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Antioxidant and *in vivo* anti-hyperglycemic activity of *Muntingia calabura* leaves extracts

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ABSTRACT

The present investigation, describes the phytochemical analysis, in vitro antioxidant and antidiabetic property of Muntingia calabura leaves extracts along with estimate the inorganic components of the leaves. The inorganic components present in the leaves were estimated by Narendhirakannan method. Total phenolic and flavonoid content in the extracts were estimated by Singleton and Zhishen method. Four complementary assays, total reductive power, total antioxidant activity, 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and metal chelating ability for ferrous ions were used to screen the antioxidant property of extracts and anti-hyperglycemic activity of extracts was screened in Streptozotocin-Nicotinamide induced type II diabetic in rat model. Ethanolic extract of leaves revealed the presence of high phenolic (33.33±0.13µg GAE/mg) and flavonoid (123.31±0 .54µg CHE/mg) content. All extracts showed significant antioxidant activity in correlation to phytoconstituents of the extracts. Results showed that the ethanolic and aqueous extracts exhibited a higher antioxidant activity, while the chloroform and petroleum ether extracts was the least. Treatment of Streptozotocin-Nicotinamide induced type II diabetic rats with extracts caused significant reduction in fasting blood glucose level in a dose dependent manner. All the crude extracts showed dose dependent antioxidant and anti-hyperglycemic activity, capable of offering protection against free radical mediated damages.

Key words: *Muntingia calabura*, free radical, antioxidant activity, phytoconstituents.

INTRODUCTION

In most of the living organisms, oxidation is essential for the production of energy in biological processes and hence frequently generating free radicals during the normal course of producing energy and also during bacteria, fungi, viruses and parasites neutralizing mechanisms. Free radicals, radical derivatives and non radical reactive species are hazardous to living organisms at high concentration and damage all major cellular constituents in our body [1]. The reactive oxygen species in our body have been implicated many diseases, such as cancer, diabetes, heart diseases and they damage the body organs like eyes, lungs, brain, kidney, heart, pancreas, etc [2]. On the other hand antioxidants significantly prevent tissue damage by giving up their electrons more easily than the tissue and stimulate wound healing [3].

Diabetes mellitus is an endocrine metabolic disorder characterized by hypoglycemia results microvascular and macrovascular complications [4]. In diabetes the hyperglycemia generates reactive oxygen species, in turns these species damage the cell membrane and cause lipid peroxidation, which in turns leads to the secondary

complications, such as heart attack, kidney failure, retinotherapy etc. The sulfonylureas, biguanide, thiazolidinedione and a-glycosidase inhibitors are currently available antidiabetic agents, used to control the hyperlipidemia and hyperglycemia, but these drugs have undesirable side effects and high rates of secondary failure [5, 6]. Thus, it's a challenging task to finding out new, more effective antidiabetic agents with minimal side effects.
The literature survey revealed that the flavonoids, tannins and polyphenolic compounds found in plants have been reported to have multiple biological effects, including antioxidant activity and antidiabetic properties [7, 8, 9]. On the other hand plants, rich in bioactive compounds have been increasing interest in food industry and research because they retard oxidative degradation of lipids, improve the nutritional value of food and have broad spectrum chemical and diverse biological properties [10, 11].

The species of *M.calabura* belong to the family *Elaeocarpaceae*, it is one of the Philippine medicinal plants and widely distributed throughout the world. *M. calabura* is commonly known as Jamaican Cherry tree and is also known as capulin or capuli in Latin America. In Southern Taiwan, *M. calabura* plant was cultivated in gardens and along the road side for edible and ornamental purposes. The various parts of the *M. calabura* plant have been documented for several medicinal uses. In traditional medicine, flowers can be used as an antiseptic and treat abdominal cramps. The leaf infusion can be drunk as tea like beverage. It can be used for the treatment of cold and headache. The fruits are widely eaten by children as it is sweet and also cooked in tarts and made into jam.

The *M. calabura* have nutritional values, scientifically a number of flavonoids and phenolic compounds have been isolated from various part of the plant and structures were elucidated by spectroscopic analysis. 8- Hydroxy-7, 3, 4, 5- tetramethoxyflavone and 8, 4-dihydroxy-7, 3, 5-trimethoxyflavone were isolated from the stem bark and root and screened the cytotoxic activities against A549 and HT-29 cells respectively [12, 13].

