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Research Article

Chemical Composition and In-Vitro Study for Analysis of Potential Neuroprotective and Antidiabetic Activity in *Mimusops elengi* L.

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ABSTRACT
The aim of the study was to analyze chemical composition and inhibitory potential of leaf and flower extracts of *Mimusops elengi* L. against the enzymes α-amylase, α-glucosidase and acetylcholinesterase. Inhibitions of these enzymes are considered to be effective in controlling diabetes and memory function. Methanol extracts of leaves and flowers were tested against the enzymes α-amylase, α-glucosidase and acetylcholinesterase. Chemical composition in both the extracts were analysed by GC-MS, after derivatization. Leaf extract had stronger activity against the enzyme α-glucosidase than the flower extract. But the flower extract showed higher activity against the enzymes α-amylase and acetylcholinesterase. Total 41 components (7 organic acids, 9 sugars and sugar alcohols, 5 amino acids, 6 fatty acids, 8 phenols, 2 flavonoids, 1 inorganic acid, porphine, phytol and phenylethylamine) could be identified from both the extracts. The flower extract contained lesser metabolites both qualitatively and quantitatively. The study suggests potential beneficial effects of the extracts which needs further analysis in vivo for bioactivity and for identification of active constituents.

Keywords: *Mimusops elengi*, α-amylase, α-glucosidase, acetylcholinesterase, metabolites

INTRODUCTION

Plants are considered as chemical factories for synthesizing biochemicals and these bioconstituents are responsible for various types of disorder controlling activities towards animal system. Natural products are known to play important roles in pharmaceutical biology. Medicinal plants are the valuable and cheap source of unique phytochemicals which are frequently used in the development of drugs against various diseases. A large fraction of the world population, especially in the developing and underdeveloped countries still depends mainly on the traditional system of medicine. The use of plants and plant products in medicines is getting popularized because the herbal medicines are cheap, easily available and in having higher safety margins and lesser or no side effects[1]. *Mimusops elengi* L., Bullet wood tree and also called Spanish cherry, which is an Indian native plant and is used for a long time in the history of the medicine and also considered as sacred plant among Hindus. There are many reports on *M. elengi* as astringent, stomachics and some on its chemical constituents. Biological, pharmacological and phytochemical properties of *Mimusops elengi* L. has recently been reviewed[2,3]. During the present study we mainly concentrated on the activity of extracts from leaves and flowers in inhibiting the enzymes related to hyperglycemia and neural disorder along with the phytochemical constituents present in the extracts.

α-Amylase and α-glucosidase are key enzymes related to hyperglycemia which hydrolyze food carbohydrates to glucose and other monosaccharides. α-Amylase and α-glucosidase inhibitions are the powerful interventions to reduce the blood glucose levels[4,5]. Acetylcholinesterase (AChE), an enzyme present in the synapse, hydrolyses the ester bond within acetylcholin (ACh) by which electrical impulses carried by nerve cells are transmitted to another nerve cell or to voluntary and involuntary muscles, stored in the vesicles of nerve terminals, thus leading to loss of stimulatory activity. Inhibition of AChE, therefore, results in a prolongation of the existence and therefore the activity, of ACh[6].

MATERIALS AND METHODS

Plant material

Leaves and flowers were collected from Kolkata, India during the month of April, 2011 from the same plant. The collected materials were properly identified botanically (voucher no. JA-33228). The samples were then washed under running water and sun dried.

Extraction

The dried flowers and leaves were powdered and extracted with methanol by reflux for 5 hours. The organic solvent, after filtration, was evaporated to dryness to obtain crude extracts and preserved at -20° C for future use.

