

# Hydroalcoholic and Alkaloidal Extracts of *Murraya koenigii* (L.) Spreng Augments Glucose Uptake Potential against Insulin Resistance Condition in L6 Myotubes and Inhibits Adipogenesis in 3T3L1 Adipocytes

Parameswari Royapuram Parthasarathy<sup>1</sup>, Janani Murthy<sup>1</sup>, Dinesh Murugan Girija<sup>1</sup>, Srivani Telapolu<sup>1</sup>, Chamundeeswari Duraipandian<sup>2</sup>, Thyagarajan Sadras Panchatcharam<sup>3\*</sup>

Parameswari Royapuram Parthasarathy<sup>1</sup>, Janani Murthy<sup>1</sup>, Dinesh Murugan Girija<sup>1</sup>, Srivani Telapolu<sup>1</sup>, Chamundeeswari Duraipandian<sup>2</sup>, Thyagarajan Sadras Panchatcharam<sup>3\*</sup>

<sup>1</sup>SRMC Centre for Indian Systems of Medicine, Quality assurance and Standardization, Central Research Facility, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, INDIA.  
<sup>2</sup>Department of Pharmacognosy, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, INDIA.  
<sup>3</sup>Central Research Facility, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, INDIA.

## Correspondence

Thyagarajan Sadras Panchatcharam

Central Research Facility, Sri Ramachandra University, Porur, Chennai – 600 116, Tamil Nadu, INDIA.

Phone: 044 – 4592 8665

E-mail: deanresearch@sriramachandra.edu.in

## History

- Submission Date: 06-11-2017;
- Review completed: 22-12-2017;
- Accepted Date: 03-01-2018

DOI : 10.5530/pj.2018.4.104

## Article Available online

<http://www.phcogj.com/v10/i4>

## Copyright

© 2018 Phcog.Net. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



## ABSTRACT

**Background:** *Murraya koenigii*, commonly known as “curry leaves” is native to India. The highly valued part of the plant is the leaves which possess various biological activities. **Objective:** The present study aimed to investigate the antidiabetic effect of *Murraya koenigii* (MK) leaf extracts, of two different solvent ratios. **Materials and methods:** 70% hydroalcoholic and alkaloidal extracts of MK leaves were prepared by cold maceration method. Preliminary phytochemical analysis was carried out for both the extracts. *In vitro* anti diabetic activity was screened by inhibitory action on  $\alpha$  – amylase,  $\alpha$  – glucosidase enzymes. Further, the 70% hydroalcoholic and alkaloidal extracts were assessed for glucose uptake potential, anti - adipogenic property, as well as inhibitory activity on diabetes associated complications. HPTLC quantification of major phytoconstituent was carried out. **Results:** The study showed presence of various phytoconstituents such as, polyphenols, alkaloids, tannins, reducing sugars etc. The 70% hydroalcoholic and alkaloidal extracts of MK leaves exhibited >90% inhibition against carbohydrate metabolising enzymes compared to aqueous and absolute alcohol extracts. Both the extracts showed enhanced glucose uptake in L6 myotubes attenuating the effect of Palmitate induced insulin resistance. Significant inhibition on adipogenesis was exerted by both 70% hydroalcoholic and alkaloidal extracts of MK leaves. Besides, marked inhibition of advanced glycation end products was exhibited by the extracts. HPTLC quantification analysis of the aforementioned extracts showed the presence of major phytoconstituent, Mahanine, in it. **Conclusion:** The results of the present study showed that MK possesses significant antidiabetic property and also exhibited considerable effect in preventing diabetes associated complications. The potent antidiabetic activity of MK could be attributed to the presence of Mahanine, the major active constituent, which is a carbazole alkaloid. **Key words:** Diabetes, Mahanine,  $\alpha$  - amylase, glucose uptake, L6 myotubes, complications.

## INTRODUCTION

Insulin resistance (IR) is a key contributor for the onset of obesity and type 2 diabetes (T2D). IR is considered as pre - diabetic state, manifested by reduced insulin sensitivity and decreased glucose uptake in peripheral tissues such as skeletal muscle and adipocytes with increased hepatic glucose production, resulting in defective insulin secretion.<sup>1</sup> The imbalanced condition of elevated hepatic glucose production, reduced insulin secretion and compromised insulin action comprehensively lead to oxidative stress which ultimately paves way to long – term complications such as, cardiovascular disease, diabetic retinopathy, diabetic nephropathy and neuropathy.<sup>2</sup> Therefore drugs that can counteract IR have become the focus of research for the management of T2D. Subsequent to develop-

ment of unwanted and severe side- effects on long – term usage of various classes of oral hypoglycemic agents and insulin secretagogues, dependence on medicinal plants enriched with bioactive phytoconstituents has geared during recent years to counteract the effects of IR.<sup>3</sup>

*Murraya koenigii* (Linn.) Spreng, belonging to *Rutaceae* family, known commonly as Curry leaves in English is used as an edible spice in Indian kitchens; it possesses significant medicinal values and has been used in traditional medicine either in isolation or in formulations.<sup>4</sup> The plant is enriched with carbazole alkaloids such as, Mahanine, Mahanimbine, Mahanimbine and essential oil.<sup>5</sup> The aerial part of MK has been prescribed for diabetes in Ayurveda

