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RESEARCH ARTICLE

Phytochemical Analysis by HR-LCMS and *In vitro* Anti-diabetic Potential of *Michelia champaca* Bark

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Abstract

The current analysis was aimed to study the phytochemical profile and *in vitro* antidiabetic capacity of HEMC bark. HR-LCMS[1] was used to identify the phytochemicals present in the extract. The outcomes of HR-LCMS showed the presence of 15 phytochemical compounds. DL-Carnitine, Catechin, D-α-Tocopherol, Colchicine, Myricetin, Epicatechin, Quercetin, Epigallocatechin gallate, Quercetin-3β-D-glucoside, Kaempferol, Sorbic acid, Apocynin, Epigallocatechin gallate, myricetin 3-O-beta-D-galactopyranoside, Naringeninchalcone are the main compounds identified. The inhibition of enzymes like α-amylase and α-glucosidase delays the rate of glucose absorption thus reducing blood glucose levels in the experimental models. The IC50 values of α-amylase and α-glucosidase inhibitory activity of HEMC were acquired to be 88.65 µg/mL and 71.28 µg/mL correspondingly. Positive control acarbose displayed IC50 assessment of 52.94 µg/mL and 50.01 µg/mL correspondingly. Consequently, the current study confirms that HEMC had remarkable antidiabetic activity and hence holds future potential as nutraceuticals in the treatment of diabetes and related ailments.

Keywords: HR – LCMS, Antidiabetic, *Michelia champaca*, Bark, α-amylase, α-glucosidase

Abbreviations

HEMC - Hydro alcoholic extracts of *Michelia champaca* Linn. (Magnoliaceae) bark.
Gms – Grams
Hrs - Hours
HRLCMS - High Resolution Liquid Chromatography Mass Spectrometry
PH – Potential of hydrogen
PPA – Porcine Pancreatic Amylase
PBS – Phosphate Buffer
NaCl – Sodium chloride
DNSA – 3, 5 – Dinitro salicylic acid
PNPG – P – nitro phenyl – α - glucopyranoside
IC50 – Half maximal inhibitory concentration

1. Introduction

There are a number of oral hypoglycaemic agents accessible for the management of TYPE II diabetes; tranquil there is an augmented ultimatum by patients to practice natural products through the antidiabetic activity as of the side effects of the synthetic drugs. Diabetes mellitus is a serious complex multifactorial disorder characterized by hyperglycemia and glucose intolerance, either due to relative deficiency in insulin secretion or impaired the effectiveness of insulin's action to enhance glucose uptake.

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Michelia champaca Linn. belongs to the family Magnoliaceae[2], commonly known as champaca, and is a medicinally important plant. It encompasses 12 genera and 220 species of evergreen trees and shrubs, intuitive to tropical and subtropical South and Southeast Asia, encompassing Southern China[3]. In India, it is highly disseminated in the Eastern Himalayan tract, Assam, Myanmar, Western Ghats, South India, Arunachal Pradesh, and Bihar. Different parts of this plant are used in various ailments in folk medicine. The bark of *Michelia champaca*[4,5] Linn has been proved to contain phenolics, tannins, terpenoids, and flavonoids. By tradition, this plant bark is utilized to treat diabetes, and leaves are used for the therapy of fever, colic, leprosy, postpartum fortification, and eye illnesses. Juice of the leaves is indicated with honey in colic[6,7]. Therefore, determination of its efficacy is very important as this plant play a significant role in the management of Type II Diabetes Mellitus.

The current study aimed to explore the primary phytochemicals and HRLCMS was executed to discrete and isolate the phytoconstituents present in *Michelia champaca* established on their retention time and M - cloud best match. The HEMC was appraised for their *in vitro* antidiabetic activity exploiting α-amylase and α-glucosidase inhibitory activity.

