*Journal of Biochemistry, Biotechnology and Biomaterials, 1(1):69-75, 2016*

*Journal Home page: http://molcare.org/jbcbb*

# Antidiabetic Efficacy of Various Organic Fractions of *Mangifera indica* Leaves

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**Abstract**

Medicinal plants are efficient ameliorator of oxidative stress associated with diabetes mellitus. The objective of the present study is the phytochemical screening and determination of antioxidant and antidiabetic potency of *M. indica* leaves in methanolic and other solvent fractions. Total phenolic and flavonoid contents ranged from 9.4±3.1-21.6±2.4 GAE (g/100 g dry weight) and 5.0±2.3-7.8±1.9 CE (g/100 g dry weight), respectively. Ethyl acetate was most potent fraction as it extracted highest phenols and flavonoids. Ethyl acetate fraction exhibited strong radical scavenging activity (77.3 %) compared to methanol, *n*-hexane, *n*-butanol and chloroform fractions. Extract and fractions exhibited almost same noticeable ($p< 0.05$) yet time-dependent antiglycation patterns (30.65-66.85%). However, their overall inhibitory strengths was meager than that of synthetic inhibitor aminoguanidine (75%). Decline in enzyme activity by *M. indica* was concentration-dependent. Ethyl acetate fraction attained greatest (71.5%) amylase inhibitory activity as compared to other test fractions. *M. indica* extract and solvent partitions reflected diverse efficacy for the parameters investigated. Such variable characteristics may be attributed to the presence of potent components in respective solvents as well as in specified bioassay. This study endorses further studies directed at isolation, purification and characterization of active components that may reveal new avenues in functional foods.

**Key words:** *Mangifera indica*, Phytoconstituents, Antioxidants, Glycation, Enzyme inhibition

**Full length article:** Received: 09 Jan, 2016 Revised: 25 Jan, 2016 Accepted: 06 Feb, 2016 Available online: 15 Feb, 2016
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## 1. Introduction

Natural products as avenue for drug discovery are being investigated since ancient times. Most of these products are used as crude extracts and about 80% of the world's population depends on these traditional medicines for health care. Plants are the rich bio-resources in traditional medicinal system, food supplements and chemical entities for synthetic drugs and pharmaceutical intermediates (Pal, 1998; Tiwari *et al*., 2011). Medicinal plants are efficient ameliorator of oxidative stress associated with diabetes mellitus. *Mangifera indica* L. (family *Anacardiaceae*) is a widely eaten tropical fruit due to its attractive color, unique taste, and nutritional qualities. It is a rich source of certain volatile compounds, organic acids, vitamins, carbohydrates, phenolic acids (caffeic acid, tannic acid, gallic acid) and amino acids (Wauters *et al*., 1995; Singh *et al*., 2004; Pino *et al*., 2005).

*Mangifera indica* fruit produce a resin which contains mangiferic acid, mangiferene, maniferol as well as resinol. It is reported that *M. indica* leaves contain unsaturated saponins, sterols, glycosides, euxanthin acid, polyphenols, mangiferine and tannins. The extracts and ashes of the *M. indica* leaves are used to treat wounds, abscesses, sores, cough, scald, diarrhea, burns and other infections (Ojewole, 2005; Bbosa *et al*., 2007; Somkuwar and Kamble, 2013). Pharmacological properties of *M. indica* are attributed to the presence of phenolic acids. These phenolic compound possess potent antioxidant activity that play an important role as anti-mutagen, anti-inflammatory and anti-carcinogenic agents (Kim *et al*., 2003; Chiou *et al*., 2007). The management of oxidative stress due to free radical-mediated pathophysiology has become a central focus for scientific efforts designed to prevent tissue injury. Many investigations have been carried out to discover antioxidant phytochemicals from medicinal plants or natural products for the treatment or prevention of free radical induced diseases (Oboh and Irondi, 2013). It has been reported that *M. indica* has free radical scavenging entities, which enhance antioxidant enzymes and reduce lipid peroxidation (Bafna and Balaraman, 2005).

