IJPSR (2020), Volume 11, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 January 2020; received in revised form, 04 March 2020; accepted, 26 March 2020; published 01 April 2020

THERAPEUTIC EFFECT OF *MORINGA OLEIFERA* LEAF EXTRACTS ON OXIDATIVE STRESS AND KEY METABOLIC ENZYMES RELATED TO OBESITY

Ganjayi Muni Swamy and Balaji Meriga *

Department of Biochemistry, Sri Venkateswara University, Tirupati - 517102, Andhra Pradesh, India.

Keywords:

Moringa oleifera, Metabolic enzymes, Metabolic disorders, Obesity

Correspondence to Author:
Dr. M. Balaji

Assistant Professor,
Department of Biochemistry,
Sri Venkateswara University, Tirupati - 517102, Andhra Pradesh, India.

E-mail: balaji.meriga@gmail.com

ABSTRACT: Obesity ailments, commonly known as "New World Syndrome," has drawn the attention of the scientific community to explore novel natural product-based therapeutics as effective alternatives. In this study, different solvent extracts of *Moringa oleifera* (*M. oleifera*; Drumstick tree) leaves were prepared, and their phytochemical analysis was done. The therapeutic effect of *M. oleifera* leaf extracts on key enzymes related to obesity and against oxidative stress was studied through standard assays (DPPH, ABTS, NO, FRAP). Among different solvent extracts, the *M. oleifera* leaf hydroalcoholic extract (MOHE) contained a high amount of polyphenols (135.45 ± 3.435 µg/mL) and flavonoids (129.52 ± 3.102 µg/mL). The IC_{50} values of MOHE for α-glucosidase, α-amylase, and pancreatic lipase were 373.6 µg/mL, 339.8 µg/mL and 437.1 µg/mL respectively which were much lower than rest of the *M. oleifera* extracts. The GC-MS analysis of MOHE showed the presence of many glycosides, oils and polyphenolic compounds, as mentioned in the results section. In conclusion, our data suggest that *M. oleifera* leaves are rich in several bioactive phytochemicals that qualify it as an effective therapeutic alternative to attenuate oxidative stress and obesity ailments.

INTRODUCTION: Excess energy intake and sedentary lifestyle are the primary causes of the growing incidence of obesity and associated ailments, generally referred to as "New world syndrome". New world syndrome in its cluster includes obesity, hypertension, diabetes, cardiovascular diseases (CVDs), *etc*. Overweight and obesity lead to low-grade inflammation, oxidative stress, and certain forms of cancers [1-3]. In spite of the enormous global demand for anti-obesity drugs, there exist very few foods and drug administration (FDA) / European Union (EU) approved medications in the market, and some of them have considerable side effects and hence failed to receive wider public acceptance.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(4).1949-57

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1949-57

Moreover, increased public awareness about the ill effects of synthetic drugs through articles published in local/national newspapers and programs aired in TV channels and social media have also considerably influenced the public thinking and their attitude in favor of Natural product-based medications. Therefore, the scientific and research community is looking to develop low cost and side-effects free drugs/molecules from natural products. In this context, the role of plant-based phyto-chemicals and, more particularly, nutraceutical based products gained significance. Previous studies on functional foods highlighted their beneficial role in mitigating oxidative stress and other metabolic complications [3-5].

Indian and Chinese traditional and folk medicine have emphasized the therapeutic effects of several plants and herbs in curing many human ailments. Decades of scientific research have also validated the medicinal claim of many natural products against several diseases [6]. It is, therefore, reasonable to extend our search to develop

healthier alternatives from such plant species to treat metabolic disorders.

Moringa oleifera, a native of Indian soil, grows in the tropical and subtropical regions of the world. It is commonly known as 'Drumstick tree' or 'Horseradish tree' with its high nutrient values; every part of the tree is suitable for either nutritional or pharmaceutical purpose. The leaves are rich in minerals, vitamins, and other essential phytochemicals [7, 8]. While the drumsticks are widely used to prepare popular recipe "sambar" in India, the leaves of *M. oleifera* are used in south India to make different dishes. Different parts of this plant are reported to have anti-allergic, anti-amyloidogenic, hypolipidemic, antibacterial, antioxidant, anti-inflammatory and antitumor properties [9-15]. In view of the above, the present study was focussed on preparing different solvent extracts of M. oleifera leaves and evaluating their antioxidant efficacy and its role on key metabolic enzymes related to obesity.

MATERIALS AND METHODS:

Chemicals and Reagents: Gries reagent, 2,2-diphenyl-2-picrylhydrazyl radical (DPPH), (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS), ascorbic acid, pancreatic α-amylase, pancreatic lipase, α-Glucosidase, acarbose, 3,5-dinitrosalicylic acid (DNS) color reagent, orlistat, p-NPB (P-nitro phenylbutyrate), isopropyl alcohol, gallic acid, and quercetin were obtained from Sigma. Folin-Ciocalteu's phenol reagent and other chemicals used in this study were of analytical grade.

Plant Material Collection and Extracts Preparation: *Moringa oleifera* leaves were collected from the local market, dried, pulverized, and soaked in maceration chambers with different solvents (Hexane, Ethyl acetate, Hydroalcoholic and Aqueous) at room temperature (RT). Crude solvent extracts of *M. oleifera* were filtered and concentrated in Rota-evaporator (Heidolf). The dark brown sticky material was collected and stored at -20 °C for further experimental purposes.