**Cite this article:** Parameswari RP, Janani M, Dinesh, MG, Srivani T, Chamundeeswari D, Thyagarajan SP. Hydroalcoholic and Alkaloidal Extracts of *Murraya koenigii* (L.) Spreng Augments Glucose Uptake Potential against Insulin Resistance Condition in L6 Myotubes and Inhibits Adipogenesis in 3T3L1 Adipocytes. *Pharmacogn J.* 2018;10(4):633-9

systems of medicine.<sup>6</sup> The crude leaf extract of MK was reported for its antioxidant,<sup>7</sup> anti – bacterial,<sup>8</sup> anti – inflammatory,<sup>9</sup> and topo–isomerases inhibition.<sup>10</sup> While studies have revealed the anti – diabetic property of MK<sup>11,12,13</sup> inconsistent results on the effect of MK extracts have also been reported.<sup>14,15,16</sup> In the present study, we show reduced hyperglycemia and amelioration of insulin sensitivity induced by fatty acid Palmitate and adipogenic inhibitory action of two different solvent ratio *Murraya koenigii* leaf extracts, besides preventing from diabetes associated complication.

## MATERIALS AND METHODS

### Collection of plant material and authentication

The leaves of *Murraya koenigii* was collected from Tirunelveli district by Dr. V. Chelladurai, Retd Research officer, CCRAS. The leaves were authenticated by Prof. P. Jayaraman, Plant Anatomist Botanists, Plant Anatomy Research Centre, Tambaram (PARC/ 2015/3167). Further, the leaves were washed thoroughly, shade dried and ground into coarse powder.

### Preparation of Extracts

Extraction of leaf (1g) involved cold maceration with 70% ethanol and 30% chloroform in methanol for 72h and the filtrate was collected. Filtered contents were distilled, evaporated and concentrated in rotary vacuum evaporator at 60°C. The extracts were used for chemo profiling and further *in vitro* assays.

### Phytochemical analysis

Qualitative phytochemical analysis was carried out for 70% hydroalcoholic and alkaloidal extracts of *Murraya koenigii* as per the method of Harbone, 1984.

### $\alpha$ – amylase inhibitory activity

*In vitro* amylase inhibition was studied by the method of Bernfeld, 1955.<sup>17</sup> Briefly, 100 $\mu$ L of different concentrations (1, 3, 10, 30, 100, 300 and 1000 $\mu$ g/ml) 70% hydroalcoholic (HA) and alkaloidal MK extracts could react with 200 $\mu$  of  $\alpha$ -amylase enzyme (Hi media RM 638) and 100 $\mu$ L of 2mM of phosphate buffer (pH-6.9). After 20-min incubation, 100 $\mu$ L of 1% starch solution was added. The same was performed for the controls where 200 $\mu$ L of the enzyme was replaced by buffer. After incubation for 5 min, 500 $\mu$ L of Dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling water bath for 5 min. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of  $\alpha$ -amylase enzyme was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

### $\alpha$ – glucosidase inhibitory activity

The enzyme inhibition activity for  $\alpha$ -glucosidase was evaluated according to the method previously reported by Shruti *et al.* (2011) with minor modifications.<sup>18</sup> The reaction mixture consisted of 50 $\mu$ L of 0.1M phosphate buffer (with pH of 7.0), 25 $\mu$ L of 0.5mM 4-nitrophenyl  $\alpha$ -D-glucopyranoside (dissolved in 0.1M phosphate buffer, with pH of 7.0), 10 $\mu$ L of different concentrations (1, 3, 10, 30, 100, 300 and 1000 $\mu$ g/ml) 70% hydroalcoholic (HA) and alkaloidal MK extracts and 25 $\mu$ L of  $\alpha$ -glucosidase solution (a stock solution of 1mg/mL in 0.01M phosphate buffer, with pH of 7.0 was diluted to 0.1Unit/mL with the same buffer, with pH of 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100 $\mu$ L of

0.2M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410nm using microplate reader. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffer. Controls were conducted in an identical manner replacing the plant extracts with methanol. Acarbose was used as positive control. All experiments were carried out in triplicates.

### Cell Culture Studies

#### Preparation of cell culture

L6, a monolayer myoblast culture (obtained from NCCS, Pune – Passage no- 15) and 3T3L1 pre-adipocytes (obtained from NCCS, Pune – Passage no - 19) were cultured in DMEM with 10% foetal bovine serum (FBS) and supplemented with penicillin (120units/ml), streptomycin (75 $\mu$ g/ml), gentamycin (160 $\mu$ g /ml) and amphotericin B (3 $\mu$ g/ml) in a 5% CO2 environment. For differentiation, the L6 cells were transferred to DMEM with 2% FBS for 4 days, post-confluence. The extent of differentiation was established by observing the multinucleate of cells. 3T3L1 pre-adipocytes grown in 48 well plates until 2 days post-confluence and the cells were induced by the differentiation medium (combination of 0.5mM/l of IBMX, 0.25  $\mu$ M/l of DEX and 1mg/l of insulin in DMEM medium with 10% FBS) to differentiate into adipocytes. Three days after induction, the differentiation medium was replaced with medium containing 1 mg/ml insulin alone. The medium was subsequently replaced again with fresh culture medium (DMEM with 10% FBS) after 2 days the extent of differentiation was measured by monitoring the formation of multi nucleation in cells.