Glycation stress involves attachment of glucose with protein and end product of glycoxidation cause oxidative stress that is responsible for cellular damage. Numerous phytomedicines are known to possess hypoglycemic potency (Wang *et al*., 2012). For instance, antiglycation activity is correlated significantly with the phenolic content of the tested plant extracts (Peng *et al*., 2008; Hori *et al*., 2012). Inhibition of α-glucosidase and αamylase is another efficient preventive preference for diabetes mellitus management. Solubility of phytoconstituents in organic solvents is varying and their reactivity fluctuates by the experimentation setup. Although research articles on numerous aspects of *M. indica* are already published, but this project was undertaken to compile selected bioactivities of *M. indica* leaves in different solvent fractions. The objective of the present study was the phytochemical screening and determination of antioxidant and antidiabetic potency of *M. indica* leaves in methanolic and other solvent fractions.

## 2. Material and Methods

### *2.1. Preparation of solvent extract and fractions*

*M. indica* leaves were collected from botanical garden of University of Agriculture, Faisalabad, Pakistan and authenticated by Dr. Mansoor Hammed, department of Botany, University of Agriculture, Faisalabad, Pakistan. Leaves were shade dried and homogenized to coarse powder. Powdered plant material was extracted with methanol at room temperature for 4 days. Methanolic extracts were evaporated and subsequent viscous greenish residues were subjected to fractions by using separating funnel. Fractionation was done into *n*-hexane (120 g), ethyl acetate (55 g), *n*-butanol (40 g), chloroform (60 g) and aqueous extract (85 g) fractions.

### 2.2. *Antioxidant activity*

#### *2.2.1. Total Phenolic Contents (TPC)*

Following Folin-Ciocalteu (FC) assay (Chahardehi *et al*., 2009) with slight modifications, *M. indica* extract was prepared in dimethylsulfoxide and mixed with Folin- Ciocalteu reagent (1 mL). $Na_2CO_3$ (3 mL of 1% w/v) was added and retained at 25 ºC for 2 hours. The absorbance was measured at 760 nm and results were expressed in terms of g gallic acid/100 g dry weight.

#### *2.2.2. Total Flavonoid Contents (TFC)*

Using spectrophotometric technique (Siddique *et al*., 2010), extract (0.5 mL of 1:10 g/mL) was added in respective solvent (1.5 mL), 10% aluminium chloride (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (2.8 mL). The absorbance was measured at 510 nm using catechin standards. TFC concentrations were represented as gram catechin equivalent/100 g dry weight.

#### *2.2.3. DPPH Radical scavenging assay*

The fractions were analyzed for their abilities to scavenge 2, 2-diphenyl l-l-l-picrylhydrazyl (DPPH) (Souri *et al*., 2008). The stock solution of DPPH radical was diluted in 80% methanol. About 50 uL aliquot of various concentrations of the samples were added to 5 mL of methanol solution of DPPH. After 25 minutes incubation period at room temperature, the absorbance was taken against a blank at 517 nm. BHT (Butylated hydroxy toluene) was used as standard control. The experiment was repeated for three times.

### *2.3. Antidiabetic activity*

#### *2.3.1 Antiglycation Assay*

*M. indica* fractions were reconstituted in DMSO. Bovine serum albumin (BSA, 10 mg/mL) and anhydrous glucose (50 mg/mL) were made in sodium phosphate buffer (67 mM, pH 7). Sample mixture contained equal amounts (100 µL) of fraction, BSA and glucose solution. Glycated (positive) control had sodium phosphate buffer instead of plant extract, while blank (negative control) contained BSA and sodium phosphate buffer only. After incubation at 37ºC for 7 days, 100 µL of 100% trichloroacetic acid was added and centrifuged for 4 minutes at 4 ºC. Pellets containing advanced glycation end product were dissolved in phosphate buffer saline (pH 10). Absorbance was taken at 440 nm. Same procedure was repeated after 3 and 5 weeks (Ayatollahi *et al*., 2010). The results were compared to those of the synthetic inhibitor aminoguanidine.

#### *2.3.2. Enzyme Inhibition Assay*

The alpha amylase inhibition assay was carried out by the method described by Apostolidis *et al*. (2006). Extract fractions (100, 300, 500 µL) and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing porcine pancreatic α-amylase (EC 3.2.1.1; Sigma Aldrich) solution (0.5 mg/mL) were incubated at 25ºC for 10 minutes. Then 500 µL of a 1% starch solution was added. The mixture was incubated at 25 ºC for 10 minutes and reaction was stopped by the adding dinitrosalicylic acid (1 mL) reagent. Heated

in boiling water bath for 5 minutes, cooled and diluted with distilled water. Absorbance was noted against blank control at 540 nm. For control (negative), 0.02 M sodium phosphate buffer (pH 6.9) was used. Whereas, positive control was metformin drug.