2. Materials and Methods

2.1 Collection of Plant Specimens

The plant specimen for the proposed report was accumulated from the Tirupati District of Andhra Pradesh in the month of February 2018. It was recognized and then authenticated by Prof. Jayaraman, Director of Plant Anatomy Research Centre, West Tambaram, and Chennai. The plant specimen no. PS-01 was stored in our laboratory for future reference.

2.2 Preparation of Plant Extract

About 500 gms of bark powder remained to Soxhlet extraction with 1500 ml of the hydro alcohol (30:70) for 8 to 10 hrs (60–70 ^{0}c). The extract was then subjected to distillation to remove excess solvent. The semisolid mass obtained was then dried in a rotary evaporator to get dry powder. The dried hydroalcoholic extract of *Michelia champaca* bark (HEMC) was used for the present study.

2.3 HRLCMS Analysis of Hydro Alcoholic Extracts of *Michelia champaca Bark*

The HR-LCMS of HEMC was fetched out in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, and Mumbai. Chemical fingerprints of HEMC were developed by Agilent high-resolution liquid chromatography and mass spectroscopy model. The compounds were recognized via their mass spectra and their unique mass fragmentation patterns[8].

2.4 *In Vitro* Antidiabetic Activity

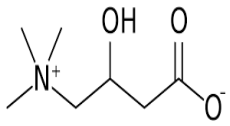
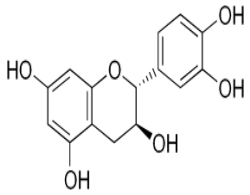
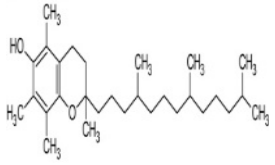
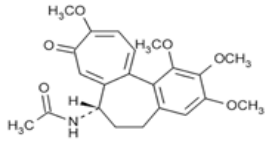
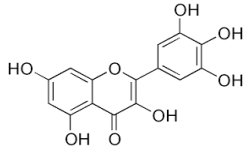
2.4.1 α-Amylase Inhibitory Assay

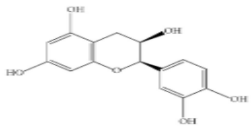
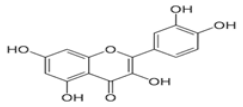
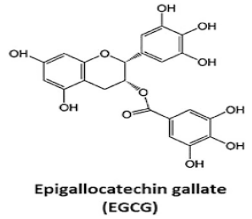
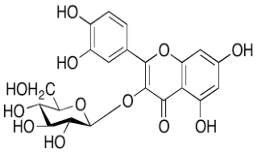
A 1% w/v of the starch solution was concocted by assimilating 1 g of starch in 100 mL of 20 mM of phosphate buffer (pH 6.9) comprising 6.7 mM salts. The enzyme solution was concocted by mixing 27.5 mg of pancreatic Porcine amylase (PPA) into 100 mL of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7 mM of NaCl. To 100 µL of (20-100 µg/mL) the HEMC, 200 µL porcine pancreatic amylase was swarmed, and the mixture was nurtured at 37 ^{o}C for 20 min. The reaction mixture 100 µL of 1% starch solution was combined and incubated at 37 ^{o}C for 10 min. The reaction was ended by placing 200 µl DNSA and setting it aside in a hot water bath for 5 min. The reaction mixture was distilled with 2.2 mL of water and absorbance was read at 540 nm. For each concentration, blank tubes were primed by replenishing the enzyme solution with 200 µL in distilled H_2O. Control, representing 100% enzyme activity, was prepared similarly, without extract. Acarbose was used as the reference standard. The conducted test was recapped thrice using a similar protocol[9].