#### Measurement of 2-deoxy-D-[1-<sup>3</sup>H] glucose<sup>19</sup>

L6 myoblasts grown in 24 well plate was subjected to glucose uptake as reported by Anand *et al.*, 2010. In brief, differentiated cells were serum starved for 5h and were incubated with different concentrations (1, 3, 10 and 30 $\mu$ g/ml) of 70% hydroalcoholic and alkaloidal extracts of *Murraya* for 24h and then cells were either stimulated with 10 $\mu$ M insulin or left untreated for 20min. After experimental incubation, cells were rinsed once with HEPES buffered Krebs Ringer phosphate solution (pH 7.4) and were subsequently incubated for 15 min in HEPES buffered solution containing 0.5 $\mu$ Ci/ml 2-deoxy-D-[1-<sup>3</sup>H] glucose. The uptake was terminated by aspiration of media. Cells were washed thrice with ice cold HEPES buffer solution and lysed in 0.1% sodium dodecyl sulphate (SDS). The lysates were transferred to the plate with glass fibre paper and allowed to air dry. This plate was used to measure the cell-associated radioactivity by liquid scintillation counter. All the assays were performed in duplicates and repeated thrice for concordance. Results have been expressed as % glucose uptake with respective control.

#### Palmitate induced insulin resistance assay<sup>20</sup>

L6 cells were seeded in 96 well plates. Cells were maintained and differentiated for 9 days. Cells were starved for overnight and pre-treated with different concentrations ranging from 1, 3, 10 and 30 $\mu$ g/ml of aqueous extract of 70% hydroalcoholic and alkaloidal extracts of *Murraya* for 1h at 37°C. 0.75mM Palmitate was added and incubated for 4h. During the period of incubation L6 cells were stimulated with 100nM insulin and 0.5 $\mu$ Ci of 2-deoxy-d-<sup>3</sup>[H] glucose uptake was analyzed following similar protocol of measuring radiolabelled glucose uptake.

#### Anti - adipogenesis assay<sup>21</sup>

3T3L1 pre-adipocytes were induced by the combination of IBMX, DEX and insulin to differentiate into adipocytes as previous described (day 0). 72 h after induction, the differentiation medium was replaced with 10% FBS–DMEM containing 1mg/l insulin for 48 h (day 5). The medium was replaced again with fresh culture medium for 48 h (day 7). The degree of the differentiation of the cells was investigated by adding different

solvent extracts of 70% hydroalcoholic and alkaloidal extracts of *Murraya* at doses of 3, 10 and 30 µg/ml day 0, a period which covered the entire induction and post induction stages. Before staining with oil red O, cells were washed twice with PBS, fixed with 10% formaldehyde for 15 min at room temperature, washed twice with distilled water and once with 70% isopropanol. Next, cells were stained for 1 h at room temperature with filtered oil red O at a ratio of 60% oil red O stock solution (0.5% w/v in isopropanol) to 40% distilled water. The cells were washed twice with distilled water, twice with PBS, and examined under a microscope. Photomicroscopic evaluation was also carried out for the comparison of triglyceride accumulation using invertoscope (Nikon) was used for imaging at room temperature. For quantitative analysis, oil red O was extracted with isopropanol for 2min, and optical density of each sample was determined at 540 nm.

#### Advanced Glycation end product (AGE) assay<sup>22</sup>

Bovine Serum Albumin (BSA) (10 mg/ml) was incubated with 1.1 M fructose in 0.1 M phosphate buffered-saline (PBS), pH 7.4 containing 0.02% sodium azide in darkness at 37°C for 7 days. Before incubation, the solution containing different concentrations of 70% hydroalcoholic and alkaloidal extracts of *Murraya* were added to the mixtures. The glycated BSA formation was determined using fluorescent intensity at an excitation wavelength 355 nm and emission wavelength 460 nm. Amino guanidine was used as a positive control for this study.

#### HPTLC Standardization of Mahanine<sup>23</sup>

The 70% hydroalcoholic and alkaloidal extracts of MK were prepared at a concentration of 25mg/ml in Methanol. Standard Mahanine was prepared at concentration of 100 µg/ml in Methanol. Standard Mahanine was spotted at a range of 200ng – 1000ng and samples at two concentrations of 50 µg/ml and 100 µg/ml were spotted using CAMAG Linomat 5 applicator. The method was optimized by selecting appropriate mobile phase of n-hexane: ethyl acetate: glacial acetic acid (3:1:0.5) and developed in a twin trough chamber, 10 x 10 cm at 25°C. The plates were dried using hair dryer. The developed plates were scanned at 298nm using CAMAG TLC scanner 3 and photo-documented using CAMAG REPOSTAR 3 at 254nm and 366nm.

#### Statistical analysis

All the experiments were performed in triplicate and the results were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) was used to calculate the statistical significance of differences between groups for cell line based studies, followed by Tukey's test for multiple comparisons among groups using Graph Pad Prism v.5.0. P<0.05 were considered as statistically significant. Linear regression analysis was done for *in vitro* extract based assays.

## RESULTS

#### Preliminary phytochemical analysis

Preliminary phytochemical analysis showed the presence of phenolic compounds, flavones, and tannins, alkaloids, reducing sugars, proteins and carbohydrates in both the extracts (Table 1).

#### *In-vitro* α-amylase and α-glucosidase inhibitory activities

The, 70% hydroalcoholic and alkaloidal extracts of MK were evaluated for α-amylase and α-glucosidase inhibitory activity (Figure 1). The 70% hydroalcoholic extract of MK showed potent inhibitory activity against α-amylase enzyme with a maximum inhibition percentage of 92.23% at 1000 µg/ml which compared with standard Acarbose. The IC<sub>50</sub> of 70% hydroalcoholic extract of MK was found to be 37.09 µg/ml which was found to be close enough to standard Acarbose whose IC<sub>50</sub> is 34.83 µg/ml. The alkaloidal extract showed its maximum inhibition percentage of 91.79% at 1000 µg/ml and its IC<sub>50</sub> value was found to be

48 µg/ml. A similar inhibitory pattern was observed for MK extracts in case of α-glucosidase. The maximum inhibition of α-glucosidase of 70% HA and alkaloidal extracts of MK was found to be 95.57% and 99% respectively whereas for Acarbose it was 97.29%. The corresponding IC<sub>50</sub> was found to be 21.56 µg/ml, 40.22 µg/ml and 42.11 µg/ml for 70% HA MK extract, alkaloidal extract and Acarbose respectively.