The leaves of *M. calabura* have potential antibacterial activity [14], free radical scavenging activity [15], antinociceptive, antipyretic, anti-inflammatory[(16] andantistaphylococcal activity [17]. It was able to internal transport of Hg, it reduces the soil pollution [18] and the fruits can be used as carbon source for glutamic acids [19].
In view of the above facts, in the present investigation, we have carried out the phytochemical analysis and antioxidant activity of extracts of *M. calabura* leaves.

MATERIALS AND METHODS

Instruments and Chemicals

UV-160A spectrophotometer (Shimadzu Corporation, Japan) was used for the measurement of absorbance of solution mixtures. Ferrozine, Ferrous chloride, Ascorbic acid, Foline-Coline reagent, Aluminum chloride and Sodium carbonate were obtained from HiMedia chemicals, Mumbai, India. Catechin, Ethylenediaminetetraaceticacid (EDTA),1,1-diphenyl-2-picrylhydrazyl (DPPH) and Streptozotocin were purchased from Sigma Chemical Co, Bangalore, India. Nicotinamide was purchased from Ranbaxy Chemicals Ltd., Mumbai,India. Glucometer and Glucometer strips for measurement of fasting blood glucose were purchased from a local vendor, manufactured by Accu-check Advantage, Roche diagnostics Mannheim, Germany. All other chemicals and solvents used were of analytical grade.

Plant material and extracts preparation

The young leaves of *M. calabura* were collected from the Kuvempu University campus during the month of December. Plant was identified by faculty of Department of Applied Botany, Kuvempu University, Shankaraghatta and the specimen voucher was kept in our laboratory for further reference. 500 gm of leaves powder was sequentially extracted with pet. ether, chloroform, double distilled ethanol and water in soxhlet apparatus and extracts were referred as *M. calabura* pet ether extract (MCPE), *M. calabura* chloroform extract (MCCE), *M. calabura* ethanolic extract (MCEE) and *M. calabura* aqueous extract (MCAE) respectively. The extracts were filtered and concentrated *in vacuo* using rotary flash evaporator (Buchi Rotavapor R-200) and obtained crude extract was stored in refrigerator until further studies. The yield and physical appearance of extracts were tabulated in Table 1.

Table 1: Yield and physical appearance of extracts

Extracts	Yield (Gm.)	Color	Physical appearance
MCPE	3.1	Green	pasty
MCCE	2.5	Dark	pasty
MCEE	10	Green	pasty
MCAE	4.6	Brown	Amorphous

Estimation of inorganic constituents in plant material

The inorganic components in *M. calabura* leaves were estimated by the method described by Narendhirakannan et, al. [20]. Briefly, the shade dried and pulverized plant material was placed overnight in a vitreosil crucible in an electric muffle furnace maintained at temperature between 400 to 420˚C, because loss of zinc might occur at >450˚C and potassium loss might occur if the temperature is too high (>480°C). Ashing will destroy all the organic materials present in the samples. The crucible containing pure ash was then taken out of the muffle furnace and kept in a desiccator. Then, 5 gm of the ash was digested with the triple acid mixture of nitric acid: sulfuric acid: perchloric acid (11: 6: 3). The digested sample was dissolved in 100 ml distilled water, filtered through Whatman filter paper and the filtrate was used for the assay of trace elements by atomic absorption spectrophotometer (AAS-Varian 200AA) using suitable hollow-cathode lamps.

Qualitative phytochemical screening

All crude extracts were qualitatively tested for the presence of chemical constituents by performing various tests like Mayer's test for Alkaloid, steroids with Salkowaski test, glycosides with Keller-killiani test, carbohydrates with Molisch's test, flavonoids and tannins with ferric chloride solution and saponins with water. These were identified by characteristic color change using standard tests as described by Khanna and Kannabiran and Harborne [21, 22].