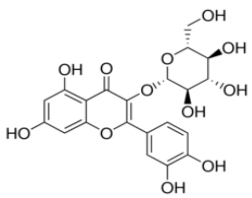
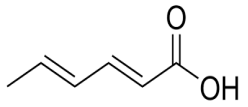
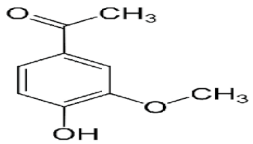
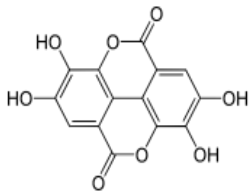
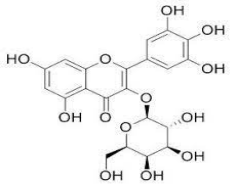
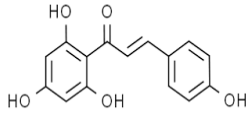
2.4.2 α-Glucosidase Inhibitory Assay

Inhibition of α- glucosidase activity of HEMC was resolved using p- nitrophenyl-α-D- glucopyranoside (pNPG) technique as described in Baron *et al*[9]., From stock solution (1 mg/mL in 5% DMSO) different concentrations of HEMC, acarbose (20–100 µg/mL) were concocted. Each of the HEMC and standard solution (500 µL) was added to 50 µL of α- glucosidase (effective concentration 1 U/mL) concocted in 0.1 M phosphate buffer (pH 6.9). Then, 250 µL of 0.1 M phosphate buffer was added to get the final concentrations. We have pre-incubated the mixture for 20 min at 37°C. Then added 10 µL of 10 mM pNPG (0.1 M

Table 1. HR-LCMS of HEMC

S.NO.	Name of the compound	Formula	Structure	Molecular weight	Retention time	M- cloud best match	Compond class	Activity
1	DL - Carnitine	$C_7 H_{15} N O_3$		161.10483	1.092	86.5	Amino acid	Acetyl-L-Carnitine was well endured in all of the patients and may stipulate a novel therapeutic contrivance for the treatment of arterial hypertension, and of dyslipidemia and committed be securely exploited in people with type 2 diabetes
2	Catechin	$C_{15} H_{14} O_6$		290.0782	6.969	83.9	Natural Poly phenolic compound	Catechin is one of the utmost flavonoids through moderately high antioxidant content. Some experimental readings testified antidiabetic, hypolipidemic, and antioxidative properties
3	D-α-Tocopherol	$C_{29} H_{50} O_2$		430.38032	25.861	97.1	organic compounds	Vitamin E supplementation has an crucial role in delaying the onset of the diabetic complications as well as for decelerating down the progression of the complications
4	Colchicine	$C_{22} H_{25} N O_6$		399.1675	13.856	90.2	Alkaloid	Colchicine could ominously reduce blood glucose levels, both fasting and post-prandial
5	Myricetin	$C_{15} H_{10} O_8$		318.03667	11.406	96	poly phenolic compound	Myricetin has been discerned to intensification the endeavor of glycogen synthase 1 in the hepatocytes of rats with diabetes

6	Epicatechin	$C_{15}H_{14}O_6$		290.0782	5.058	97.7	poly phenolic compound	Epicatechin has been revealed to diminish blood glucose levels in diabetic patients, during which is anticancer effect was indorsed to its antioxidant possessions, antiangiogenic and unswerving cytotoxicity to cancer cells
7	Quercetin	$C_{15}H_{10}O_7$		302.04195	13.205	60.4	Flavonoid	Quercetin ameliorates hyperglycaemia and dyslipidaemia and convalesce antioxidant significance in type 2 diabetes.
8	Epigallocatechin gallate	$C_{22}H_{18}O_{11}$		458.08391	9.161	97.3	poly phenolic compound	EGCG detrimentally curbs glucose and lipid metabolism in H4IIE cells and prominently enriches glucose tolerance in diabetic rodents. Dietary supplementation with EGCG might potentially subsidise to nutritional stratagems for the prevention and therapy of type 2 diabetes mellitus
9	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$		464.09572	11.814	80.3	Flavonoid	Quercetin-3β-D-glucoside as an auspicious agent critical of type 2 diabetes. Copious prospective cellular, animal, and clinical studies provide significant substantiation to consider bioflavonoids as hypothetical therapeutic agents for the therapy of diabetes and its impediments