Determination of Total Phenolic Content: Total phenolic compounds present in *M. oleifera* leaves was estimated by using the Folin-Ciocalteu method [16]. Briefly, the sample solution (0.6 ml) at different concentrations (ranging from 100 to 500 µg/mL) was mixed with 2.58 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 0.3 ml of a saturated sodium carbonate solution was added to the mixture. The reaction mixtures were incubated at room temperature (25 °C) for 20 min, and the absorbance was measured at 760 nm with a spectrophotometer. A dose-response linear regression curve was generated by using the gallic acid standard absorbance, and the concentration of phenolic compounds was expressed as gallic acid equivalents (mg of GAE/gm of extract). The estimation was performed in triplicate, and the results are expressed as mean ± SD.

Determination of Total Flavonoid Content: Total flavonoid content was estimated by the aluminum chloride method [17]. *M. oleifera* extracts (0.5 µl) were mixed with 2.5 ml of distilled water and 150 µl $NaNO_2$ solution (5 %). The contents were vortexed for a few seconds and kept at room temperature for 5 min. Then, 300 µl $AlCl_3$ (10 %), 1 ml NaOH (1 mM), and 550 µl of distilled water were added. The solution was mixed well and kept for 15 min. The absorbance of the sample was measured at 510 nm. The total flavonoid content was calculated using the quercetin standardization curve. The results were expressed as mg of quercetin equivalent (QE) per gram of sample.

Anti-oxidant Activity of *M. oleifera* Leaves:

DPPH Assay: Free radicals scavenging activity of *M. oleifera* extract against 2,2-Diphenyl-1-picryl hydroxyl radical (DPPH) was determined spectrophotometrically at 517 nm according to the procedure of Deshari [18] with some modifications using vitamin C as a standard. Briefly, a 0.3 mM solution of DPPH was prepared in methanol, and 500 µl of this solution was added to 1 ml of the extract (dissolved in 10% DMSO) at different concentrations (100-500 µg/mL). These solutions were mixed and incubated in the dark for 30 min at room temperature. The free radical scavenging activity was calculated using the formula

$$\text{Absorbance} = \text{Control} - \text{Test} \times 100 / \text{Control}$$

Nitric Oxide (NO) Radical Scavenging Activity: Sodium nitroprusside (5 µM) in phosphate buffer (pH 7.4) was incubated with different concentrations (100, and 500 µg/mL) of *M. oleifera* extracts and tubes were incubated at 25 ºC for 120 min. A control experiment was conducted with an

equal amount of solvent in an identical manner. At 5 min intervals, 0.5 ml of incubation solution was taken and diluted with 0.5 ml of Griess reagent (1 % Sulfanilamide, 0.1% N-naphthyl ethylene-diamine dihydrochloride and 2% o-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and resultant N-naphthyl ethylenediamine dihydrochloride was read at 546 nm. Nitric oxide scavenging activity was calculated by the following equation.

Absorbance = Control – Test × 100 / Control

2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Assay: The 2, 2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) assay was carried out according to the methodology described by Re [19], with some modifications. The ABTS radical was formed from the reaction of 2.45 mM potassium persulfate with 7 mM ABTS stock solution, kept in the dark at room temperature for 16 h. For the analysis, the ABTS stock solution was diluted with distilled water until the absorbance of 0.7 at 731 nm was obtained. 50 µl of test compounds (100 to 500 µg/mL) in methanol solution was then homogenized with 3mL of the ABTS radical. The absorbance of the samples was read at 731 nm after 70 min of reaction. Radical scavenging activity (RSA) was calculated as follows

$$RSA\ (\%) = (A_{control} - A_{sample}) \times 100 / A_{control}$$

Where A control was the absorbance of pure ABTS and A sample was the absorbance of ABTS in the presence of samples.

Ferric Reducing Power Assay (Frap): The reducing power of *M. oleifera* extracts was determined according to the method of Deshari [18]. Different concentrations of extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml, 1%), then the mixture was incubated at 50 °C for 20 min, trichloroacetic acid (10%) was slightly added. Different concentrations of *M. oleifera* extracts in 1 ml of distilled water was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) then taken the absorbance at 700 nm. The reference standard was ascorbic acid, and the blank solution contained a phosphate buffer. Results were expressed as µM of ascorbic acid equivalents/g dry weight of defatted samples.

Pancreatic Lipase Inhibitory Assay: The ability of *M. oleifera* to inhibit the pancreatic lipase (PL) was measured using p-NPB (*P*-nitro phenyl-butyrate) as the substrate following the procedure described by Kim [20]. An enzyme buffer was prepared by the addition of 30 µL (10 units) of PL in 10 mM MOPS and [1 Mm EDTA, pH 6.8], 850 µL of Tris-buffer (100 mM Tris HCl and 5 mM $CaCl_2$, pH7.0). 100 µL of the test sample or orlistat was mixed with 880 µl of the enzyme buffer, incubated for 15 min at 37 °C. 20 µL of substrate solution (10 mM p-NPB) was added to the reaction. The enzymatic reaction was allowed to incubate for 15 min at 37 °C. The lipase activity was determined by measuring the hydrolysis of *P*-NPB to *P*-Nitro phenol at 405 nm using an ELISA reader. The inhibition of enzyme activity was calculated as follows

% Lipase inhibition = 1 (Absorbance of sample × 100) / Control

***In-vitro* α-Amylase Assay:** The α -amylase activity was determined according to the method described by Oboh [21] with slight modifications. Briefly, 40 µL of α-Amylase (5 U/mL) was mixed with 0.36 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) and 0.2 µl of extracts or acarbose. After incubation for 20 min at 37 °C, 300 µL of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added, the mixture was re-incubated for 20 min, followed by the addition of 0.2 mL dinitrosalicylic acid (DNS). The contents were mixed well and kept in a boiling water bath for 5 min. The reaction mixture was diluted by adding 6 mL distilled water, and the absorbance was measured at 540 nm in a UV-Visible spectrophotometer. Acarbose was used as a positive control. All assays were carried out in triplicate.

