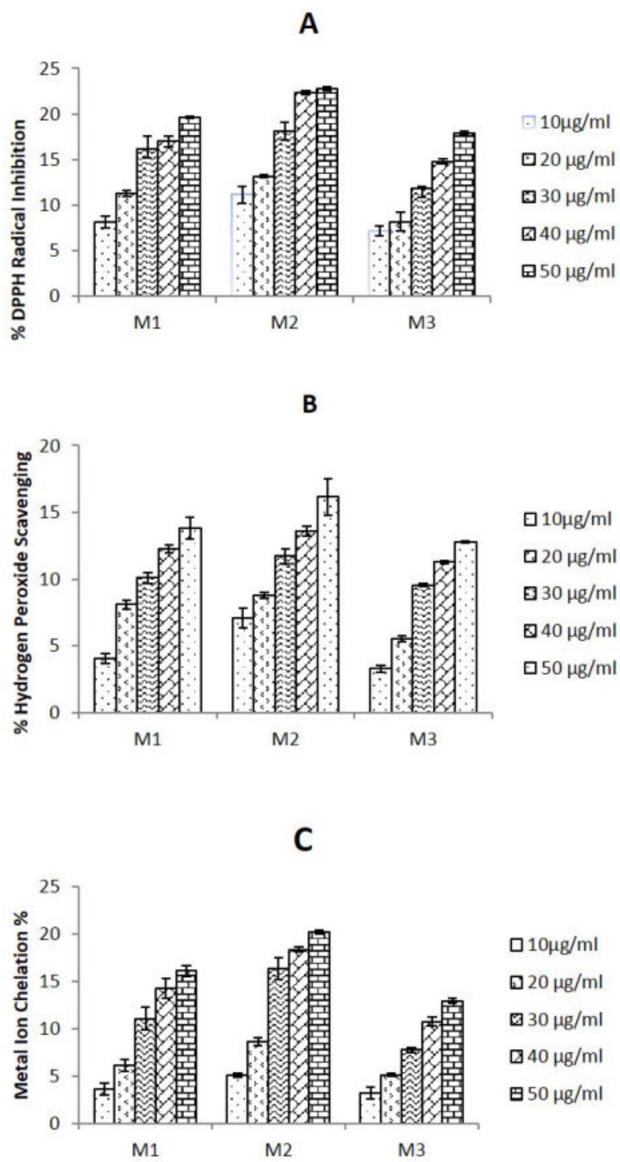

Figure 1 DPPH radical scavenging (A), hydrogen peroxide scavenging (B) and metal ion chelation (C) activities of *M. charantia* (M1), *M. foetida*(M2) and *M. cissoides* (M3) extracts. Values are expressed as mean±SEM (n=3).

Hydrogen Peroxide Scavenging

As revealed in Fig. 1B, hydrogen peroxide scavenging activity showed that M2 had the highest % inhibition (16.15±0.07) and the lowest % inhibition was exhibited by M3 (13.82±0.84). The IC $_{50}$ values for M1-3 were > 50 μg/ml while that of ascorbic acid used as the standard was 1.23 μg/ml (Table 1)

Table 1 IC_{50} values(μg/ml) from the DPPH radical scavenging, hydrogen peroxide scavenging and metal ion chelation activities of *M. charantia* (M1), *M. foetida* (M2) and *M. cissoides* (M3) extracts.

Sample	DPPH	H_2O_2	Metal ion
M1	> 50	> 50	> 50
M2	> 50	> 50	> 50
M3	> 50	> 50	> 50
Ascorbic acid	19.2	-	-
EDTA	-	-	17.6

Metal ion Chelation activity

The result of the metal ion chelation activity showed that at 50 μg/ml, M2 had the highest % inhibition (20.25±0.20) and the lowest % inhibition in M3 (12.93±0.26) (Fig.1C). Similarly at the same concentration, EDTA used as control had % inhibition (99.01±0.73) with IC_{50} 17.8 μg/ml, Table 1.

