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UNVEILING THE PHYTOCHEMICAL AND IN VITRO ANTIDIABETIC EFFECT OF MACARANGA PELTATA

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ABSTRACT

Diabetes mellitus (DM) is a chronic endocrine disorder affecting a significant portion of the global population, leading to severe complications such as cardiovascular diseases, neuropathy, and renal failure. The current study investigates the anti-diabetic potential of phytoconstituents extracted from *Macaranga peltata* using in vitro assays. The ethanolic extract of *Macaranga peltata* (EEMP) was prepared and subjected to preliminary phytochemical screening, revealing the presence of flavonoids, tannins, saponins, alkaloids, proteins, and carbohydrates. The *in vitro* anti-diabetic activity was evaluated using α-glucosidase and α-amylase inhibition assays. EEMP demonstrated significant α-glucosidase inhibition, with an IC_{50} value of 10.29 µg/mL, comparable to the standard drug acarbose (IC_{50} = 16.12 µg/mL). Similarly, α-amylase inhibition showed an IC_{50} value of 11.92 µg/mL for EEMP, indicating strong enzyme inhibitory potential. These findings suggest that *Macaranga peltata* may serve as a promising natural source for anti-diabetic drug development.

KEYWORDS: Diabetes mellitus, Ethanolic Extract of *Macaranga peltata*, *In vitro*, Flavonoid, Phytoconstituents.

1. INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder that affects more than 6% of our total population. It is caused by ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood.[1] The presence of DM shows increased risk of many complications such as cardiovascular diseases, peripheral vascular diseases, stroke, neuropathy, renal failure, retinopathy, blindness, amputations etc.[2]

The primary goal of treatment is to regulate blood sugar levels and prevent complications. Type 1 DM requires insulin therapy, while type 2 DM is managed through lifestyle modifications and oral hypoglycemic agents. However, current medications have limitations, including side effects and lifelong dependency. Medicinal plants and bioactive compounds offer alternative therapeutic potential, especially in regions with limited access to conventional treatments. Experimental models help evaluate their antidiabetic properties.[3,4]

Various plants are being used in complementary and alternative medicines for management of diabetics. Synthetic drugs available for treatment of diabetics have various adverse effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease.

Macaranga peltata is one of the important plant widely found in Western Ghats in India. *Macaranga peltata* belonging to Euphorbiaceae family is a genus of important pioneer trees widely distributed in south East Asia. It is a small tree growing to a height of about 15 m widely distributed in the primary and secondary forests of India, generally in high light environments. It belongs to the genus Macaranga with about 300 species distributed worldwide. About 12 species are found in

India.[5] There are evidences that show that bergenin derivatives and polyphenols have been recorded from *Macaranga peltata* and other flavonoids as well as diterpenoids are suspected to be present. Leaf extract showed the presence of carbohydrates, steroids and sterols, glycosides, flavonoids, tannins, proteins and amino acids while Stem Bark extract showed positive results for carbohydrates, glycosides, saponins, flavonoids, tannins, proteins and amino acids.[6]

Figure no.1: Whole plant of *Macaranga peltata.*

2. MATERIALS AND METHODS

- **Description of plant material**

Table No 1: Description of plant material.

SL NO.	PLANT NAME	PART USED	FAMILY	COLLECTION PERIOD	PLACE OF COLLECTION
1.	*Macaranga peltata*	Leaf, stem, flower, root.	Euphorbiaceae	December	Thiruvananthapuram

- **Reagents used in phytochemical analysis**

Table No 2: Reagents used in phytochemical analysis.

SL NO.	REAGENTS	USED FOR
1.	Ethanol	Extraction
2.	Magnesium chips	Test for flavonoids
3.	Lead acetate	Test for flavonoids
4.	Alkaline reagent	Test for flavonoids
5.	Ferric chloride	Test for tannin
6.	Distilled water	Test for saponin
7.	Dragendorff's reagent	Test for alkaloid
8.	Fehling's solution	Test for carbohydrate
9.	Nitric acid	Test for proteins

2.1 METHODS

2.1.1 Collection and authentication of *Macaranga peltata*

The whole plant of *Macaranga peltata* is collected from Thiruvananthapuram in the month of December. The plant material was identified and authenticated from Post Graduate and Department of Botany, Christian College, kattakada, Thiruvananthapuram. The whole plant were thoroughly washed with water to remove dust and air-dried at room temperature in the shade for approximately two weeks until moisture-free. The dried leaves were then powdered using a mechanical grinder and stored in an airtight container to maintain their integrity.