% α - Amylase inhibition = 1 (Absorbance of sample × 100) / Control

***In-vitro* α-glucosidase Assay:** The α-glucosidase inhibitory activity of *M. oleifera* extracts was determined according to the method described by

Ademiluyi and Oboh [21] with slight modifications. Briefly, 250 µL of each extract or acarbose at different concentrations (100-500 µg/mL) was incubated with 500 µL of 1.0 U/mL α-glucosidase solution in 100 mM phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250 µl of p-nitrophenyl D-glucoside solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added, and the mixture was further incubated at 37 °C for 20 min. The absorbance of the released p-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as a percentage of the control without inhibitor. All assays were carried out in triplicate and the calculation was done according to the following formula.

% α - Glucosidase inhibition = 1 (Absorbance of sample × 100) / Control

GC-MS Analysis: *Moringa oleifera* hydro-alcoholic extracts (MOHE) were analyzed using GC-MS (Gas Chromatography-Mass Spectroscopy). The analysis was conducted with a JEOL GC MATE II, GC coupled with a quadruple double-focusing mass analyzer, and a photon multiplier tube detector was used HP 5 ms Column was used (30 m-0.25 mm internal diameter, 0.25 mm film thickness). The ultrapure helium was used as a carrier gas at a flow rate of 1mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250 °C. The initial oven temperature was 50 °C, which was ramped up to 250 °C at a rate of 10 °C/min with a hold time of 3 min. Injections of 1mL sample were made in the splitless mode.

The mass spectrometer was operated in the electron ionization mode at 70 eV with the electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 250 °C, solvent delay 4 min, and scan range 50 to 600 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology library (NIST) Ver.2.1 MS data library and comparing the spectrum obtained through GC–MS compounds present in the plant extracts were identified.

Statistical Analysis: Data are expressed as mean values ± standard deviation (SD). Comparisons between control and test groups were performed with (Tukey's honest significant difference multiple range post hoc test $p < 0.05$ IBM SPSS Ver.23). The experiments were performed in triplicate.

RESULTS AND DISCUSSION:

Total Polyphenols and Flavonoids Content: The results of total polyphenols (TPC) and flavonoids content (FC) in different solvent extracts of *M. oleifera* are presented in **Table 1**. The content of phenolic and flavonoid compounds extracted from biological substances is dependent on location, soil, season, and extraction conditions [22].

The TPC was determined by the Folin-Ciocalteu method and expressed as milligram gallic acid equivalent (GAE) per 100 g of dry weight. Among different extracts of *M. oleifera* leaves, the hydroalcoholic extract (MOHE) showed the highest yield of polyphenols (135.45 mg/g GAE), as shown in **Table 1**. Also, MOHE had higher flavonoids (129.52 mg/g QE) compared with the rest of the solvent extracts of *M. oleifera*.

TABLE 1: TOTAL PHENOLIC AND FLAVONOID CONTENTS

Sample	Total polyphenols (mg/g GAE)	Total flavonoids (mg/g QE)
Hexane	8.34 ± 0.132^{a}	9.14 ± 0.123^{a}
Ehtylaacetate	14.115 ± 0.21^{b}	13.152^{b}
Hydroalcoholic	135.45 ± 3.435^{c}	129.52 ± 3.102^{c}
Aqueous	98.34 ± 2.135^{d}	89.241 ± 8.102^{d}

Data are presented as mean ± SD of triplicate determinations. Different superscript letters within a column for a given parameter are significantly different from each other ($p<0.05$). GAE = gallic acid equivalent; QE = quercetin equivalent.

Evaluation of Antioxidant Capacity of *M. oleifera* Leaves: Therapeutic agents derived from plants/nutraceuticals have been used as new leads to treat metabolic disorders without any adverse effect due to the presence of a diversity of secondary metabolites [23].

According to Cai [24], there is a correlation between polyphenolic, flavonoid content, and antioxidant capacities in medicinal plants since medicinal plants are generally a rich source of antioxidant compounds [25].

In the present study, we evaluated the antioxidant capacity of *M. oleifera* by using different methods like DPPH, ABT, Nitric oxide (NO), Ferric reducing antioxidant power (FRAP) assays with different solvent extracts of *M. oleifera* **Table 2**.

TABLE 2: IC_{50} VALUES OF *M. OLEIFERA* LEAF EXTRACTS ON *IN-VITRO* ANTIOXIDANTS

Sample	IC_{50} Values µg/mL			
	DPPH	ABTS	O-2	NO
Hexane	1243.897[a]	811.525[c]	1634.931[b]	1019.273[a]
Ehtyl acetate	431.875[d]	648.7977[c]	1037.970[a]	862.109[c]
Hydroalcoholic	249.149[e]	235.862[e]	198.657[e]	244.4462[e]
Aqueous	424.422[d]	455.9[d]	594.966[c]	383.913[d]
Ascorbic acid	249.402[e]	-	145.913[f]	154.540[f]
BHT	-	246.617[e]	-	-

Data are presented as mean ± SD, N=3. [a-f] Different superscript letters within a column for a given parameter are significantly different from each other ($p < 0.05$)

Effect of *M. oleifera* Leaf Extracts on DPPH Radicals: *In-vitro* DPPH assay is extensively used in evaluating the antioxidant activity of various components. The results of DPPH Free radical scavenging activity by different solvent extracts are presented in **Fig. 1**. MOHE showed significantly higher ($p<0.05$) inhibitory activity when compared with other solvent extracts in a dose depended on manner. Maximum antioxidant capacity (68.86%) was observed at the concentration of 500 µg/mL. These results are similar to previous studies by Moyo [26]. The degree of decoloration indicates the scavenging potential of the extract due to hydrogen proton donation.