10	Kaempferol	$C_{15}H_{10}O_6$		286.04708	12.88	75.6	Flavonoid	Kaempferol diminished the prevalence of overt diabetes from 100% to 77.8%, whereas the proportion of diabetic mice in the control group persisted at 100% with glucose levels of in excess of 400 mg/d L .Oral administration of Kaempferol expanded glucose control in STZ-induced diabetic rats.
11	Sorbic acid	$C_6H_8O_2$		112.05217	1.5	48.7	polyun saturated fatty acid	Sorbic acid is an innately ensuing compound that's suited the most recurrently used as preservative.
12	Apocynin	$C_9H_{10}O_3$		166.06268	7.829	68.7	Natural organic compound	Insulin intransigence
13	Ellagic acid	$C_{14}H_6O_8$		302.00554	12.504	95.9	Polyphenol ic	Ellagic acid (EA) has been recently complicated with type 2 Diabetes utilizing anti-diabetic activity through conflict on pancreatic β-cells resulting in augmented size and number of β-cells, amplified antioxidant status, diminished blood glucose and increased serum insulin
14	Myricetin 3-O-beta-D-galactopyranoside	$C_{21}H_{20}O_{13}$		480.08931	10.534	84.9	Flavanoid	Myricetin 3-O-beta-D-galact – pyranoside has been monitored to intensification the activity of glycogen synthase 1 in the hepatocytes of rats with diabetes
15	Naringeninchalcone	$C_{15}H_{12}O_5$		272.06774	11.505	51.4	Polyphenol compound	Naringeninchalcone supplementation is expedient for the controlling of obesity, diabetes mellitus, hypertension, and metabolic condition.

phosphate buffer pH 6.9) and incubated again for 30 min at 37°C. 650 µL of 1 M sodium carbonate was augmented to stop the reaction and spectrophotometric absorbance was taken at 405 nm against the blank reagent. Acarbose was used as the reference standard. The experiment was repeated thrice using the same protocol.

The concentration of the extract required to inhibit 50% of α-amylase and α-glucosidase activity beneath the assay conditions was defined as the IC_{50} value. IC_{50} was calculated by using the percentage inhibition activities at various concentrations of HEMC, using linear regression analysis[11].

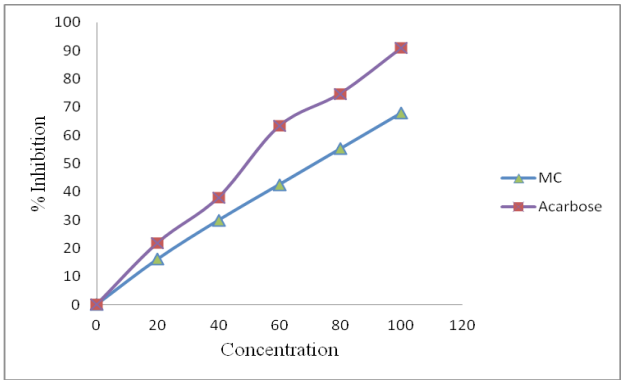


Figure 1. α-Amylase inhibitory activity.

3. Results and Discussion

3.1 Yield

The percentage yield of HEMC was originate to be 69.3 gms (w/w).

3.2 HR-LCMS Interpretation

In HR-LCMS elucidation, the compounds like DL-Carnitine, Catechin, D-α-Tocopherol, Colchicine, Myricetin, Epicatechin, Quercetin, Epigallocatechin gallate, Quercetin-3β-D-glucoside, Kaempferol, Sorbic acid, Apocynin, Ellagic acid, myricetin 3-O-beta-D-galactopyranoside, Naringeninchalcone were present in *Michelia champaca* (Bark) on their retention time and M - cloud best match. The results are presented in Table 1.