Quantitative analysis of crude extracts

Determination of total phenolic content

Total phenolic content in the extracts were estimated by Folin-Ciocalteu method using Gallic acid as a standard [23]. Briefly, 2 ml of each extract at different concentrations (50-150 µl) in ethanol was mixed with 2.5 ml Folin-Ciocalteu reagent (diluted 1:10 v/v) and 2 ml of Na_2CO_3 (7.5 % v/v) solution in the test tube and allowed to stand for 90 min. at room temperature and the absorbance of content in each test tube was measured against the blank at 750 nm using UV spectrophotometer. Total phenolic content of the extract was expressed in terms of µg equivalent to Gallic acid. Estimation was repeated thrice, and the results were averaged.

Determination of total flavonoid content

Total flavonoid content in the extracts was determined according to modified method of Zhishen et al., using catechin as a standard [24]. Briefly, 2 ml of extract at different concentrations (50-150 µg) in ethanol was mixed with 0.3 ml each of 5 % $NaNO_2$ and 10% $AlCl_3$ and incubated at room temperature for 6 min. To this mixture, 2 ml of 1M NaOH was added and the volume in each test tube was made up to 5 ml by adding distilled water. Absorbance was measured against the blank at 510 nm using UV spectrophotometer and total flavonoid content in the extract was expressed in terms of µg equivalent to Catechin. Test was repeated thrice and the results were averaged.

***In vitro* antioxidant activities**

Total antioxidant capacity

Total antioxidant capacity of extract was determined as described by Prieto et al., [25]. Briefly, extracts in 3000 µl of ethanol at different concentrations (20–100 µl) were taken in separate test tubes. To this, 3 ml of reagent mixture containing 4 mM ammonium molybdate, 0.6 M sulfuric acid and 28 mM of sodium phosphate was added. Then, test tubes were kept for incubation at 95˚C for 90 min. and allowed to cool. Absorbance of the content of each test tube was measured at 695 nm against a blank.

Total reductive capability

Total reductive capacity of each extract was determined according to the method of Oyaizu [26]. Briefly, 1 ml of extract solution at different concentrations (20-100 µl) in ethanol was mixed with 2.5 ml, 0.2 mol/L phosphate buffer (pH 6.5) and 2.5 ml 1% potassium ferricyanide solution. Then the mixture was incubated at 50˚C for 20 min. At the end of the incubation, 2.5 ml, 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm

for 10 min. The upper layer of solution was collected and mixed with 2.5 ml distilled water and 0.5 ml, 0.1% ferric chloride solution. The absorbance was measured at 700 nm against a blank.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

All the extracts were screened for free radical scavenging activity by DPPH method of Braca et al., [27]. Extracts in methanol at different concentrations (20-100 µg/ ml) were added to each test tubes and volume was made up to 4 ml using methanol. To this, 3 ml of 0.004% DPPH in methanol was added and the mixtures were incubated at room temperature under dark condition for 30 min. The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm and radical scavenging activity was calculated using the formula:

% of radical scavenging activity = ([($A_{control}$ - A_{test})/$A_{control}$]) × 100

Where $A_{control}$ is the absorbance of the control sample (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution + test compound). The DPPH radical scavenging activity of butylated hydroxyl anisole (BHA) was also assayed for comparison. Test was performed in triplicate, and the results were averaged.

Metal chelating activity

The chelating ability of ferrous ions by the extract and standard (EDTA) was estimated by the method of Dinis et al., [28]. Briefly, 3ml of extract solution at different concentrations (20-100 µg/ml) was taken and 0.05 ml of 2 mM $FeCl_2$ was added to it. The reaction was initiated by adding 0.2 ml, 5 mM ferrozine and the content was mixed vigorously and incubated at room temperature for 10 min. Absorbance of the solution was measured at 562 nm. The control contains $FeCl_2$ and ferrozine and complex formation molecule. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated by using the formula:

% of inhibition = ([($A_{control}$ - A_{test})/$A_{control}$]) × 100

Where $A_{control}$ is the absorbance of the control sample and A_{test} is the absorbance of the test sample. Test was performed in triplicate, and the results were averaged.

Oral glucose tolerance test in normal rats

Overnight fasted normal rats were divided into eleven groups of six rats each. They were orally administered with vehicle, MCCE, MCEE and MCAE (250, 500 and 1000 mg/kg) and glibenclamide (60mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extract [29]. Blood was withdrawn through the tail vein at 0, 30, 60 and 120 min of glucose administration.