Chemicals

Acetylcholinesterase (AChE) from *Electrophorus electricus* (electric eel) was purchased from Sigma. Acetylthiocholine iodide (ACI) was obtained from Sisco Research Laboratories PVT. Ltd., India. α-amylase, α-

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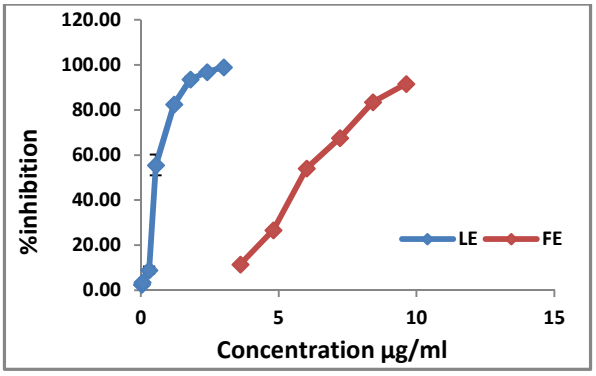

Figure 1: α-Glucosidase inhibition

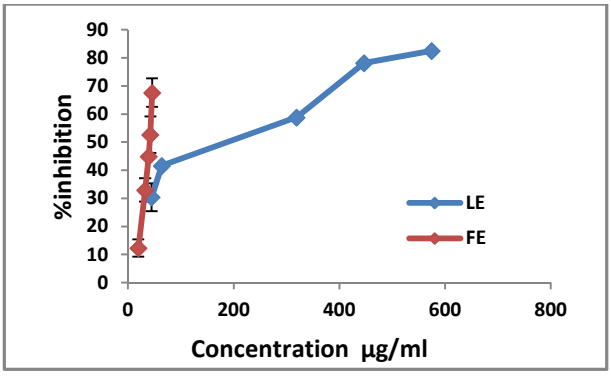

Figure 2: α-Amylase inhibition

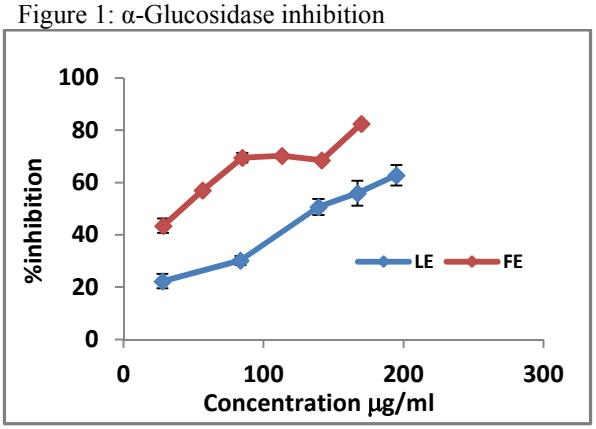

Figure 3: Acetylcholinesterase inhibition

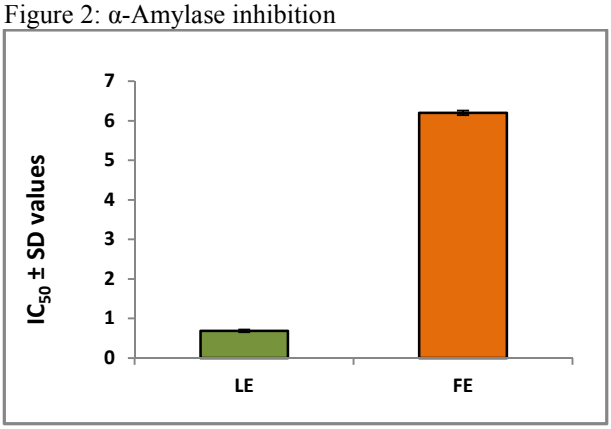

Figure 4: IC_{50} values for α-glucosidase inhibition

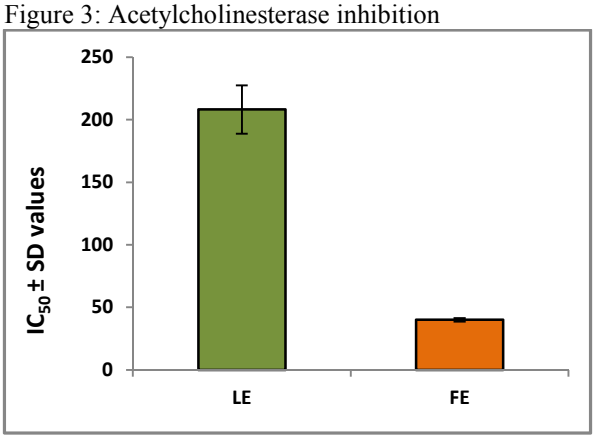