Figure No. 2: Dried Powdered Plant of *Macaranga peltata.*

2.1.2 Preparation of ethanolic extract of *Macaranga peltata*[7]

- Whole plant of *macaranga peltata* were washed thoroughly with fresh water and air dried at room temperature under shade for 15 days and pulverized into coarse powder by aid of mechanical grinder.
- The powdered *Macaranga peltata* leaves were subjected to maceration with ethanol (75%) in a ratio of 1:4, mixture was left inside a sealed 1000 ml conical flask for 48 hours at room temperature.
- After 1 week the solution was first filtered using a muslin cloth and then through filter paper. The filtrate was concentrated using a water bath and then dried in a vacuum oven.
- The crude EEMP was preserved in a refrigerator for further

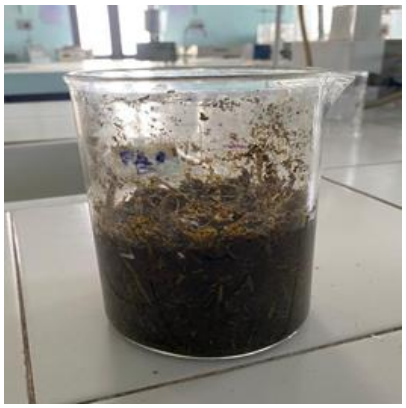

Figure No. 3: Ethanolic extract of *Macaranga peltata.*

2.1.3 Calculation of percentage yield

The percentage yield of extract was calculated using the formula;

$$\textbf{Percentage yield} = \frac{w2}{w1} x100$$

Where,

W_1- weight in grams of dried plant material

W_2-weight in grams of extract obtained

2.1.4 Preliminary Phytochemical Screening[8-11]

1. Test for flavonoids

a) Shinoda's test

About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

b) Lead acetate test

Mix 2ml of extract with 1ml of 10% of lead acetate. Formation of bulky yellow precipitation indicates a positive test.

c) Alkaline reagent test

1ml of extract is treated with 2ml of 2% of NaOH solution and add few drops of dil. HCl, formation of an intense yellow become colourless on addition of diluted acid.

2. Test for Tannin

a) Ferric chloride test

About 0.5 g each portion was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

3. Test for Saponin

a) Foam test

One gram of each portion was boiled with 5 ml of distilled water, filtered. To the filtrate, about 1 ml of distilled water was further added and shaken vigorously for about 5 minutes.

Frothing which persisted on warming was taken as evidence for the presence of saponins.

4. Test for alkaloid

a) Dragendroff's test

2 mL of extract was warmed with 2% H_2SO_4. Few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids.

5. Test for Carbohydrate

a) Fehling's Test

Boiled 1ml of the extract with 1ml of Fehling solution A and 1ml of Fehling solution B on a water bath for 30 mins. Red to yellow precipitate is formed.

6. Test for Proteins

a) Xanthoproteic test

2ml of extract was boiled with 1ml of con. HNO_3. Cooled the solution and made alkaline with 40% sodium hydroxide. Yellow colour changes to orange colour.

2.1.5 *IN VITRO* STUDY OF *MACARANGA PELTATA*

1. α -GLUCOSIDASE INHIBITION ASSAY[12]

Principle

Under specified conditions (pH = 6.8; T = 37 °C), α -glucosidase enzyme will catalyse the conversion of the substrate 4-nitrophenyl-α-D-glucopyranoside to α-D-glucopyranoside and p-nitrophenol. The yellow colour of the latter product is measured spectrophotometrically at 400 nm.