Antidiabetic assay

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

Diabetes mellitus was induced in overnight fasted adult Wistar strain albino male rats by a single intraperitoneal injection of freshly prepared STZ (60mg/kg b. wt) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 ml/kg b.wt.After15 min the intraperitoneal injection administration of 120 mg/kg of nicotinamide was dissolved in normal saline [30]. Diabetes was confirmed by the elevated glucose levels in plasma, determined at 72 h. Rats with a fasting plasma glucose range of 280–350 mg/dl were considered diabetic and used for the study.

Experimental design

Animals were divided into twelve groups of six rats each. Group I: normal control rats administered gum acacia (2%) daily for 14 days; Group II: diabetic control rats administered gum acacia (2%) daily for 14 days; Group III: diabetic rats administered MCCE (250 mg/kg); Group IV: diabetic rats administered MCCE (500 mg/kg); Group V: diabetic rats administered MCCE (1000 mg/kg); Group VI: diabetic rats administered MCEE (250 mg/kg); Group VII: diabetic rats administered MCEE (500 mg/kg); group VIII: diabetic rats administered MCEE (1000 mg/kg); group IX: diabetic rats administered MCAE (250 mg/kg); group X: diabetic rats administered MCAE (500 mg/kg); group XI: diabetic rats administered MCAE (1000 mg/kg) ;group XII: diabetic rats administered glibenclamide (0.25 mg/kg).The extract and drug was administered for 14 days. The effects of administration of extracts to normal and diabetic rats were determined by measuring fasting plasma glucose levels by enzymatic glucose oxidase method using a commercial glucometer. Fasting plasma glucose was estimated on days 0, 1, 3, 5, 7, and 14 of extract administration. The change in the body weight at initial and final was noted.

STATISTICAL ANALYSIS

Data was statistically expressed as mean (SEM, Statistical evaluation was done using one-way analysis of variance (ANOVA)), P<0.05. Difference was considered to be significant at P<0.05.

RESULTS AND DISCUSSION

Most of the organisms are well protected against free radical damage by enzymes such as superoxide dismutase, catalase and by natural antioxidant compounds like ascorbic acid, tocopherols and glutathione [31]. In our body, the cooperative defense system protects the body from free radical damage that includes antioxidant nutrients and enzymes. Hence the antioxidant compounds present in food is an important health protecting factor because of their presumed safety, potential nutritional and therapeutic effects [32, 33].

The inorganic component analysis in the sample indicated the presence of Cd, Mn, Cu, Ni, Pb, Zn, K and Na. Table 2, represents the concentration of elements found in plant material. These elements play a pivotal role in secondary metabolites formation and which in turn responsible for pharmacological actions of medicinal plants.

Table 2: Concentration of Inorganic constituents in leaves

Sl. No	Metal	Wave length (nm)	Concentration (ppm)
1	Cadmium	228.8	1.233
2	Manganese	279.5	51.327
3	Cupper	240.7	478.99
4	Nickel	232.0	11.24
5	Lead	217.0	0.737
6	Zinc	213.9	18.940
7	Sodium	589.0	55.35
8	Potassium	766.5	228.70

Phytochemical analyses of all fractions were tabulated in the Table 3. The preliminary phytochemical screening of crude extracts indicated the presence of flavonoids, glycosides, saponins, and tannins in the crude extracts and these active components present in the plant extracts may be responsible for their biological activities.

The phenolics, tannins and flavones constitute one of the major groups of compound acting as primary antioxidants. Therefore, it is worthwhile to determine their total amount present in the extracts. The concentration of phenolic and flavonoid contents in the extracts, expressed as µg GAE/mg sample and µg Catechin /mg sample, has been shown in Table 4. The antioxidant properties of flavonoids are due to the chelating of metal ions, such as iron and copper. It has been recognized that, phenols and flavonoids showed antioxidant activity and their effects on human nutrition and health are considerable.

Table 3: Preliminary phytochemical screening of *M. calabura* leaves extracts.