Figure 5: IC_{50} values for α-amylase inhibition

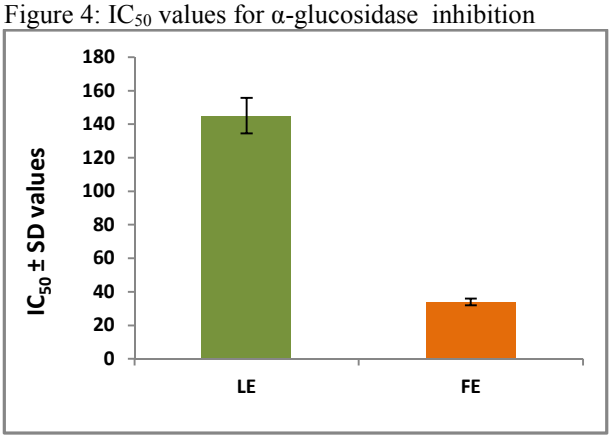

Figure 6: IC_{50} values for acetylcholinesterase inhibition

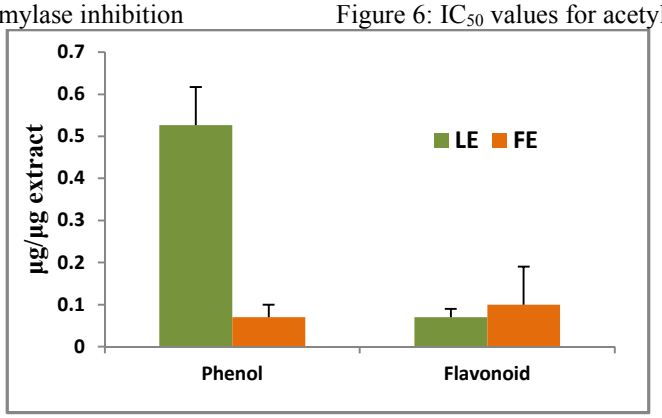

Figure 7: Total phenol and flavonoid contents in LE and FE

glucosidase, para-nitrophenyl-α-D-glucopyranoside (PNPG) were from Sisco Research Lab. Ltd., India. N-methyl-N-(trimethylsilyl) trifiuoroacetamide (MSTFA), methoxamine hydrochloride, ribitol and FAME (Fatty Acid Methyl Ester) were from Sigma. All other reagents were of analytical grade.

α-Amylase inhibition assay

α-Amylase inhibitory property was measured by following the modified method of Bernfeld[7]. The reaction mixture