#### [<sup>3</sup>H]-2-Deoxy glucose (Radiolabelled) uptake assay

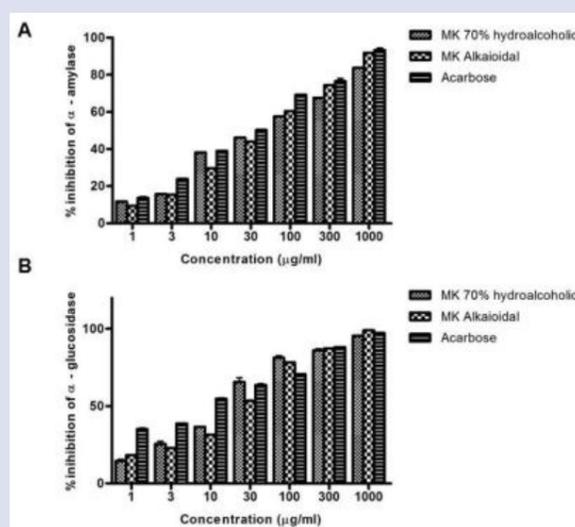
Glucose uptake assay was carried out in differentiated L6 myotubes using radio-labelled glucose [<sup>3</sup>H]-2-Deoxy glucose. Incubation of L6 myotubes with different concentrations (1, 3, 10 and 30 µg/ml) of 70% HA and alkaloidal extracts of MK exhibited a dose-dependent increase in the glucose uptake activity which was comparable with that of Insulin (Figure 2). In the absence of insulin, 70% HA extract of MK showed maximum glucose uptake of 65.80% at 30 µg/ml concentration, while the alkaloidal extract MK exhibited 55.99% glucose uptake at the same concentration. The glucose uptake activity in both 70% HA and alkaloidal groups in presence of insulin did not vary significantly compared to treatment without insulin. Hence it was revealed from the results that no additive or synergistic effect was exhibited by the MK extracts and insulin.

#### Palmitate induced insulin resistance assay

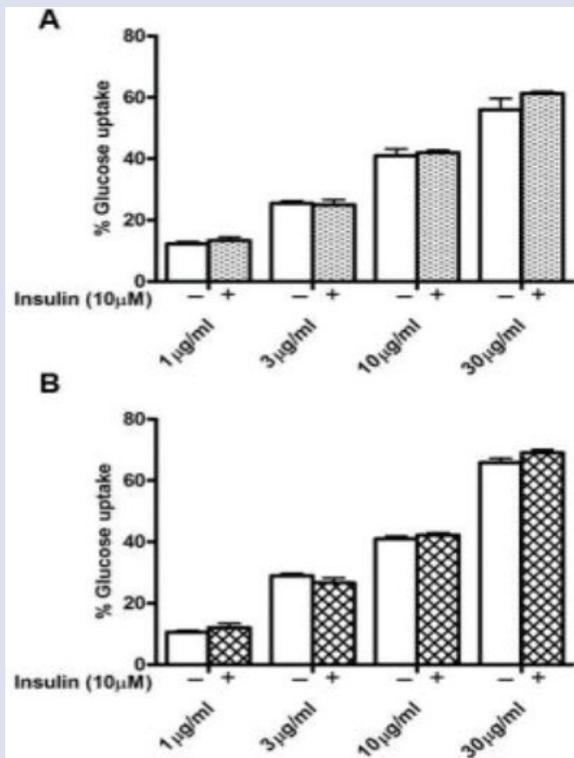
The effect of 70% HA and alkaloidal extracts of MK was evaluated against Palmitate induced insulin resistance (Figure 3). Significant attenuation (p<0.001) of glucose uptake in L6 skeletal muscle cells was observed in Palmitate induced group due to insulin resistant condition. The 70% HA and alkaloidal extracts of MK at different concentrations (1, 3, 10 and 30 µg/ml) exhibited dose-dependent increase in uptake of glucose by attenuating the effect of Palmitate. While insulin (10 µM) exhibited 85.43% uptake of glucose, 70% HA and alkaloidal extracts of MK showed maximum uptake of 67.23% and 61.12% of glucose at 30 µg/ml.

#### Oil O red adipogenesis assay

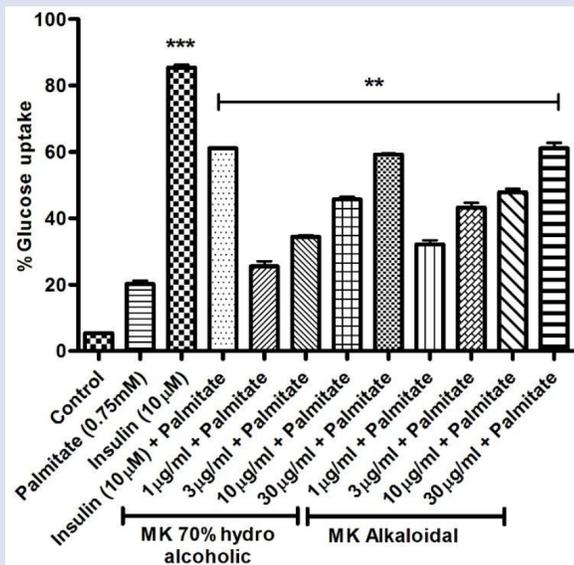
The effect of 70% HA and alkaloidal extracts of MK on lipid accumulation was investigated using 3T3L1 adipocytes at various doses of 1, 3, 10 and