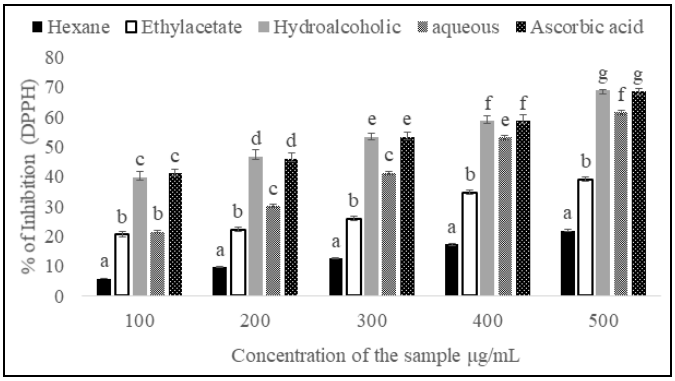

FIG. 1: ANTIOXIDANT ACTIVITY OF *M. OLEIFERA* LEAF EXTRACTS ON DPPH RADICALS. Data are presented as mean ± SD of triplicate determinations. Different superscript above (a-g) on the bars for a given concentration are significantly different from one ($p<0.05$).

ABTS Scavenging Ability: The ABTS scavenging ability of *M. oleifera* extracts is shown in **Fig. 2**. When compared to other solvent extracts, MOHE exhibited the highest scavenging capacity (71.975) in ABTS assay, which is nearer to positive control BHT (79.25 %) at 500 µg/mL concentration. The ABTS assay, which is based on the generation of a blue/green ABTS is applicable to both hydrophilic and lipophilic antioxidant systems. The results indicate that MOHE contains potent phytochemicals that are responsible for its high ABTS scavenging activity [27].

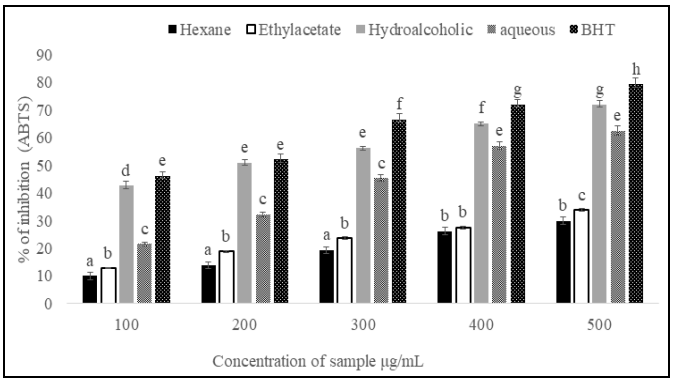

FIG. 2: ABTS SCAVENGING ACTIVITY OF THE LEAVES OF *MORINGA OLEIFERA* L. Data are presented as mean ± SD of triplicate determinations. Different superscript is above (a-g); the bars for a given concentration are significantly different from each other ($p<0.05$).

Nitric Oxide Scavenging Activity: Nitric oxide is a central agent in the physiological and pathological conditions and, when reacts with macromolecules, may induce inflammation, muscle sclerosis, arthritis, ulcerative colitis and other metabolic disorders [28]. The effect of *M. oleifera* leaf extracts against nitric oxide radicals was evaluated in this study. MOHE considerably reduced the release of nitric oxide radicals in a dose-dependent manner, and the maximum inhibition (79.99%) was noted at 500 µg/mL concentration **Fig. 3**.

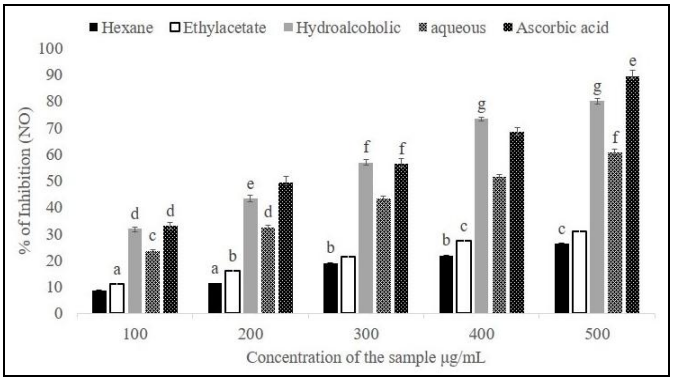

FIG. 3: NITRIC OXIDE SCAVENGING ACTIVITY OF *MORINGA OLEIFERA* LEAVES. Data are presented as mean ± SD of triplicate determinations. Different superscripts (a-g) above the bars are significantly different from each other for a given concentration (Tukey's-honest significant difference multiple range post hoc test $p<0.05$ IBM SPSS ver. 23).

FRAP Assay: The results of the Ferric reducing power assay of *M. oleifera* leaf extracts showed increased efficiency with their increasing

concentration. When compared to other solvent extracts, MOHE showed better activity, and it is nearer to ascorbic acid, the standard **Fig. 4**. It is more relevant to determine the reducing power of polyphenolic constituents to find the relationship between their antioxidant capacity and their reducing power [25]. These findings are in agreement with Moyo [25], who showed that antioxidant properties were concomitant with the development of reducing power. In the present context, phenolic compounds (Rutin, Quercitin, Kameferol, chlorogenic acid, astragalin) present in *M. oleifera* leaf extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to a stable product [7].

Indeed, these kinds of natural compounds are endowed with strong antiradical activity due to their characteristic conjugated ring structure and their hydrogen donor ability, conferred by the presence of hydroxyl groups.