Test	MCPE	MCCE	MCEE	MCAE
Alkaloids	-	-	+	-
Steroids	-	+	+	+
Flavonoids	-	+	+	+
Glycosides	-	-	-	+
Phenolics	+	+	+	+
Quinones	-	-	+	-
Saponins	-	-	+	+
Terpenoid	-	-	+	+

+ = Present, - = Absent

Total antioxidant capacity was determined by phosphomolybdenum assay, based on the reduction of metal Mo (VI) to Mo (V) by the extracts and subsequent formation of green phosphate at acidic pH. It is difficult to assign an order of antioxidant capacities to the extracts because different extracts exhibited various degrees of antioxidant capacity. Among all extracts, MCEE showed significant and MCCE and MCAE extract showed moderate antioxidant activity while MCPE shown minimal activity (Fig. 1).

Table 4: Qualitative phytochemical analysis of *M. calabura* leaves extracts

Extracts	Total phenolic (µg/mg of extract)	Total Flavonoid (µg/mg of extract)
MCPE	15.83±0.12	17.936±0.89
MCCE	22.16±2.3	47.086±0.68
MCEE	33.33±0.13	123.316±0.54
MCAE	25.00±2.52	44.833±0.21

Each value represents mean±SE, Where, n= 3.

Compounds with reducing power indicated that they are electron donors and can capable to reduce oxidized intermediates. Reductive assay based on the transformation of Fe (III) to Fe (II) in presence of samples. The reduction is based on the terminating the reaction catalyzed by free radicals either by donating hydrogen atom or reacting with certain precursors of peroxides to prevent peroxide formation. The polyphenolic compounds react with free radical by donating its electrons and convert them into more stable products. Reducing ability of all extracts compared with standard Quercetin (Fig.2).

Among all extracts, MCEE showed comparable activity as that of standard used, MCAE, shown significant reducing power and MCCE and MCPE showed temperate reducing capacity. The linear reducing power of MCEE may be due to the presence of high content of phenolic and flavanoid compounds. The reducing capacities of extracts serve as a significant indicator of its potential antioxidant activity.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay is widely used as an index to evaluate the antioxidant potential of plants. The DPPH assay is based on the ability of DPPH, a stable free radical, to delocalize in presence of antioxidants [34]. When it accept an electron donated by antioxidant compound, the electrons delocalized, which can be quantitatively measured from the absorbance change. The extracts exhibited a significant dose dependent inhibition of DPPH activity, and found to have considerable free radical scavenging activity as indicated by their IC_{50} values: 496.18±4.56 µg/mL for MCPE, 107.99±6.24 µg/mL for MCCE, 79.96±0.91 µg/mL for MCEE and 97.638± 2.06µg/mL for MCAE in comparison to ascorbic acid 40.43±3.95µg/mL (Fig.3). The mean values of MCEE were found to be significant and indicated that it had more radical scavenging ability than other fractions. The high contents of the phenolic compound in the MCEE might be explained its radical scavenging activity.

Table 5: IC_{50} values of extracts and standards

Extract	IC_{50} values of DPPH radical scavenging activity (µg/mL)	IC_{50} values of Metal chelating activity µg/mL
MCPE	496.18±4.56	247.92±1.56
MCCE	107.99±6.24	122.25±0.75
MCEE	79.96±0.91	85.61±4.42
MCAE	97.68± 2.06	96.88±3.01
AA	40.43±3.95	-
EDTA	-	35.27±3.56

The chelation of Fe^{2+} ions was estimated based on, formation of complex between ferrozine and Fe^{2+} quantitatively. The formation of complex is distressed if any chelating agents present in the solution and color of solution changed. The IC_{50} value of extracts found to be 247.92±1.56 µg/mL, 122.25±0.75 µg/mL, 85.61±4.42 µg/mL and 96.88±3.01 µg/mL for MCPE, MCCE, MCEE and MCAE respectively, while the standered EDTA has 35.27±3.56 µg/mL. Among different extracts, MCEE shown the highest ferrous ion chelating ability followed by MCAE, MCCE and MCPE (Fig. 4).

Table 6 shows the oral glucose tolerance levels of *M. calabura* leaves extracts. After glucose load, the administration of 250, 500 and 1000 mg/kg of extracts decreased the elevation of serum glucose level significantly ($P \leq 0.005$) at 120 min.