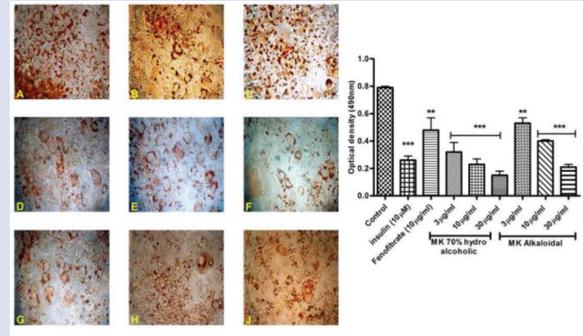
**Figure 1:** Effect of 70% hydroalcoholic and alkaloidal extracts of *Murraya koenigii* on (A) α-amylase inhibitory activity (B) α-glucosidase inhibitory activity compared with Standard Acarbose. All the data expressed as Mean ± SEM of three independent experiments.



**Figure 2:** Dose response analysis of 70% hydroalcoholic and alkaloidal extracts of *Murraya koenigii* on radiolabelled [3H]-2-Deoxy glucose uptake (A) without insulin and (B) with insulin using L6 myotubes. Cells were treated with different concentrations (1, 3, 10 and 30 µg/ml) of MK extracts. All the data expressed as Mean ± SEM of three independent experiments. P<0.05 was considered statistically significant.



**Figure 3:** Effect of Palmitate on glucose uptake in L6 myotubes. L6 myotubes cultured in high glucose media with and without different concentrations of 70% hydroalcoholic and alkaloidal extracts of *Murraya koenigii* followed by incubation with 0.75mM Palmitate for 4h. Glucose uptake was measured using radiolabelled [3H]-2-Deoxy glucose. All the data expressed as Mean ± SEM of three independent experiments. P<0.05 was considered statistically significant.



**Figure 4:** Effect of 70% HA and alkaloidal extracts of *Murraya koenigii* on differentiated adipocytes. The adipocytes were stained using Oil O red (A) Differentiated adipocytes (B) Insulin 10µM (C) Fenofibrate (10µg/ml) (D) 3µg/ml 70% HA extract (E) 10µg/ml 70% HA extract (F) 30µg/ml 70% HA extract (G) 3µg/ml alkaloidal extract (H) 10µg/ml alkaloidal extract (I) 30µg/ml alkaloidal extract. Bar graph represents relative density measured at 490nm of accumulated lipid content. All the data expressed as Mean ± SEM of three independent experiments. P<0.05 was considered statistically significant.

30µg/ml (Figure 4). Following eight days of differentiation, intracellular accumulation of lipid content was analysed by Oil O red staining. Increased lipid accumulation was visualized in control differentiated adipocytes indicated by increase in staining intensity. The 70% HA and alkaloidal extracts of MK treated cells lowered triglyceride accumulation which was exhibited by decrease in staining pattern, suggestive of anti-adipogenic property of the extracts.

**Diabetes associated complications assay – AGE product assay**

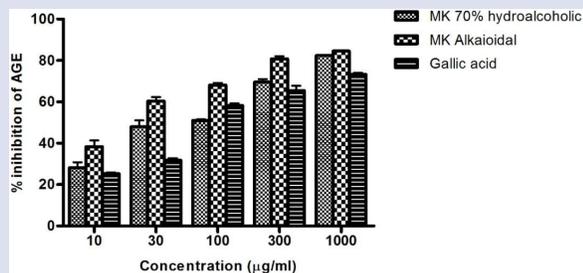
The potential of 70% HA and alkaloidal extracts of MK were studied against AGE product inhibitory activity (Figure 5). Different doses of the extracts (10, 30, 100, 300 and 1000µg/ml) were examined against AGE formation. The alkaloidal *Murraya* extract showed a considerable inhibition of 84% at a concentration of 1000µg/ml while with 70% HA extract of *Murraya*, inhibition was about 82% at the same concentration level. Gallic acid was used as positive control and it showed an inhibitory potential of about 73%.

**HPTLC quantification of Mahanine in 70% hydroalcoholic and alkaloidal extracts of MK**

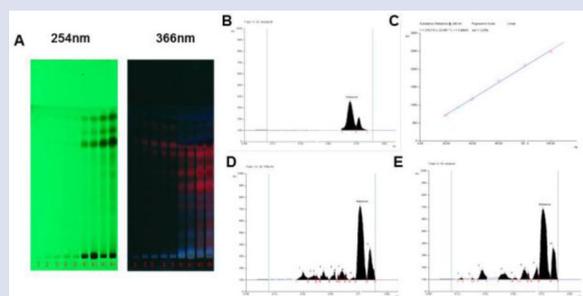
The HPTLC chromatograms of Mahanine, carbazole alkaloid are depicted in Figure 6. The retention factor ( $R_f$ ) of Mahanine in 70% hydroalcoholic and alkaloidal extracts of MK was found to be 0.73. The quantification of Mahanine at scanning wavelength of 298nm in the solvent system of n-hexane: ethylacetate: glacial acetic acid (3:1:0.5) demonstrated the presence of Mahanine in both the extracts. The percentage content of Mahanine in 70% hydroalcoholic and alkaloidal extracts of MK was found to be 19.68% and 26.59% respectively.