PNPG + α-glucosidase → α-D-glucopyranoside + PNP (yellow colour)

Procedure

400 µl of α-glucosidase (0.067 U/ml) was preincubated with different concentration of the sample for 30 min. Then 200 µl of 3.0 mM (pNPG) used as substrate dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-coloured para- nitro phenol released from pNPG at 400 nm. The results were expressed as percentage of inhibition. Same procedure was done with Acarbose (1mg/ml stock) which was used as standard.

Inhibitory activity (%) = (B-T/B-C) ×100

Where,
B is the absorbance of blank.
T is the absorbance in the presence of test substance.
C is the absorbance of control.

2. α-AMYLASE INHIBITION ASSAY[13]

Principle

The α -amylase activity is measured using a colorimetric method with using Miller's method, i.e., the DNS method (3,5-dinitrosalicylic acid reagent). In this method, starch by α – amylase is converted into maltose. Maltose released from starch is measured by the reduction of 3,5-dinitrosalicylic acid.

Procedure

500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml of α-amylase enzyme and different concentration of the test sample as enzyme inhibitor were pre-incubated at 37°C for 10 min. After the pre-incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at room temperature for 5 mins. The reaction was stopped using 1.0 ml of dinitro salicylic acid (DNSA) reagent. The test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The volume of the reaction mixture was made up to 10ml by adding distilled water, and the absorbance was measured at 540 nm using UV-Visible spectrophotometer. The absorbance was compared with the controls and blank that contained buffer instead of test sample.

Calculation

$$\textbf{Percentage inhibition} = \frac{(B-A)\ X\ 100}{(B-C)}$$

Where,
C- Absorbance of the Control with starch and without alpha amylase
B- Absorbance of the Control with starch and alpha amylase
A- Absorbance of the Test.

3. RESULTS

3.1 Preparation of whole plant extract of *Macaranga peltata* and the calculation of percentage yield

The whole plant were collected from certain areas of Thiruvananthapuram district, Kerala, India. The powdered plant material extracted with ethanol for 72 hours by using maceration. Then it is stored in an air tight container until further use.

Table no. 3: Percentage yield of *Macaranga peltata.*

Name of Plant	Plant Part Used	Method of Extraction	Solvent Used For Extraction	Percentage Yield (% w/w)
Macaranga peltata	Leaf, Stem, Bark, root.	Maceration	Ethanol	6% w/w

3.2 Preliminary phytochemical screening

The whole plant extract of *Macaranga peltata* were subjected to screening for its phytochemical constituents. The presence of flavonoids, tannins, phenol, saponins, alkaloids, proteins, carbohydrates are determined by conducting chemical test such as Shinoda test, ferric chloride test, foam test, etc.

Table no. 4: Result of phytochemical screening.

Sl no.	Name of The Constituents	Name of The Test	Inference
1.	Flavonoids	Shinoda's test	+
		Lead acetate test	+
		Alkaline reagent test	+
2.	Tannins	Ferric chloride test	+
3.	Saponins	Foam test	+
4.	Alkaloids	Dragendorff's test	+

5.	Carbohydrate	Fehling's test	+
6.	Proteins	Xanthoproteic test	+

(+) – positive
(-) – Negative

3.3 *In vitro* study of *Macaranga peltata*

1. Alpha glucosidase inhibition assay

The α-glucosidase inhibition assay was conducted to evaluate the inhibitory effects of a test sample and compare it with Acarbose. The assay measured the conversion of PNPG to PNP using α-glucosidase and determined the percentage inhibition at various concentrations. The results showed that Acarbose exhibited increasing inhibition with higher concentrations, achieving 97.64% inhibition at 100 µg/ml and an IC50 of 16.12 µg/ml. The test sample EEMP showed 39.88% inhibition at 6.25 µg/ml and 71.95% inhibition at 100 µg/ml, with an IC50 of 10.29 µg/ml. These findings suggest that EEMP demonstrates significant α-glucosidase inhibitory activity, comparable to Acarbose, and has potential as an anti-diabetic agent.