Total phenolic content, tannin, ABTS (TEAC), FRAP and flavonoid content.

The total phenolic content (TPC) and tannin is expressed as Gallic acid equivalents in mg/100g of extract (Table 2). M3 had the highest phenolic content (38.50±1.73) while M2 had the least (16.38±1.15). The result of the tannin content followed a similar pattern. M3 had the highest value (81.94±3.47) while M2 (27.60±0.87) had the lowest.

The ABTS (TEAC) result showed that M2 had the highest scavenging ability (11.96±1.28) mg/100g while the lowest was observed in M3 (8.55±0.43) mg/100g (Table 2).

The FRAP assay is expressed as AAEmg/100g as shown in Table 2. M3 had the highest reducing capability with 1020.00±5.00 while M2 646.25±3.75 had the lowest value.

The result of the flavonoid content of the plant extract as expressed in QEmg/100g showed that it ranged between 49.2±8.46 – 111.7±4.10. M3 had the highest amount of flavonoid while M2 had the least content.

Alpha-amylase and glucosidase inhibition activity

Alpha-amylase inhibitory potential of the methanolic extracts obtained from the three *Momordica* species; *M. charantia* (M1), *M. foetida* (M2) and *M. cissoides* (M3) was determined (Fig.

Table 2 Other antioxidant parameters (mean ± SEM) measured in three different extracts of leaves of Momordica species

Extract	Phenolics (GAE mg/100g)	Tannin (GAE mg/100g)	ABTS (TEAC) (mg/100g)	FRAP (AAE mg/100g)	Flavonoids (QAE mg/100g)
M1	23.1 ±4.04	74.8±4.55	8.55±0.43	821.3±1.25	77.5±1.92
M2	16.4± 1.15	27.6±0.87	11.96±1.28	661.3 ± 3.75	49.1±2.46
M3	38.5±1.73	81.9± 1.53	7.56±0.28	990.0 ±1.36	111.1±3.10

M.charantia (M1),*M. foetida* (M2) and *M. cissoides*(M3).

2A), at the highest concentration 50 μg/ml M3 had the highest % inhibition (31.45±1.04) while M2 had the lowest (21.66±0.46). The IC_{50} values were calculated (Table 3). M1-M3 had IC_{50} values > 50 μg/ml. The standard positive control Acarbose showed an IC_{50} of 45.2 μg/ml and the maximum % inhibition of 55.89± 0.43. Similarly, the result of the α-glucosidase inhibitory potential of M1, M2 and M3 (Fig. 2B, Table 3) showed that at 50 μg/ml, the maximum dose, M3 had the highest % inhibition (24.11±0.43) while M2 had the lowest (15.67±0.64). M1-M3 IC_{50} values were > 50 μg/ml. Acarbose, the control had the % inhibition of 52.72±0.31, IC_{50} value 46.2 μg/ml.