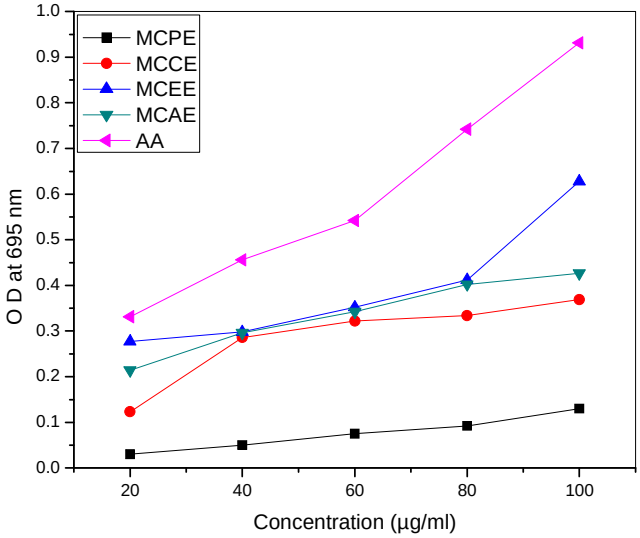

Figure 1: Total Antioxidant activity of *M. calabura* extracts

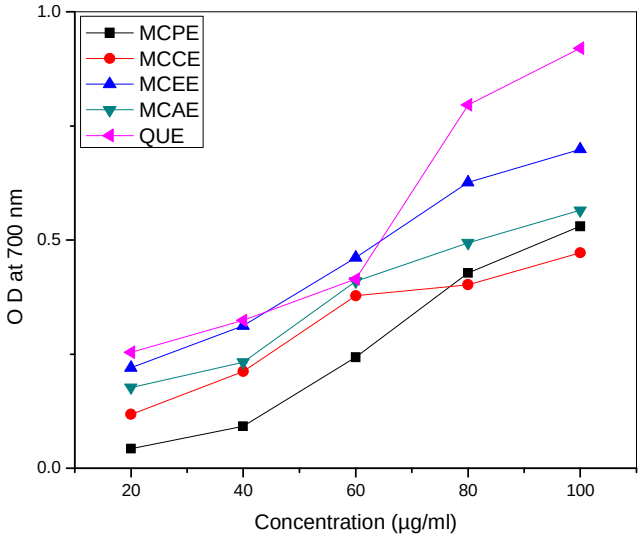

Figure 2: Reductive Capability of *M. calabura* extracts

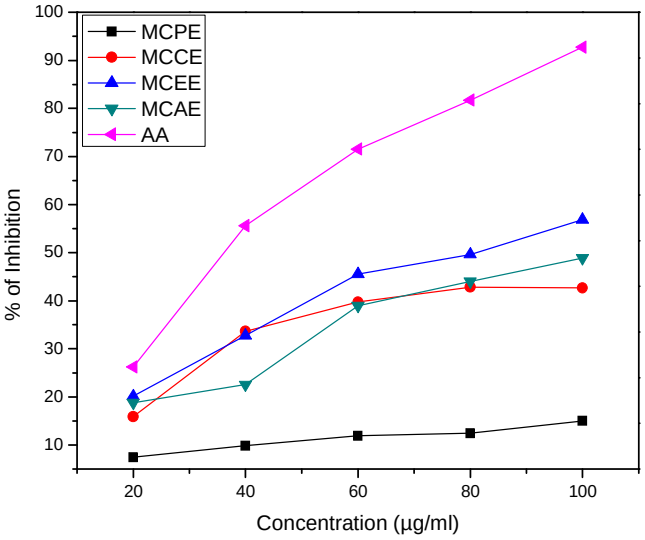

Figure 3: DPPH radical scavenging activity of *M. calabura* extracts

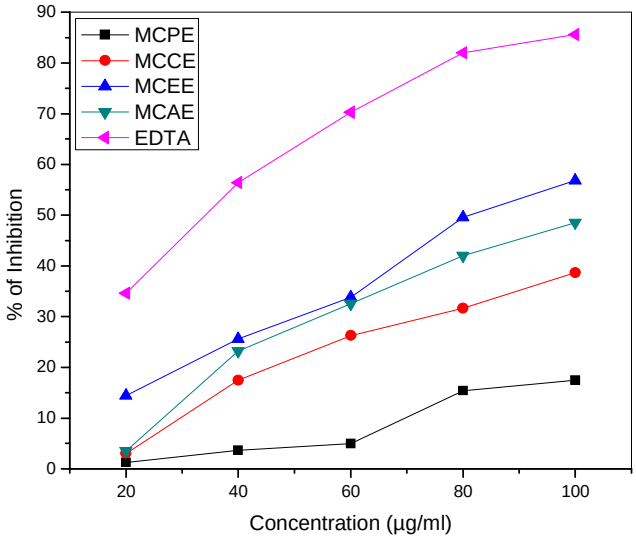

Figure 4: Metal chelating activity of *M. calabura* extracts

Table 6: Effect of *M. calabura* bark extracts on oral glucose tolerance.

Group	Blood glucose levels (mg/dl)			
	0min	30 min	60 min	120 min
Normal control	99.87 ± 1.05	119.30 ± 3.02	116.07 ± 2.05	101.71 ± 1.21
MCCE (250 mg/kg)	99.80 ± 2.15	118.04 ± 2.63	113.54 ± 0.95	112.94 ± 1.81
MCCE (500 mg/kg)	97.02 ± 2.22	117.05 ± 2.80	115.04 ± 2.15	112.80 ± 2.25
MCCE (1000 mg/kg)	99.32 ± 2.85	116.86 ± 0.92	106.52 ± 1.40	100.86 ± 2.46
MCEE (250 mg/kg)	95.05 ± 2.08	119.75 ± 1.05	118.12 ± 3.09	116.90 ± 1.98
MCEE (500 mg/kg)	96.88 ± 1.04	118.75 ± 2.51	117.20 ± 2.01	105.44 ± 2.21
MCEE (1000 mg/kg)	98.45 ± 1.85	123.03 ± 0.81	113.28 ± 1.56	99.05 ± 1.75
MCAE (250 mg/kg)	100.8 ± 0.15	118.64 ± 1.73	113.04 ± 2.75	112.02 ± 1.86
MCAE (500 mg/kg)	97.25 ± 1.71	117.32 ± 1.86	115.70 ± 2.45	112.34 ± 2.91
MCAE (1000 mg/kg)	99.13 ± 2.70	116.11 ± 2.08	106.35 ± 1.36	100.41 ± 2.34
Glibenclamide (60mg/kg)	96.62 ±2.44	115.34 ± 2.66	99.03 ± 1.26	90.09 ± 2.06

The obtained results clearly indicated that the blood glucose level in diabetic control group increased significantly by comparing with normal group, which indicate that the diabetic animal mode was successfully established. In STZ-Nicotinamide induced diabetic rats, the body weight was significantly decreased. 