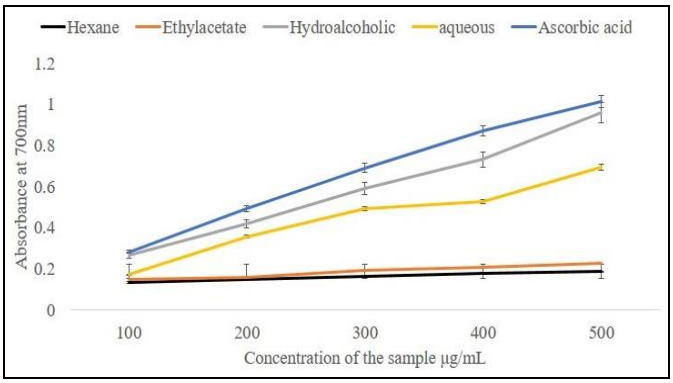

FIG. 4: FRAP ASSAY OF THE LEAVES OF *MORINGA OLEIFERA* L. Data are presented as mean ± SD of triplicate determinations. Different superscripts (a-g) above the bars are significantly different from each other ($p<0.05$).

Effect of *M. oleifera* Extracts on Pancreatic Lipase Activity: Further, we evaluated the inhibitory effect of *M. oleifera* extracts on lipase activity. In the digestive system, pancreatic lipase is the most active enzyme in the digestion of dietary fats and empowers absorption across the intestine. Control of pancreatic lipase is one of the significant marks to combat obesity and other metabolic disorders [29]. Pancreatic lipase (PL) inhibitory activity is depicted in **Fig. 5A**. MOHE has shown potent anti-lipase activity in a dose-dependent manner, which is higher than other solvent extracts. Maximum inhibition of PL (64.34%) was recorded at 500 µg/mL when compare with orlistat (62.19%). Toma [30] in their studies reported the potential benefits of sitosterol derived from a species of Moringacea family against hormone-sensitive lipase, adipogenesis, and lipolysis mechanism in rat adipocytes.

TABLE 3: IC_{50} VALUES OF DIFFERENT SOLVENT EXTRACT OF *M. OLEIFERA* ON METABOLIC ENZYMES

Sample	IC_{50} Values µg/mL		
	Lipase	α-Amyalse	α-Glucosidase
Hexane	1104.3249	1386.8381	843.9375
Ehtyl acetate	606.3736	546.7447[a]	697.7735[a]
Hydroalcoholic	437.153[a]	339.8526	373.6093b
Aqueous	825	486.609[a]	513.6986[a]
Orlistat	584.003[a]	-	-
Acarbose	-	252.196[e]	322.934[b]

Data are presented as mean ± SD, n = 3. Different superscript (a-b) letters within a column for a given parameter are significantly different from each other ($p<0.05$).

Effect of *M. oleifera* leaf Extracts on α-Amylase and α-Glucosidase Activity: Elevation of blood sugar causes insulin resistance and diabetes, a metabolic disorder, which leads to atherosclerosis, hypertension, other cardiovascular diseases and obesity [31]. A systematic approach for controlling diabetes can be regulating post-prandial plasma glucose rise through inhibition of carbohydrate-hydrolyzing enzymes (α-amylase, α-glucosidase) in the digestive tract [32]. Among different solvent extracts of *M. oleifera* leaves, the MOHE showed the highest inhibition in a dose depended manner. **Fig. 5B** explains the pancreatic α-amylase inhibitory activity of MOHE. The maximum inhibition of α-amylase (90.43%) was recorded at 500 µg/mL of MOHE, which is higher than standard drug acarbose (86.47%).

Fig. 5C explains the inhibitory activity of *M. oleifera* leaf extracts against the α-glucosidase activity. Among different extracts, the hydroalcoholic extract was found to be a potential inhibitor against α-glucosidase when compared to other extracts. The maximum inhibition of 83.14% was recorded with MOHE at 500 µg/mL, which is higher than the standard drug acarbose (76.149%). **Table 3** show the IC50 values of metabolic enxymes. The obtained results are similar to the observation made by Adejoh [33]. Adisakwattana and Chanathong [34], reported that tea polyphenols might act as anti-nutritional factors on digestive enzymes. These properties of medicinal plants may be considered in alternative adjuvant therapy with other oral hypoglycemic drugs for treating metabolic disorders.