**DISCUSSION**

Diabetes is a chronic long – term metabolic disorder which is associated with persistent complications. Review of glycaemic control at regular time points with proper therapeutic treatment can develop control over the disorder.<sup>24</sup> To devoid the adverse effects caused by modern medicines, options for complementary and alternative medicines have emerged. In this background, the results of the present study exemplify the effect



**Figure 5:** Effect of different concentrations of 70% hydroalcoholic and alkaloidal extracts of *Murraya koenigii* on inhibition of Advance glycation end products compared with standard amino guanidine. All the data expressed as Mean ± SEM of three independent experiments. P<0.05 was considered statistically significant.



**Figure 6:** HPTLC chromatogram of Mahanine in 70% HA and alkaloidal extracts of *Murraya koenigii*. Lanes 1 - 5 – Standard Mahanine 2µl – 10µl; Lane 6 – 2µl of 70% HA extract; Lane 7 – 4µl of 70% HA extract; Lane 8 – 2µl of alkaloidal extract; Lane 9 – 4µl of alkaloidal extract. (A) Photo document showing presence of Mahanine at 254nm and 366nm (B) Standard Mahanine (C) Std. Calibration Curve (D) Peak area of Mahanine in 70% HA extract (E) Peak area of Mahanine in alkaloidal extract. Mobile phase: n-hexane: Ethyl acetate: Glacial acetic acid [3:1:0.5]; Scanning wavelength: 298 nm; R<sub>f</sub> value: 0.73.

of *Murraya* extracts on carbohydrate metabolizing enzymes, glucose uptake potential, insulin resistance, and adipogenesis as well as on diabetes-associated complications.

Hyperglycemia is one of the hallmark features of T2D; hence regulating plasma glucose plays a crucial role in preventing T2D. α – amylase and α – glucosidase are the two key enzymes that are responsible for postprandial hyperglycemia. Hence inhibiting the activity of these enzymes would be one of the best approaches in the management of diabetes, as it delays the absorption of ingested carbohydrates.<sup>25</sup> The results of our present study exhibited that aqueous, absolute alcohol, 70% hydroalcohol and alkaloidal extract of MK have effectively inhibited these enzymes which suggests that *Murraya koenigii* possess carbohydrate metabolism regulating property, thereby beneficial in reducing postprandial glucose. Skeletal muscle is the predominant regulatory site for insulin stimulated glucose uptake which helps in maintenance of glucose homeostasis. Major portion of glucose is stored in muscle cells as glycogen; it is considered as target tissue to investigate on T2D and its complications. Impaired insulin action concomitant with decreased glucose uptake is

**Table 1: Preliminary Phytochemical analysis of 70% hydroalcoholic (HA) and alkaloidal MK extracts showing presence of various phyto-constituents.**

S.NO	CHEMICAL CONSTITUENTS	<i>Murraya koenigii</i>	
		70% Hydroalcohol extract	Alkaloidal extract
1	Phenolic compounds	+++	+++
2	Reducing sugars	+++	+++
3	Carbohydrates	+++	+++
4	Flavones	++	++
5	Glycosides	+++	+++
6	Saponins	++	++
7	Steroids	-	+
8	Alkaloid	+++	+++
9	Quinones	+++	+++
10	Proteins	++	++
11	Tannins	++	++

+++ = High; ++ = Moderate; + = Mild; - = absent

characteristic of skeletal muscle insulin resistance.<sup>26</sup> Quite a few reports on anti – diabetic effect of alkaloids, especially Carbazole alkaloids from *Murraya* have been reported.<sup>27-30</sup> Corroborating the previous reports, the present study results revealed the potential of 70% hydroalcoholic and alkaloidal extract of MK in augmenting glucose uptake by attenuating the effect of Palmitate induced insulin resistance, proposing that Carbazole alkaloids present in the extracts might be the contributing factor for the anti – hyperglycemic effect.

Synthesis and storage of lipids, in the form of triglycerides is the primary function of adipocytes. Adipogenesis is a process that encompasses increased growth of adipocyte mass, adipocyte differentiation and accumulation of lipid in adipocytes, factors that leads to obesity condition.<sup>31</sup> Hence inhibiting adipogenesis would be one of the best strategies for the management of obesity. Herbal extracts enriched with polyphenols, alkaloids etc., possess potent anti – adipogenic and hypoglycemic activities.<sup>32</sup> Our study results exhibited decreased lipid content in 70% HA and alkaloidal extracts treated group, indicative of adipogenesis inhibition in 3T3L1 adipocytes, which signifies the anti – obesity effect of MK.

Metabolic imbalance in T2D results in generation of reactive oxygen species (ROS) which decreases the antioxidant levels. This leads to increased oxidative stress which in turn, activates the polyol pathway. Activation of polyol pathway leads to increased production of intracellular sorbitol and fructose, thereby increasing the production of AGEs.<sup>33</sup> Hence, any compound which possesses the property of inhibiting both these activities may be beneficial. Studies have demonstrated that plants rich in flavonoids possess aldolase inhibitory property,<sup>34,35</sup> whereas plants enriched with alkaloids have shown potent AGE inhibitory property.<sup>36</sup> 70% hydroalcoholic and alkaloidal extracts of MK effectively inhibited the formation of AGE, which may be due to the presence of carbazole alkaloids.