14th days of treatment with extracts and standard drug, the body weight was significantly increased compared to the initial days. All the three doses showed significant improvement in body weight compared to diabetic control (Table 7). Extracts administered at three different doses of 250mg/kg, 500mg/kg, 1000mg/kg to STZ-Nicotinamide treated diabetic rats caused significant (P<0.001) reduction of blood glucose levels which was related to dose and duration of treatment (Table 8). The MCEE showed promising activity while MCAE and MCCE showed moderate activity by comparing standard.

Table 7: Effect of extracts of *M. calabura* leaves on body weight in STZ-Nicotinamide induced diabetic rats.

Group (n=6)	Treatment	Body weight	
		0th day	14th day
I	Normal control	195.01 ± 4.08	224.09 ± 9.94
II	Diabetic control	190.56 ± 9.45^{c}	168.13 ± 15.83^{c}
III	Diabetic + MCCE (250mg/kg)	204.01 ± 3.42	216.60 ± 11.08
IV	Diabetic + MCCE (500mg/kg)	198.01 ± 5.12	231.45 ± 14.01
V	Diabetic + MCCE (1000mg/kg)	203.03 ± 5.50	234.17 ± 10.76
VI	Diabetic + MCEE (250mg/kg)	199.33 ± 9.78	222.32 ± 20.45$^{a\,b\,c}$
VII	Diabetic + MCEE (500mg/kg)	192.02 ± 7.05	232.88 ± 2.77
VIII	Diabetic + MCEE (1000mg/kg)	203.78 ± 16.22^{a}	243.85 ± 4.55$^{a\,b\,c}$
XI	Diabetic + MCAE (250mg/kg)	195.00 ± 2.98	217.04 ± 6.01
X	Diabetic + MCAE (500mg/kg)	187.11 ± 14.52	228.06 ± 9.01
XI	Diabetic + MCAE (1000mg/kg)	199.88 ± 12.44$^{a\,b}$	240.77 ± 12.05$^{a\,b\,c}$
XII	Diabetic + glibenclamide (0.25 mg/kg)	195.65 ±13.73$^{a\,b}$	216.88 ± 16.45 $^{a\,b}$