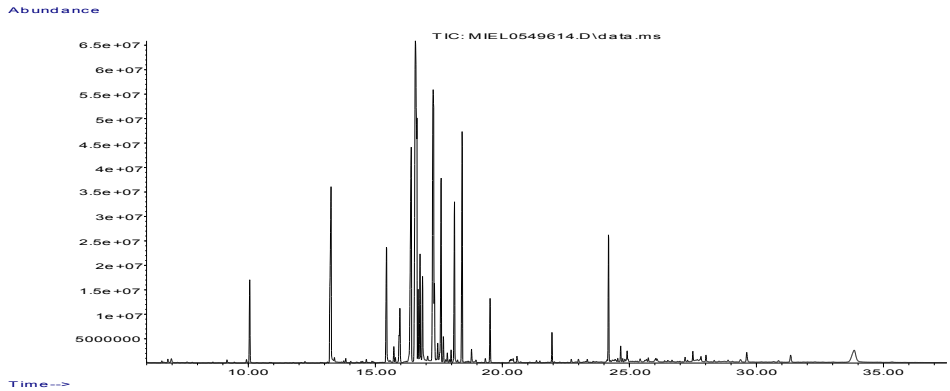

Figure 8: Total ion chromatogram of LE

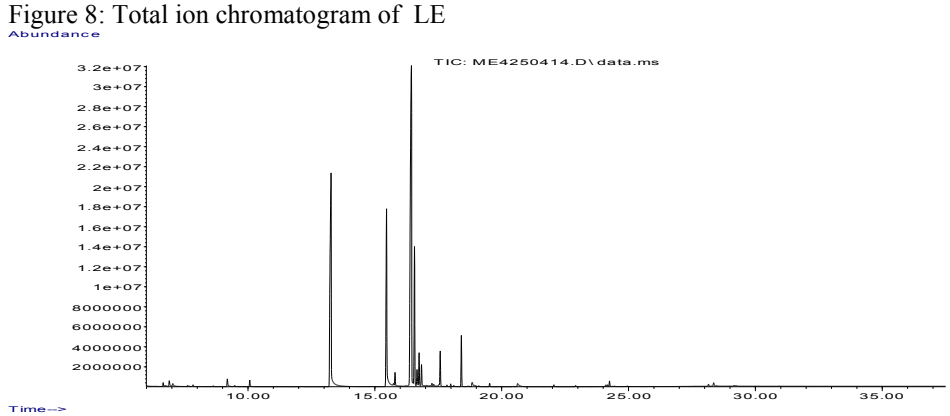

Figure 9: Total ion chromatogram of FE

consisted of extract and 0.05 ml of enzyme (ex porcine pancreas) (0.2 ml 0.003% solution) dissolved in 0.02 M phosphate buffer (pH 6.9). After incubation at 37°C for 20 minutes, 0.025 ml of starch solution was added and again incubated for 3 min at 37° C. Then 0.05 ml DNSA (Dinitrosalisylic acid) was added and the tubes were well plugged and heated in water bath for 5 min at 100° C. After cooling the mixture, 1 ml of distilled water was added and the absorbance was measured at 540 nm spectrophotometrically. Percentage inhibition was calculated by using a formula [(Ao-Ae)/Ao] x100 (Ao = Absorbance without extract; Ae = absorbance with extract).

α-Glucosidase inhibition assay

α-Glucosidase inhibitory property was measured by modifying the method of Kwon et al.[8].PNPG was used as a substrate. α-Glucosidase (ex microorganisms) solution (0.006%) was prepared in 0.02 M phosphate buffer (pH 6.3). In each set 0.034 ml of methanolic extract, 0.11 ml of phosphate buffer (pH 6.3) and 0.034 ml of enzyme were mixed. After 1 hour of incubation at 25° C, 0.17 ml of PNPG was added. Again the reaction mixture was incubated for 30 min at 30° C. The enzyme reaction was stopped by adding 0.5 ml of 1(M) Na_2CO_3 solution. Blank set consisted of 0.034 ml of buffer instead of enzyme. The control set prepared by adding methanol instead of extract. The optical density was measured at 405 nm spectrophotometrically. Percentage inhibition was calculated by using a formula [(Ao-Ae)/Ao] x100 (Ao = Absorbance without extract; Ae = absorbance with extract).

Acetylcholinesterase inhibition assay

Acetylcholinesterase (AChE) inhibitory property was measured following the modified method of Ellman[9]. AChE from electric eel was used for assay. Different concentrations of methanolic extracts (0.01ml) were added to 0.02 ml AChE and 1ml of buffer. The reaction was started by adding 0.01 ml 0.5 mM 5,5' dithiobis (2 nitrobenzoic acid) (DTNB) and 0.02 ml. 0.6mM ACI solution. The reaction mixture was incubated at 37° C for 20 min. The optical density was measured at 412 nm immediately. Percentage inhibition was calculated by using a formula [(Ao-Ae)/Ao]x100 (Ao = Absorbance without extract; Ae = absorbance with extract).