## CONCLUSION

Results of our study demonstrate multiple effects of *Murraya koenigii* in the management of diabetes as well as its associated complications,

thereby suggesting that the effective bioactive ingredients present in MK can be used for future research, both *in vitro* and *in vivo* and evaluate its antidiabetic activity by exploring the underlying mechanisms.

## ACKNOWLEDGEMENT

The authors wish to thank the Drugs and Pharmaceutical division, Department of Science and technology, New Delhi for the research grant.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

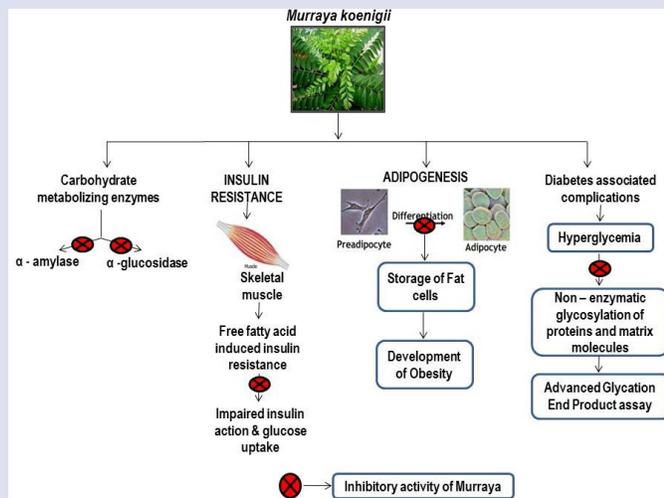
**IR:** Insulin resistance; **T2D:** Type 2 diabetes; **MK:** *Murraya koenigii*; **HA:** hydroalcoholic; **CCRAS:** Central Council for Research in Ayurveda and Siddha; **NCCS:** National Centre for Cell Science; **DMEM:** Dulbecco's Modified Eagle's Medium; **FBS:** Fetal bovine serum; **IBMX:** 3-isobutyl-1-methylxanthine; **DEX:** Dexamethasone; **PBS:** Phosphate buffered Saline; **AGE:** Advanced Glycation End Product; **HPTLC:** High Performance Thin Layer Chromatography; **ROS:** Reactive oxygen species.