Values are mean±SEM of 6 animals in each group. a: P<0.05 comparing with the normal; b: P<0.05 comparing with diabetic control: c: P<0.05 comparing with glibenclamide treated group.

Table 8: Effect of extracts of *M. calabura* leaves on fasting blood glucose levels (mg/dl) of normal and diabetic rats.

Group n=6	Treatment	Fasting plasma glucose concentration (mg/dl)					
		0th day	1st day	3^{ed} day	5th day	7th day	14th day
I	Normal control	83.50 ± 2.11	101.45 ±2.10	95.03 ± 3.07	82.42 ± 3.78	98.16 ± 4.21	90.01 ± 4.77
II	Diabetic control	345.64 ± 1.22^{ac}	360.94 ±10.27$^{a\,c}$	404.78 ±3.88^{ac}	390.18 ±13.08^{ac}	373.05 ±15.24$^{a\,c}$	382.43 ± 8.90^{ac}
III	Diabetic + MCCE (250mg/k)	310.42±11.47	363.66±10.02	342.12±4.14	385.44±9.45	327.46±7.46	386.78±12.30
IV	Diabetic + MCCE (500mg/k)	310.42±12.7	343.34±18.68	362.39±18.48	375.71±11.15	387.42±45.07	356.13±21.02
V	Diabetic + MCCE (1000mg/kg)	320.45±45.43	333.42±35.04	312.46±9.34	305.15±17.78^{a}	321.56±20.75	300.8±18.2^{abc}
VI	Diabetic + MCEE (250mg/k)	310.0±23.45	318.0±08.45	320.0±11.31	353.0±52.49	347.0±12.04	301.0±36.65^{ac}
VII	Diabetic + MCEE (500mg/k)	330.0±11.05	322.0±27.04^{ab}	300.0±16.02	285.0±15.38	297.0±15.64^{a}	261.0±13.03^{ac}
VIII	Diabetic + MCEE (1000mg/kg)	345.00±14.01^{a}	311.0±19.66	335.0±17.07	335.0±15.30^{a}	279.0±12.03^{a}	225.0±13.84^{abc}
IX	Diabetic + MCAE (250mg/k)	320.0±19.22	367.0±23.26	391.0±15.51	408.0±25.49	428.0±29.02	435.0±28.35^{ac}
X	Diabetic + MCAE (500mg/k)	320.0±21.55	344.0±14.85^{ab}	321.0±16.22	297.0±23.50	312.0±16.44^{a}	307.0±13.23^{ac}
XI	Diabetic + MCAE (1000mg/kg)	331.00±11.51^{a}	311.0±15.46	289.0±19.40	279.0±19.40^{a}	264.0±13.93^{a}	245.0±14.84^{abc}
XII	Diabetic + glibenclamide (0.25 mg/kg)	251.72 ± 8.07^{ab}	201.02±14.11	187.38±9.09	173.45 ± 5.62	99.03 ± 7.86$^{a\,b}$	102.87 ± 6.20^{ab}

Values are mean±SEM of 6 animals in each group. a: P<0.05 comparing with the normal; b: P<0.05 comparing with diabetic control: c: P<0.05 comparing with glibenclamide treated group.

CONCLUSION

Variations in antioxidant capacity of different extracts may be attributed to differences in their chemical composition such as phenolics, flavonoid and other phytoconstituents and also the structure of the antioxidants present in them.

From the present study it is clear that, all the extracts showed an increase in antioxidant capacity with increase in dose of sample. In conclusion, the screening results of antioxidant activity and hyperglycemic activity of *M. calabura* extracts showed that they are endowed with potentially exploitable free radical scavengers and hence it can be considered as promising source of natural antioxidant for medicinal and commercial uses.

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