Determination of total phenol content

Total phenolic content in the extracts was determined by the modified Folin–Ciocalteu method[10]. The extract (3ml) was mixed with 0.5 ml Folin–Ciocalteu reagent. 2 ml (20%) sodium carbonate was added after 3 min of incubation at room temperature. The mixture was then placed in a boiling water bath for 1 min and then reading counted at 650 nm. Total phenol content was expressed as gallic acid equivalent (µg/µg crude extract).

Determination of total flavonoid content

Total flavonoid content was determined using the method of Kim et al.[11]. A volume of 0.4 ml of water, 0.03 ml $NaNO_2$ (5%) and 0.1 ml of sample (in methanol) were added and kept for incubation for 5 min at room temp. Then 0.03 ml $AlCl_3$ (10 %) was added and again the mixture was allowed to stand for 1 min of incubation. Then after addition of 0.2 ml NaOH (1 M) and 0.24 ml H_2O the absorbance was measured at 510 nm. Total flavonoid content was calculated as catechin equivalent (µg/µg crude extract).

NATURE OF COMPOUNDS	NAME OF COMPOUNDS	RT EXPECTED	RT OBTAINED	LE	FE
SUGAR AND SUGAR ALCOHOLS	Galactinol	26.479	26.453		
	Glycerol	9.941	10.057		
	Lactitol	24.877	24.828		
	Lactose	24.534	24.327		
	D-mannitol	17.81	17.984		
	D-(+) trehalose	24.752	24.949		
	D-threitol	12.954	13.125		
	Sucrose	23.988	24.179		
	Xylitol	15.376	15.469		
AMINO ACIDS	L-alanine	7.495	7.573		
	L-glutamic acid 3 (dehydrated)	13.232	13.347		
	DL-isoleusine	8.576	8.709		
	L-tyrosine	17.856	17.918		
	L-valine	9.151	9.213		
FATTY ACIDS	4-guanidinobutyric acid	13.348	13.395		
	Lauric acid	14.789	14.75		
	Linoleic acid	20.399	20.336		
	Oleic acid	20.504	20.447		
	Palmitic acid	18.864	18.849		
	Stearic acid	20.675	20.656		
PHENOLS	Arbutin	23.393	23.569		
	Benzoic acid	9.594	9.777		
	Gallic acid	18.012	18.113		
	Quinic acid	17.076	17.253		
	Shikimic acid	16.433	16.606		
	4-hydroxybenzoic acid	14.505	14.533		
	4-hydroxycinnamic acid	17.854	17.892		
	2-(4-hydroxyphenyl)ethanol	13.841	13.987		
FLAVONOIDS	(-)- epicatechin	25.472	25.678		
	Myricetin	27.941	28.014		
ORGANIC ACIDS	Glyceric acid	10.735	10.844		
	Glycolic acid	7.049	6.899		
	L-(+) lactic acid	6.851	6.945		
	Lactobionic acid	24.583	24.745		
	D-malic acid	12.794	12.911		
	Oxalic acid	7.883	7.674		
	Succinic acid	10.509	10.573		
INORGANIC ACID	Phosphoric acid	9.966	10.071		
OTHERS	Phenylethylamine	13.766	13.841		
	Phytol	20.026	20		
	Porphine	10.77	10.941		

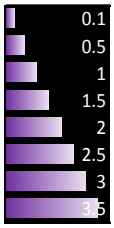

Figure 10: Comparison of log of relative response ratios of metabolites identified in LE and FE