3.3 *In vitro* Antidiabetic Activity

3.3.1 α-Amylase Inhibitory Activity

Percentage inhibition paraded by *Michelia champaca* (Bark) IC_{50} value 88.65 µg/mL and acarbose concealed IC_{50} value of 52.94 µg/mL. The outcomes were presented in Table 2.

The percentage inhibition exhibited by *Michelia champaca* (Bark) extract in α-amylase inhibitory activity was shown in Figure 1, IC_{50} value 88.65 µg/mL and standard displayed lower IC_{50} value of 52.94 µg/mL.

Table 2. α-Amylase inhibitory activity (Standard and Sample)

HEMC	Standard	Sample
20	22.8	10.47
40	39.04	20.9
60	60	35.2
80	75.2	43.8
100	91.42	51.4

3.3.2 α-Glucosidase Inhibitory Activity

Table 3 shows that there was a dose-dependent escalation in the percentage inhibitory activity contrary to α-glucosidase enzyme. *Michelia champaca* (Bark) and acarbose at an extreme concentration of 100 µg/mL specified a percentage inhibition of 67.81±2.3 and 90.8±2.71 respectively. IC_{50} values of *Michelia champaca* (Bark) was constitute to be 71.28 µg/mL. The standard positive control, acarbose revealed an IC_{50} value of 50.01 µg/mL as represented in Figure 2.

It is well known that suppression of starch digestive enzymes like α-amylase and α-glucosidase condense elevation of postprandial blood glucose levels. The

Table 3. α-Glucosidase inhibitory activity (Standard and Sample)

HEMC	Standard	Sample
20	21.84	16.09
40	37.93	29.88
60	63.21	42.52
80	74.71	55.17
100	90.8	67.81

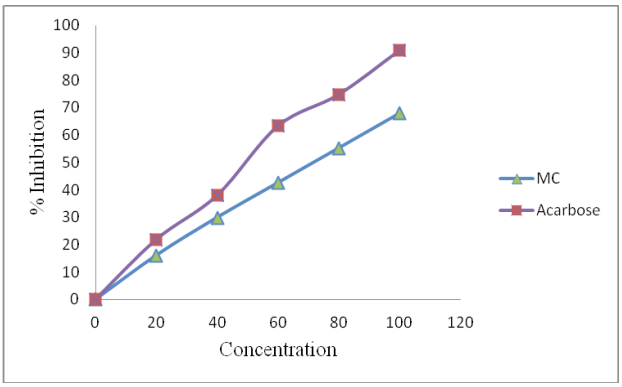


Figure 2. α-Glucosidase inhibitory activity.

contemporary study demonstrated that hydro alcoholic extracts of *Michelia champaca* bark, had substantial *In vitro* α-amylase then α-glucosidase inhibitory activity. The IC_{50} values of all the extracts were comparable to that of acarbose, which is a marketed antidiabetic drug. This consequence infers beneficial effect in minimizing postprandial blood glucose level in diabetic patients through impeding breakdown and intestinal absorption of dietary carbohydrates.

4. Conclusion

The Present study quantified that HR – LCMS and *In vitro* antidiabetic activities of *Michelia champaca* bark extract which influence due to the occurrence of secondary plant metabolites like phenolic compounds, flavonoids, and tannins are the main compounds identified which has been narrated to influence antidiabetic properties. The existing data suggested that the extracts arises direct and potent antioxidant activities through multiple mechanisms. *Michelia champaca* Linn. bark exert its hypoglycaemic activity independent of insulin and through restoring or maintaining the health and proper functioning of the beta-cell and the pancreas. The possible mechanisms of antidiabetic action of *Michelia champaca* Linn. bark linked to strong proliferative and antioxidative effects and interactions with insulin receptors. This observed antidiabetic activity of this extract might be due to their phytochemical constituents reported by HR - LCMS. The findings from this study therefore support the folkloric usage of *Michelia champaca* Linn. bark in the treatment of diabetes.

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