### *2.4. Phytochemical analysis*

Phytochemical analyses of *M. indica* was conducted as described by Krishnaiah *et al*. (2009).

### *2.5. Statistical analysis*

Data was presented as averaged means. Analysis was performed by SPSS (SPSS Inc. Chicago, IL, USA) software (version 16.0) with 0.05 level of significance.

## 3. Results and Discussion

### *3.1. Antioxidant Contents and Activity*

The total phenol contents (TPC) and total flavonoid contents (TFC) of *M. indica* leaf extract fractions are summarized in table 1. Total phenolic and flavonoid contents ranged from 9.4±3.1-21.6±2.4 GAE (g/100 g dry weight) and 5.0±2.3-7.8±1.9 CE (g/100 g dry weight) respectively. Considerable variances in TPC were apparent. TPC were significantly higher in ethyl acetate fraction than others ($p < 0.05$).

**Table 1.** Antioxidant Profile

| **Extract/fractions** | **Antioxidant components** | | |
|---|---|---|---|
| | **TPC (GAE g/100 g DW)** | **TFC (CE g/100 g DW)** | **DPPH, $IC_{50}$ (µg/mL)** |
| **Methanol** | 17.8 ± 2.1 | 6.2 ± 2.5 | 70.1 ± 1.3 |
| ***n*-hexane** | 15.4 ± 1.4 | 5.6 ± 1.0 | 65 ± 1.56 |
| ***n*-butanol** | 10.7± 1.2 | 6.5 ± 1.07 | 59 ± 1.6 |
| **Chloroform** | 9.4 ± 3.1 | 5.0 ± 2.3 | 55 ± 1.78 |
| **Ethyl acetate** | 21.6 ± 2.4 | 7.8 ± 1.9 | 77.3 ± 2.34 |
| **BHT** | - | - | 21.5 ± 0.27 |

Data (Mean ± SD) are average of three samples of each extract/fraction, analyzed individually in triplicate. (n = 1x3x3), DW: dry weight, TPC: total phenolic contents expressed g gallic acid equivalent /100 g dry weight; TFC: total flavonoid contents expressed as g catechin equivalents/100 g dry weight; DPPH: 2, 2-diphenyl l-l-l-picrylhydrazyl, BHT: Butylated hydroxy toluene.