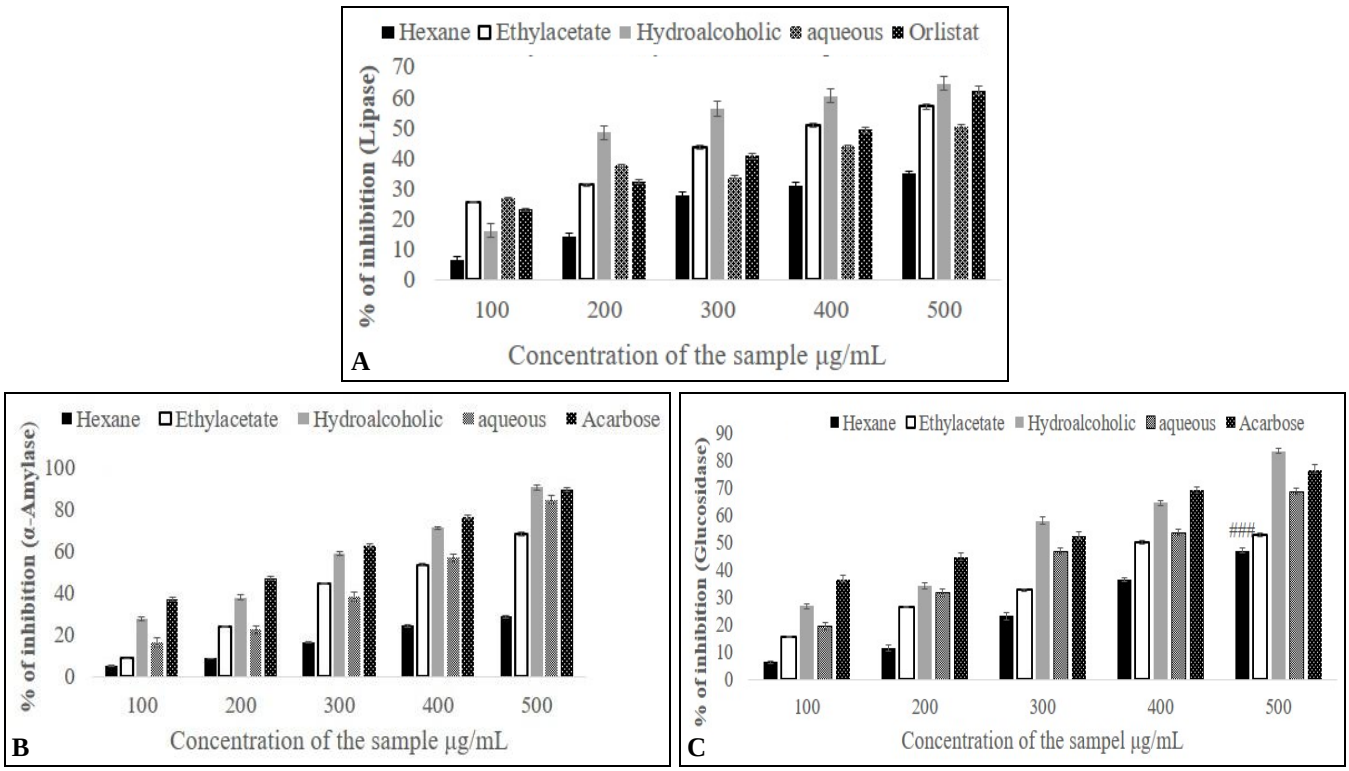

FIG. 5: *IN-VITRO* METABOLIC ENZYME INHIBITION ACTIVITY OF *MORINGA OLEIFERA* LEAVES. Data is presented as mean ± SD, N=3. A. Pancreatic lipase, B. α- Amylase, C. α-Glucosidase. The bars are significantly different from each other ($p<0.05$).

GC/MS Analysis: GC-MS analysis of *Moringa oleifera* hydroalcoholic extract shows the presence of several bioactive compounds, as represented in **Fig. 6a** & **b**.

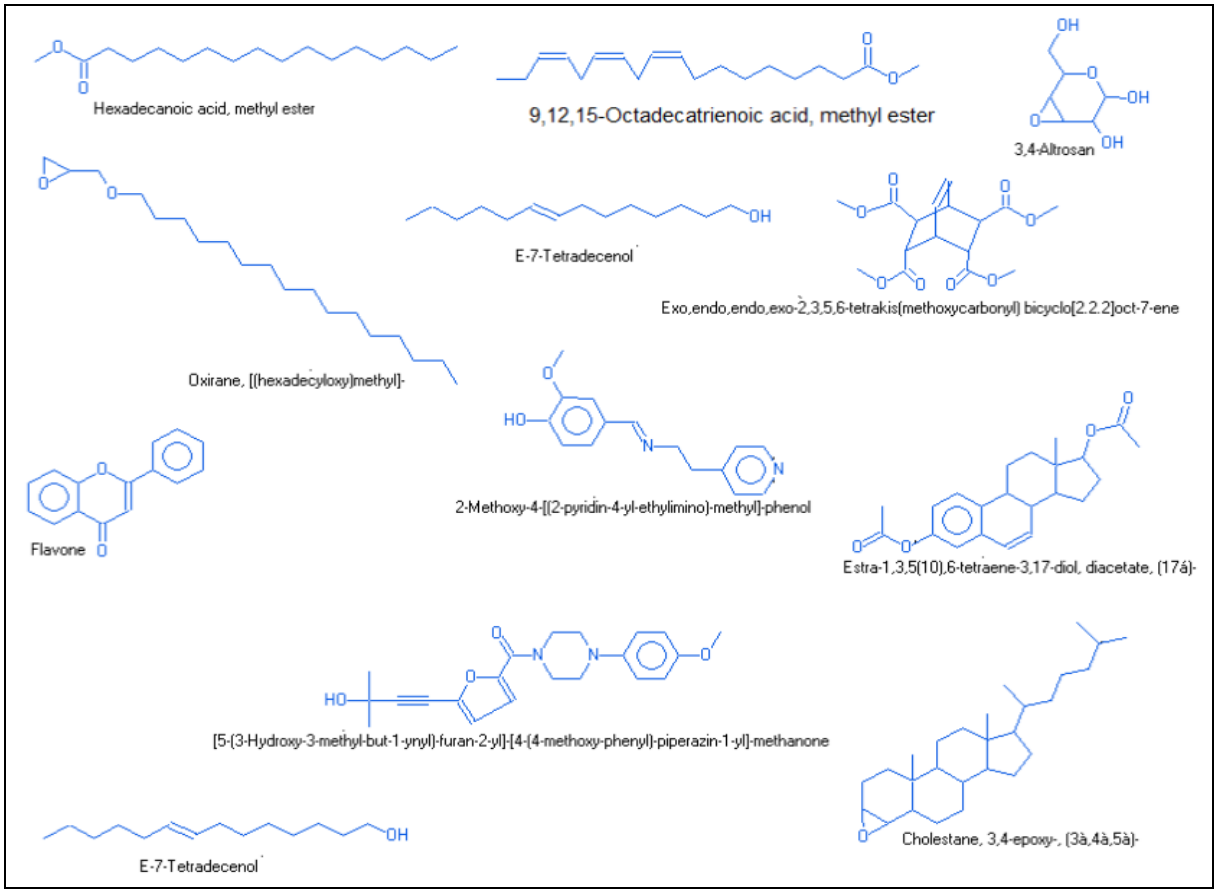

FIG. 6A: CHEMICAL STRUCTURES OF COMPOUNDS IDENTIFIED IN MOHE BY GC-MS

The compounds include hexamethanoic acid, 9,12,15-octodecatrieonic acid, tetradecanoic acid, 5-hydroxymethylfurfural, malic acid, cis-vaccenic acid, flavone, Palmitoyl chloride, trans phytol, n-hexadecanoic acid, stearic acid, [5,(3-hydroxy-3-methyl-but-1-ynyl)-furan-2-yl-(4-[4-methoxy-phenyl-peragin-1-yl]-methaone, pyran-4-one, cyclohexa-none, and cholestran 3,4-epoxy. The retention times, molecular ion peaks or other prominent peaks (where molecular ion peaks were absent) and percentage area of peaks are presented in **Fig. 6b**.