GC-MS analysis

GC-MS analysis was carried out following the method of Kind *et al*.[12]after modification. HP-5MS capillary column [Agilent J & W; GC Columns (USA)] (length 30 m plus Duraguard 10 m, diameter 0.25 mm narrow bore, film 0.25 µm) was used. The analysis was performed under the following oven temperature programme: Injection with fast plunger speed without viscosity delay or dwell time, oven ramp 60ºC (1 minute hold) to 325ºC at 10ºC/minute, 10 minute hold before cool – down, 37.5 minute run time. The injection temperature was set at 250º C; the MS transfer line at 290º C and the ion source at 230º C. Helium was used as the carrier gas at a constant flow rate of 0.723ml / min (carrier linear velocity 31.141 cm/sec). The dried extracts were derivatized after using methoxamine hydrochloride (20 mg/ml in Pyridine) and subsequently with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) to increase volatility of metabolites. 2 µl FAME (Fatty Acid Methyl Esters) markers [a mixture of internal Retention Index (RI) markers was prepared using fatty acid methyl esters of C8, C10, C12, C14, C16, C18, C20, C22, C24 and C26 linear chain length, dissolved in chloroform (HPLC) at a concentration of 0.8 mg/ml (C8-C16) and 0.4 mg/ml (C18-C26)] was added. Derivatized samples (1 µl) were injected via the split mode (Split ratio 10:1) onto the GC column. Automated Mass Spectral Deconvolution and Identification System (AMDIS) was used to deconvolute GC-MS to identify chromatographic peaks. Identification of the metabolites was carried out by comparing the fragmentation patterns of the mass spectra and retention times (Rt) and retention indices (RI) with entries of mass spectra, Rt and RI in Agilent Fiehn Library. The relative response ratios of all the metabolites were calculated after normalizing the peak areas of the compounds by extract dry weight and peak area of internal standard.

Statistical analysis

All the experiments were performed at least thrice. Mathematical calculations like means, standard deviations were calculated from replicas within the experiments and analyses have been done using Microsoft Excel 2007.

RESLTS AND DISCUSSION

The present work was carried out in continuation to our search for enzyme inhibitors from medicinal plants[13-16]. The methanol extracts of the leaf and flower of *M. elengi* were analysed for their activities against these enzymes α-glucosidase, α-amylase and acetylcholinesterase. Both the extracts inhibited the key enzymes α-glucosidase and α-amylase, related to diabetes, in a dose dependent manner (Fig. 1 and Fig. 2). Both the extracts also inhibited AChE in a dose dependent manner (Fig. 3). The IC_{50} values to determine the concentrations required for inhibition of the enzymes by 50% were calculated from the regression equations prepared from concentrations versus percentage inhibitions. It is apparent that leaf extract (LE) has significantly lower value indicating higher activity against the enzyme α-glucosidase (Fig. 4). However the LE showed significantly higher IC_{50} value than flower extract (FE) depicting lower activity against α-amylase inhibition (Fig. 5).The activity as measured by the IC_{50} value (Fig. 6) indicates that FE had significantly higher activity than that of LE against the enzyme AChE. Total phenol content was detected much higher in LE in comparison to FE although total flavonoid content was found to be lesser in quantity in LE than in FE (Fig. 7).

Both the LE and the FE were analysed following a metabolomic approach using Gas Chromatography and Mass Spectrometry (GC-MS). Total ion chromatograms of LE (Fig. 8) and FE (Fig. 9) indicate that LE contains more constituents than that of FE. Total 41 components (7 organic acids, 9 sugars and sugar alcohols, 5 amino acids, 6 fatty acids, 8 phenols, 2 flavonoids, 1 inorganic acid, porphine, phytol and phenylethylamine) could be identified. Log values of relative response ratios expressed per gm dry weight of extracts have also been compared in the form of heat map (Fig. 10). From the comparative bars, it is clear that LE contains more fatty acids and phenols than that of FE. LE contains more sugar than that of FE. Less organic acids and no detectable amino acids in FE is noteworthy. Along with GC–MS analysed data LE showed high total phenol content too.

The flower extract demonstrated higher α-amylase and acetylcholinesterase inhibitory activity than leaf extract in spite of having lower level of chemical constituents identified and total phenol content. So, there may be some other unidentified constituents in the methanol extract and their combined effects impart higher activity against the said enzymes by the flower extracts.

CONCLUSION

The leaf and flower extracts of *Mimusop elengi* inhibited the enzymes α-amylase, α-glucosidase and acetylcholinesterase. However no correlation could be established between activity and chemical composition, which suggests that there may be some unknown compounds responsible for such activities. So further analysis is necessary to identify the active principles and to establish the activity in vivo.

ACKNOWLEDGEMENTS

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