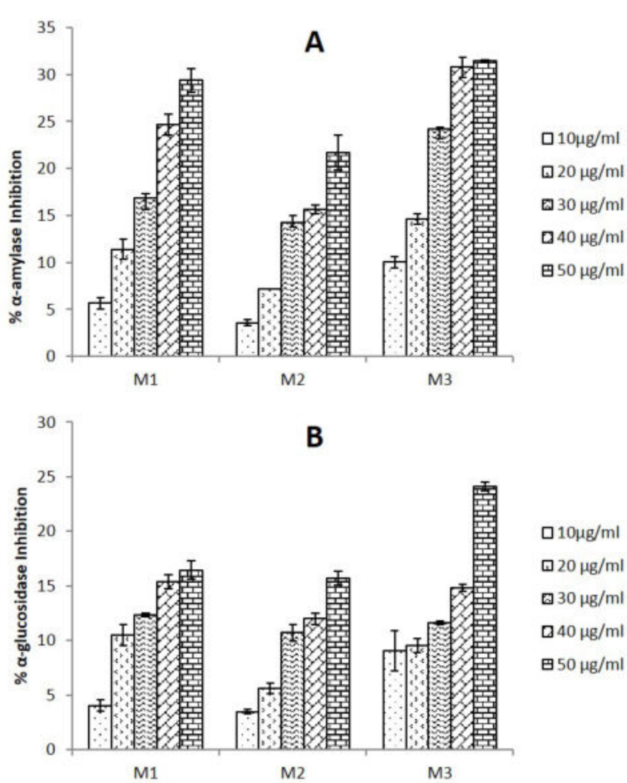

Figure 2 Inhibitionof α- amylase (A) and α-glucosidase enzymes (B) by *M. charantia* (M1), *M. foetida* (M2) and *M. cissoides* (M3) methanol extracts. Values are expressed as mean±SEM (n=3).

Table 3 IC_{50} values(μg/ml) from inhibition of α- amylase and α-glucosidase enzymes by *M.charantia* (M1), *M. foetida* (M2) and *M. cissoides* (M3) methanol extracts.

Sample	α- amylase	α-glucosidase
M1	> 50	> 50
M2	> 50	> 50
M3	> 50	> 50
Acarbose	45.2	46.0

Discussion

There is a growing trend in the screening of medicinal plants for antidiabetic activity. This has also necessitated the discovery of new effective drugs for the disease. Although the leaf extracts of *M. charantia* and *M. foetida* have exhibited antidiabetic activities [8, 10, 12]. This study would be the first report on detailed comparative study of antioxidant, α- amylase and α- glucosidase inhibitory activity study on the three *Momordica* species. It is also the first report of α- amylase and α- glucosidase inhibitory activity on *M.cissoides* to the best of our knowledge.

The comparative α-amylase and glucosidase inhibitory studies performed demonstrated that the extracts of *M.cissoides* had the highest inhibitory potentials. The inhibitory activity may likely be due to the greater number of phytochemicals present in the plant as reported by Akinwumi [13]. Previous report on α- amylase and α- glucosidase inhibitors isolated from medicinal plants suggest that several prospective inhibitors belong to flavonoid class [20, 21] . It may be necessary to isolate the biologically active compounds responsible for the activity.

It has been predicted that diabetic complications occur as a result of the oxidative stress due to the formation of free radicals with the glucose oxidation and the subsequent oxidative degradation of glycated proteins. Consequently, the use of antioxidants alongside with anti-diabetic drugs is frequently recommended to evade such complications [14]. The antioxidant activity of a compound has been attributed to various mechanisms, namely; prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging ability [22].

Generally, all the extracts do possess mild antioxidant activities. The antioxidant activities measured by DPPH scavenging properties, metal ion chelation, hydrogen peroxide scavenging and ABTS revealed that *M. foetida* had the highest inhibition potential, followed by *M. charantia* and the least being *M. cissoides*. In contrast, the antioxidant activities measured by FRAP, the total phenolic content , flavonoids and tannins revealed that *M. cissoides* had the best antioxidant potential, while *M. foetida* had the least activity. This is fully in agreement with the phytochemical analysis result as reported by Akinwumi [13] , which clearly indicated that *M. cissoides* is very rich in phenols, saponin and tannin. The least activity observed in *M. foetida* could be as a result of absence of flavonoid in the extract. Other phytochemicals like; tannin and phenols were also present in small quantities when compared with *M.cissoides*.

Conclusions

This study demonstrates that the methanol extracts of *M. charantia*, *M. foetida* and *M. cissoides* has variable antioxidant activity that can be ascribed to variability in the concentration of various phytochemicals like; phenols, saponin, tannin and flavonoids. *M. cissoides* extract exhibited the best α-amylase and glucosidase inhibitory activity. Hence the leaves of *M.cissoides* have the potential to be used in the treatment of Type II diabetes mellitus. Furthermore, this study has opened opportunities for future research in the search for novel effective drugs for diabetes that possess both antioxidant and anti-diabetic activities.

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