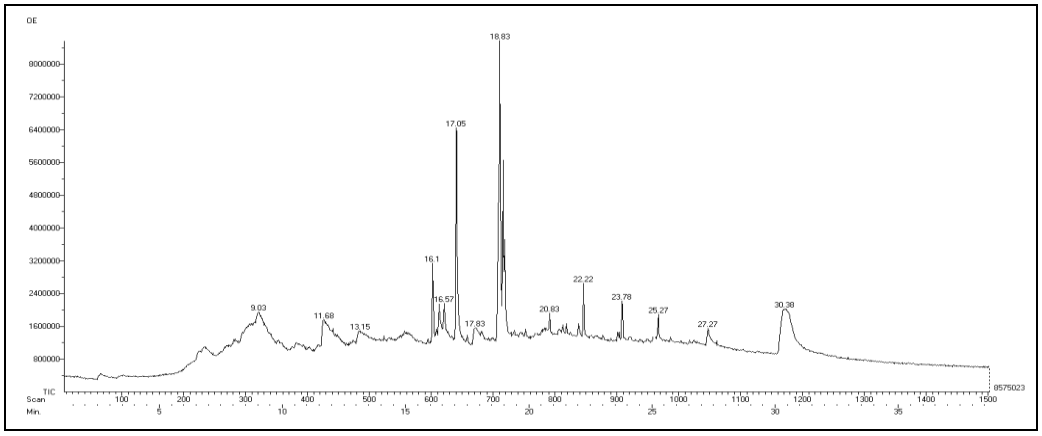

FIG. 6B: GC-MS CHROMATOGRAM OF HYDROALCOHOLIC EXTRACT OF MORINGA OLEIFERA LEAVES

CONCLUSION: The results clearly demonstrate that *Moringa oleifera* leaves have potent antioxidant activity and significantly inhibited pancreatic lipase, α-amylase, and α-glucosidase, the key metabolic enzymes associated with obesity. Hence, *M. oleifera* leaves may be considered as an accessible nutraceutical source for attenuating obesity and associated metabolic disorders and adds scientific validation to its use in folk medicine.

ACKNOWLEDGEMENT: Authors acknowledge the financial support from the DST-SERB (EEQ/2016/000123) and thank the DST-FIST Dept. of Biochemistry and DST-PURSE, S.V. University, Tirupati for their support and SAIF, IIT Chennai for GC-MS analysis.

CONFLICTS OF INTEREST: No conflict of interest among the authors.

REFERENCES:

1. Calle EE, Rodriguez C, Walker-Thurmond K and Thun MJ: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. New England Journal of Medicine 2003; 348(17): 1625-38.
2. Marie NG, Fleming T, Robinson M, Thomson B, Graetz N, Margono C and Abraham JP: Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet 2014; 384(9945): 766-81.
3. Balaji M, Ganjayi MS, Kumar GEH, Parim BN, Mopuri R and Dasari S: A review on possible therapeutic targets to contain obesity: The role of phytochemicals. Obesity Research & Clinical Practice 2016; 10(4): 363-80.
4. Oboh G, Oyeleye SI, Akintemi OA and Olasehinde TA: *Moringa oleifera* supplemented diet modulates nootropic-related biomolecules in the brain of STZ-induced diabetic rats treated with acarbose. Metabolic Brain Disease 2018; 33(2): 457-66.
5. Shodehinde SA, Oyeleye SI, Olasehinde TA, Adebayo AA, Oboh G and Boligon AA: *Lasianthera africana* leaves inhibits α-amylase α-glucosidase, angiotensin-I converting enzyme activities and Fe $^{2+}$-induced oxidative damage in pancreas and kidney homogenates. Oriental Pharmacy and Experimental Medicine 2017; 17(1): 41-49.
6. Lawal B, Shittu OK, Inje OF, Berinyuy EB and Muhammed H: Potential antioxidants and hepato-protectives from African natural products: a review. Clin Phytosci 2017; 2(23): 1-66.
7. Saini RK, Shetty NP and Giridhar P: Carotenoid content in vegetative and reproductive parts of commercially grown *Moringa oleifera* Lam. cultivars from India by LC–APCI–MS. European Food Research and Technology 2014; 238(6): 971-78.
8. Hekmat S, Morgan K, Soltani M and Gough R: Sensory evaluation of locally-grown fruit purees and inulin fibre on probiotic yogurt in mwanza, Tanzania and the microbial analysis of probiotic yogurt fortified with Moringa oleifera. Journal of Health Population and Nutrition 2015; 33(1): 60.
9. Panda S: Butanolic fraction of *Moringa oleifera* Lam. (Moringaceae) attenuates isoprotrenol-induced cardiac necrosis and oxidative stress in rats: an EPR study. EXCLI Journal 2015; 14: 64.
10. Al-Malki, Abdulrahman L and El Rabey HA: The antidiabetic effect of low doses of *Moringa oleifera* Lam. seeds on streptozotocin-induced diabetes and diabetic nephropathy in male rats. BioMed Research International 2015; 1-15.
11. Wang Y, Gao Y, Ding H, Liu S, Han X, Gui J and Liu D: Subcritical ethanol extraction of flavonoids from *Moringa oleifera* leaf and evaluation of the antioxidant activity. Food Chemistry 2017; 218: 152-58.