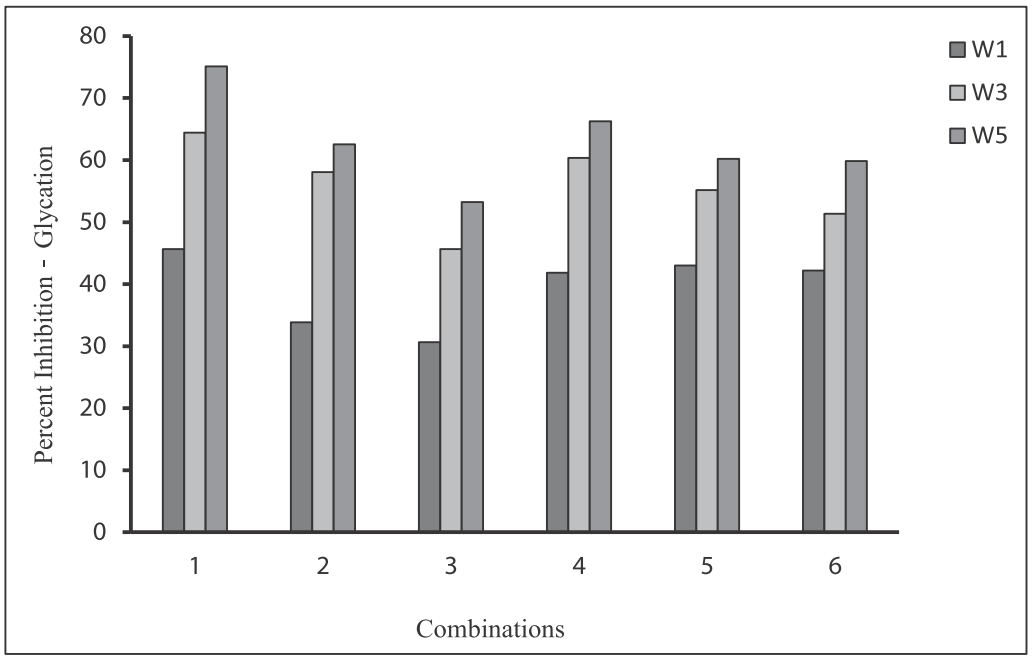


**Fig. 1.** Antiglycation activity, BSA: Bovine serum albumin, AG: Aminoguanidine, G: Glucose, HF: *n*-hexane fraction; BF: *n*-butanol fraction; MF: Methanol fraction; EF: ethyl acetate fraction; CF: chloroform fraction. Inhibition was assessed after 1st, 3rd and 5th weeks (W1, W3, W5) incubations.

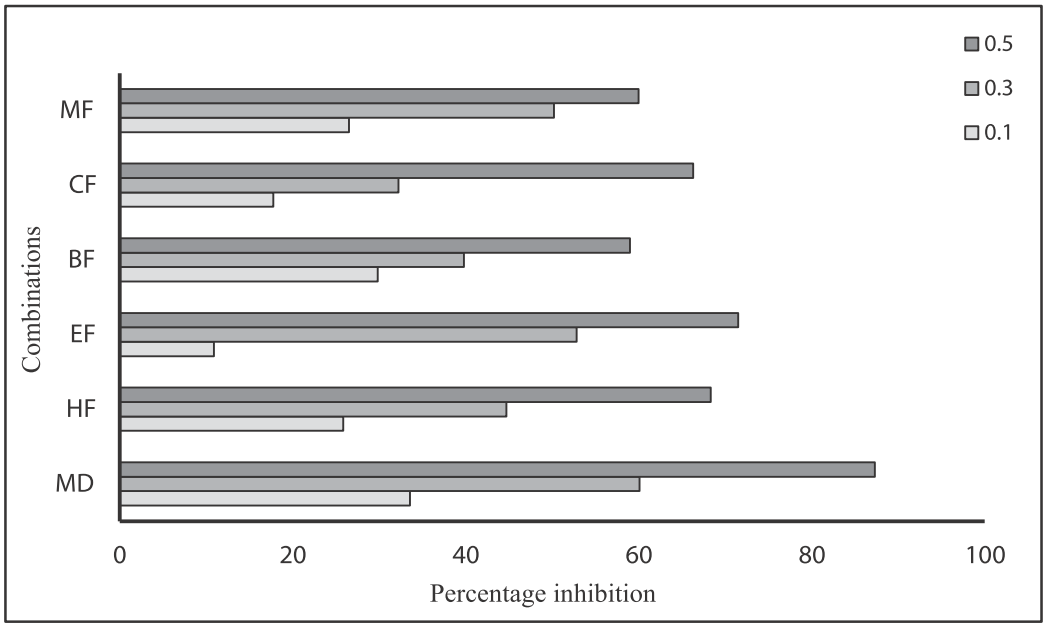


**Fig. 2.** Alpha amylase inhibition. MD: Metformin drug; HF: *n*-hexane fraction; EF: ethyl acetate fraction; BF: *n*- butanol fraction; CF: chloroform fraction; MF: Methanol fraction. Percent inhibition was calculated with 0.1, 0.3, 0.5 mL extract concentrations.

Chloroform fraction had least TPC. While other fractions had TPC in descending order as methanol > *n*-hexane > *n*-butanol. Compare to TPC, minimal disparities in TFC were observed in samples. Significantly higher TFC were measured ($p<0.05$) in ethyl

acetate fraction followed by *n*-butanol > methanol > *n*-hexane > chloroform. Ethyl acetate was found to be more effective in recovering the highest TPC and TFC. All the extract and fractions possessed free radical scavenging properties, but to a significantly varying degrees ($p< 0.05$), ranging from 55 to 77.3% DPPH scavenging (Table 1). For radical scavenging efficacy, *M. indica* ethyl acetate extract was the strongest ($p< 0.05$). For other partitions, DPPH scavenging activity in descending order was methanol > *n*-hexane > *n*-butanol > chloroform. In terms of IC50, all the extracts showed considerable activity as compared to BHT ($p< 0.05$).