## REFERENCES

- Chiasson JL, Rabasa-Lhoret R. Prevention of type 2 diabetes: insulin resistance and beta-cell function. *Diabetes*. 2004;53(3):S34-8.
- Juarez-Reyes K, Brindis F, Medina-Campos ON, Pedraza-Chaverri J, Bye R, Linares E, *et al.* Hypoglycemic, antihyperglycemic, and antioxidant effects of the edible plant *Anoda cristata*. *J Ethnopharmacol*. 2015;161:36-45.
- Perera PK, Yunman Li. Functional herbal food ingredients used in type 2 diabetes mellitus. *Pharmacogn Rev*. 2012;6(11):37-45.
- Jain V, Momin M, Laddha K. *Murraya Koenigii*: An Updated Review. *IJAHM*. 2012; 2(4):607-27.
- Nagappan T, Chandra segaran T, Abdul Wahid ME, Ramasamy P, Vairappan SC. Efficacy of Carbazole Alkaloids, Essential Oil and Extract of *Murraya koenigii* in Enhancing Subcutaneous Wound Healing in Rats. *Molecules*. 2012;17(12):14449-63.
- Satyavati GV, Gupta AK, Tandon N. Medicinal Plants of India. *ICMR*. 1999;2:289-99.
- Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N. Comparison of Anti Oxidative Properties Of Carbazole Alkaloids From *Murraya koenigii* Leaves. *J Agric Food Chem*. 2003;51(22):6461-7.
- Aqil F, Ahamad I. Broad Spectrum antibacterial and antifungal properties of certain traditionally used Indian Medicinal plants. *World J Microb Biotech*. 2003;19(6):653-7.
- Maiti AK, Dash GK, Patro CP. Anti-inflammatory and analgesic activity of leaf essential oil from *Murraya koenigii* Spreng. *Hamdard Med*. 2004;47(2):22-6.
- Powers SK, Deruisseau KC, Quindry J, Hamilton KL. Dietary antioxidants and exercise. *J Sports Sci*. 2004;2(1):81-94.
- Vinuthan MK, Girish KV, Ravindra JP, Jayaprakash, Narayana K. Effect of extracts of *Murraya koenigii* leaves on levels of blood glucose and plasma insulin in alloxan-induced diabetic rats. *Indian J Physiol Pharmacol*. 2004;48(4):348-52.
- Kesari N, Gupta RK, Watal G. Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits. *J Ethnopharmacol*. 2004;97(2):247-51.
- Tembhurne SV, Sakarkar DM. Protective effect of *Murraya koenigii* (L) leaves extract in streptozotocin induced diabetic's rats involving possible antioxidant mechanism. *J Med Plants Res*. 2010;4(22):2418-23.
- Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. *J Ethnopharmacol*. 1999;64(2):179-84.
- Adebajo AC, Olayiwola G, Eugen VJ, Iwalewa EO, Omisore NOA, Bergenthal D, *et al.* Evaluation of the Ethnomedical Claims of *Murraya koenigii*. *Pharm Biol*. 2005;42(8):610-20.
- Yadav S, Vats V, Dhunoo Y, Grover JK. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. *J Ethnopharmacol*. 2002;82(2):111-6.
- Harborne JB. *Phytochemical methods*. Chapman and Hall publications, 2<sup>nd</sup> Ed. New York. 1988;288.
- Bernfeld P. Amylases  $\alpha$  and  $\beta$  in: *Methods in Enzymology*, Clowick SP and Kaplan NO, Editors. Academic Press Inc Publishers. New York. 1955;149-58.
- Sanchetia S, Sanchetia S, Lee SH, Lee JE, Seo SY. Screening of Korean Medicinal Plant Extracts for  $\alpha$ -Glucosidase Inhibitory Activities. *Iran J Pharm Res*. 2011;10(2):261-4.
- Anand S, Muthusamy VS, Sujatha S, Sangeetha KN, Bharathi Raja R, Sudhagar S *et al.* Aloe emodin glycosides stimulates glucose transport and glycogen storage through PI3K ependent mechanism in L6 myotubes and inhibits adipocyte differentiation in 3T3L1 adipocytes. *FEBS Letters*. 2010;584(14):3170-8.
- Gupta SD, Bhattacharya S, Maitra S, Pal D, Majumdar SS, Datta A, *et al.* Mechanism of lipid induced insulin resistance: Activated PKC $\epsilon$  is a key regulator. *Biochim Biophys Acta*. 2011;1812(4):495-506.
- Harris CS, Beaulieu LP, Fraser MH, McIntyre KL, Owen PL, Martineau LC, *et al.* Inhibition of advanced glycation product formation by medicinal plant extracts correlates with phenolic metabolites and antioxidant activity. *Planta Med*. 2011;77(2):196-204.
- Wagner H, Bladt S. *Plant Drug Analysis - A Thin Layer Chromatography Atlas*. 2nd Ed. Berlin: Springer-Verlag. 1996;384.
- Deutschlander MS, Van de Venter M, Roux S, Louw J, Lall N. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *J Ethnopharmacol*. 2009;124(3):619-24.
- Tiwari AK, Rao M. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and prospects. *Curr Sci*. 2002;83(1):10.
- Tamrakar AK, Jaiswal N, Yadav PP, Maurya R, Srivastava AK. Pongamol from *Pongamia pinnata* stimulates glucose uptake by increasing surface GLUT4 level in skeletal muscle. *Mol Cell Endocrinol*. 2011;339(1):98-104.
- Cheng Z, Pang T, Gu M, Gao AH, Xie CM, Li JY, *et al.* Berberine-stimulated glucose uptake in L6 myotubes involves both AMPK and p38 MAPK. *Biochim Biophys Acta*. 2006;1760(11):1682-9.
- Gulfraz M, Mehmood S, Ahmad A, Fatima N, Praveen Z, Williamson EM. Comparison of the antidiabetic activity of Berberis lycium root extract and berberine in alloxan-induced diabetic rats. *Phytother Res*. 2008;22(9):1208-12.
- Dineshkumar B, Mitra A, Manjunatha M. Antidiabetic and hypolipidemic effects of mahanimbine (carbazole alkaloid) from *Murraya koenigii* (Rutaceae) Leaves. *Int J Phytomed*. 2010;2(1):22-30.
- Patel OP, Mishra A, Maurya R, Saini D, Pandey J, Taneja I, *et al.* Naturally Occurring Carbazole Alkaloids from *Murraya koenigii* as Potential Antidiabetic Agents. *J Nat Prod*. 2016;79(5):1276-84.
- Hsu CL, Yen GC. Induction of cell apoptosis in 3T3-L1 pre-adipocytes by flavonoids is associated with their antioxidant activity. *Mol Nutr Food Res*. 2006;50(11):1072-9.
- Li KK, Liu CL, Shiu HT, Wong HL, Siu WS, Zhang C, *et al.* Cocoa tea (Camellia ptilophylla) water extract inhibits adipocyte differentiation in mouse 3T3-L1 preadipocytes. *Sci Rep*. 2016;6:20172.
- Gupta P, Jain V, Pareek A, Kumari P, Singh R, Agarwal P, *et al.* Evaluation of effect of alcoholic extract of heartwood of *Pterocarpus marsupium* on *in vitro* antioxidant, anti-glycation, sorbitol accumulation and inhibition of aldose reductase activity. *J Tradit Complement Med*. 2016;7(3):307-14.
- Patil UH, Dattatraya KG. *Pterocarpus marsupium*: a valuable medicinal plant in diabetes management. *Int J App Bio Pharm Tech*. 2011;2:6-13.
- Hilal A, Kalyanaraman R. Pharmacology of *Pterocarpus marsupium* Roxb. *Med Plant Res*. 2015;5:1-6.
- Maqsood AS, Saima Rasheed, Quaiser Saquib, Al-Khedhairi AA, Al-Said MS, Javed Musarrat C. *In-vitro* dual inhibition of protein glycation, and oxidation by some Arabian plants. *BMC Complementary and Alternative Medicine*. 2016;16(1):276.

**Cite this article:** Parameswari RP, Janani M, Dinesh, MG, Srivani T, Chamundeeswari D, Thyagarajan SP. Hydroalcoholic and Alkaloidal Extracts of *Murraya koenigii* (L.) Spreng Augments Glucose Uptake Potential against Insulin Resistance Condition in L6 Myotubes and Inhibits Adipogenesis in 3T3L1 Adipocytes. *Pharmacog J*. 2018;10(4):633-9.

**GRAPHICAL ABSTRACT**



**SUMMARY**

- The 70% hydroalcoholic and alkaloidal extract exhibited beneficial effect against insulin resistance and adipogenesis.
- The extracts were found to be effective not only on obesity and type 2 diabetes but were also potent enough in prevention of diabetes associated complications.
- The anti-diabetic effect of *Murraya koenigii* might be attributed to the presence of major phyto constituent Mahanine, carbazole alkaloid present.