12. Waterman C, Silva PR, Tumer TB, Kuhn P, Richard AJ, Wicks S and Raskin I: Isothiocyanate rich *Moringa oleifera* extract reduces weight gain, insulin resistance, and hepatic gluconeogenesis in mice. Molecular Nutrition & Food Research 2015; 59(6): 1013-24.
13. Metwally FM, Rashad HM, Ahmed HH, Mahmoud AA, Raouf ERA and Abdalla AM: Molecular mechanisms of the anti-obesity potential effect of *Moringa oleifera* in the experimental model. Asian Pacific Journal of Tropical Biomedicine 2017; 7(3): 214-21.
14. Jaja-Chimedza A, Graf BL, Simmler C, Kim Y, Kuhn P, Pauli GF and Raskin I: Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (*Moringa oleifera*) seed extract. PloS One 2017; 12(8): e0182658.
15. Sanchez-Munoz MA, Valdez-Solana MA, Campos-Almazan MI, Flores-Herrera O, Esparza-Perusquia M, Olvera-Sanchez S and Sierra-Campos E: Streptozotocin-Induced Adaptive Modification of Mitochondrial Supercomplexes in Liver of Wistar Rats and the Protective Effect of *Moringa oleifera* Lam. Biochemistry Research International 2018; 1-13.
16. Chew YL, Lim YY, Omar, M and Khoo KS: Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Science and Technology 2008; 41(6): 1067-72.
17. Barreira JC, Ferreira IC, Oliveira MBP and Pereira JA: Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chemistry 2008; 107(3): 1106-13.
18. Dehshahri SH, Wink M, Afsharypuor S, Asghari G and Mohagheghzadeh A: Antioxidant activity of methanolic leaf extract of *Moringa peregrina* (Forssk.) Fiori. Research in Pharmaceutical Sciences 2012; 7(2): 111.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 1999; 26(9-10): 1231-37.
20. Kim JH, Kim HJ, Park HW, Youn SH, Choi DY and Shin CS: Development of inhibitors against lipase and α-glucosidase from derivatives of monascus pigment. FEMS Microbiology Letters, 2007; 276(1): 93-98.
21. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle T, Saliu JA and Schwarzenbolz U: Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin-I converting) *in-vitro*. Journal of Functional Foods 2012; 4(2): 450-58.
22. Aslam M, Anwar F, Nadeem R, Rashid U, Kazi TG and Nadeem M: Mineral composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan. Asian Journal of Plant Science 2005; 4(4): 417-21.
23. Yuan Y, Zhang J, Fan J, Clark J, Shen P, Li Y and Zhang C: Microwave assisted extraction of phenolic compounds from four economic brown macroalgae species and evaluation of their antioxidant activities and inhibitory effects on α-amylase, α-glucosidase, pancreatic lipase and tyrosinase. Food Research International 2018; 113: 288-97.
24. Cai Y, Luo Q, Sun M and Corke H: Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 2004; 74(17): 2157-84.
25. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J and Li HB: Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. International Journal of Molecular Sciences 2017; 18(1): 96.
26. Moyo B, Oyedemi S, Masika PJ and Muchenje V: Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. Meat Science 2012; 91(4): 441-47.
27. Sreelatha S and Padma PR: Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods for Human Nutrition 2009; 64(4): 303.
28. Hazra B, Sarkar R, Mandal S, Biswas S and Mandal N: Studies on antioxidant and antiradical activities of *Dolichos biflorus* seed extract. African Journal of Biotechnology 2009; 8: 3927-33.
29. Ganjayi MS, Meriga B, Hari B, Oruganti L, Dasari S and Mopuri R: Poly Phenolic rich fraction of Terminalia paniculata attenuates obesity through inhibition of pancreatic amylase, lipase and 3T3-L1 adipocyte differentiation. Journal of Nutrition & Intermediary Metabolism 2018; 10: 19-25.
30. Toma A: Evaluation of antidiabetic, antihyperlipidemic, and antiglycation effect of *Moringa stenopetala* (Baker f) Cufodontis leaves (Doctoral dissertation, Addis Ababa University). 2016.
31. Kaewnarin K, Suwannarach N, Kumla J and Lumyong S: Phenolic profile of various wild edible mushroom extracts from Thailand and their antioxidant properties, anti-tyrosinase and hyperglycaemic inhibitory activities. Journal of Functional Foods 2016; 27: 352-64.
32. Shim YJ, Doo HK, Ahn SY, Kim YS, Seong JK, Park IS and Min BH: Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. Journal of Ethnopharmacology 2003; 85(2-3): 283-87.
33. Adejoh IP, Chiadikaobi OS, Barnabas AO, Ifeoluwa AO and Muhammed HS: *In-vivo* and *in-vitro* comparative evaluation of the anti-diabetic potentials of the parts of Moringa oleifera tree. European Journal of Biotechnology and Bioscience 2016; 4(1): 14-22.
34. Adisakwattana S and Chanathong B: Alpha-glucosidase inhibitory activity and lipid-lowering mechanisms of *Moringa oleifera* leaf extract. Eur Rev Med Pharmacol Sci 2011; 15(7): 803-08.

How to cite this article:
Swamy GM and Meriga B: Therapeutic effect of *Moringa oleifera* leaf extracts on oxidative stress and key metabolic enzymes related to obesity. Int J Pharm Sci & Res 2020; 11(4): 1949-57. doi: 10.13040/IJPSR.0975-8232.11(4).1949-57.