Phenolic compounds in plants have multiple biological effects, including antioxidant activity (Choi *et al*., 2002). Our data regarding TPC and TFC was in accordance with that of the Sultana *et al*. (2009). Similarly, Palafox-Carlos *et al*. (2012) found four major phenolic compounds in *M. indica* pulp are chlorogenic, gallic, protocatechuic and vanillic acid and also found synergistic interactions between the major phenolic acids contributing total antioxidant activity. They reported 25.2-41.3 GAE (g/100 g dry weight) and 3.0-5.8 CE (g/100 g dry weight) TFC and TPC in *M. indica*. Total phenols and free radical scavenging ability have endless association. Higher the TFC, higher will be the antioxidant efficacy observable in DPPH methodology (Koleva *et al*., 2002). Likewise, DPPH activity measured in present results were analogous to DPPH activity (58-61%) stated previously (Kim *et al*., 2003).

***Antidiabetic activities***

Extract and fractions exhibited almost same noticeable ($p< 0.05$) yet time-dependent antiglycation patterns (30.65-66.85%) at the end of 5th week (figure 1). However, their overall inhibitory strengths was meager than that of synthetic inhibitor aminoguanidine (75%). Central theme in therapeutic options against diabetes induced such complications is to restrict hyperglycemia through the mediation of natural products (Fujiwara *et al*., 2011). Formation of advanced glycation end products are involved in the pathogenesis of diabetes mellitus. Therefore, targeting glycation should have a broad and beneficial impact on treating diabetes and related manifestations. Several herbal formulations have been shown to possess not only *in vitro* antioxidant but also antiglycation effects (Povichit *et al*., 2010). In vitro glycation inhibition portrayed by all fractions in present study was consistent with earlier data. Gupta and Gupta (2011) stated that *M. indica* possessed potent antiglycation activity. Our results further endorse the opinion that indigenous medicinal plants are potent antidiabetic remedying source.

Porcine pancreatic alpha amylase inhibitory activity is shown in the figure 2. Ethyl acetate fraction attained greatest (71.5%) amylase inhibitory activity as compared to other test samples. Least nonetheless significant ($p< 0.05$) enzyme inhibition was shown by *n*-butanol (59%). While, methanol, *n*-hexane and chloroform reduced amylase activity by 60-68% ($p< 0.05$). Decline in enzyme activity by *M. indica* was concentration-dependent. With 0.5mL extracts, percent inhibition was enhanced. Contrary to these inferences, synthetic inhibitor metformin exhibited remarkable enzyme inhibition (87%).

The inhibitory effects of dietary polyphenols against α-amylase have attracted great interest among researchers (Xiao *et al*., 2013). Alpha amylase inhibition by *M. indica* extracts in the current report are in agreement with the findings of Prashanth *et al*. (2001). They mentioned that ethanolic extracts of *M. indica* exhibited interesting α-amylase inhibitory activity.

**Table 2.** Phytochemical constituents

| Phytochemicals | Methanol extract | Water extract |
|---|---|---|
| **Tannins** | + | + |
| **Saponins** | + | + |
| **Phlobatannins** | - | - |
| **Terpenoids** | + | + |
| **Flavonoids** | + | + |
| **Alkaloids** | + | + |
| **Glycosides** | + | + |

Key: + = present, - = absent

Preliminary phytochemical analysis is presented in table 2. Imperative chemicals such as tannins, saponins, terpenoids, phenols, flavonoids, glycosides and alkaloids present in the *M. indica* leaves may be responsible for the antioxidant and antidiabetic

activities. Current inference was in agreement with the results of other studies. El-Mahmood (2009) revealed the presence of alkaloids, cardiac glycosides tannins, phenols, saponins in methanolic stem bark extract of *M. indica*. Similarly, tannins, glycosides, phenols, flavonoids and saponins were detected in leaf extracts of *Mangifera indica* (Farrukh *et al*., 2006; Doughari and Manzara, 2008; Luka and Mohammed, 2012). Ethanolic extract of *M. indica* flowers had alkaloids, phenols, flavonoids and carbohydrates (Parvathi *et al*., 2012). Future work directed at isolation, purification and characterization of active components may reveal new avenues in functional foods.

Our research exertion clearly indicates the considerable antioxidant and antidiabetic action of *M. indica* leaves extracts and fractions. This study endorses further studies directed at isolation, purification and characterization of active components that may reveal new avenues in functional foods.

**CONFLICT OF INTEREST**

Authors have no conflict of interest.

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