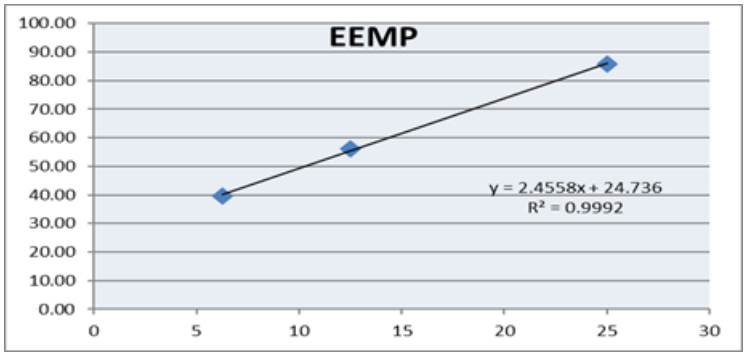

Figure no. 4: Effect of EEMP in alpha glucosidase inhibition assay.

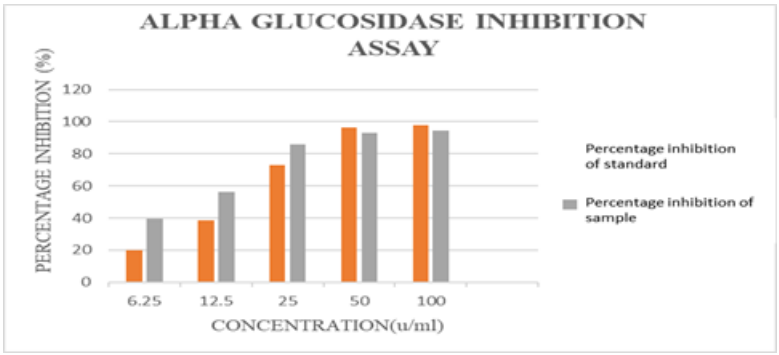

Figure no. 5: Comparison of percentage inhibition of acarbose and EEMP.

Table no. 5: Percentage inhibition of standard drug.

Sample code	Concentration (µg/ml)	Absorbance at 400nm	Percentage of Inhibition (%)
Blank	-	0.704	-
Control	-	0.026	-
Acarbose (standard)	6.25	0.567	20.20
	12.5	0.442	38.64
	25	0.208	73.15
	50	0.050	96.46
	100	0.042	97.64
	IC 50		**16.72 µg/ml**

Table no. 6: Percentage inhibition of EEMP.

Sample code	Concentration (µg/ml)	Absorbance at 400nm	Percentage of Inhibition (%)
Blank	-	1.355	
Control	-	0.031	
EEMP	6.25	0.831	39.58
	12.5	0.611	56.19

	25	0.218	85.88
	50	0.125	92.90
	100	0.102	94.64
	IC 50		**10.29 µg/ml**

2. Alpha amylase inhibition assay

The α-amylase inhibition assay was performed to assess the anti-diabetic potential of test substances by evaluating their ability to inhibit the α-amylase enzyme. The assay utilized the DNS method to measure the release of maltose from starch, with absorbance measured at 540 nm. Acarbose, a standard anti-diabetic drug, showed 89.11% inhibition at 100 µg/ml and an IC50 of 36.44 µg/ml. The test sample EEMP exhibited 71.95% inhibition at the same concentration, with an IC50 of 11.92 µg/ml, indicating strong α-amylase inhibitory activity. The results suggest that EEMP could be a promising candidate for anti-diabetic applications, with inhibition comparable to the standard drug Acarbose.

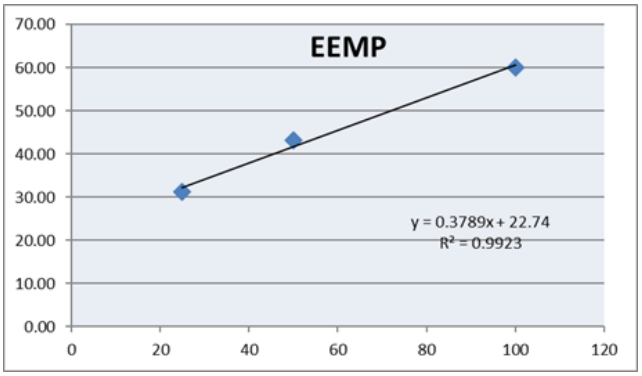

Figure no 6: Effect of ethanolic extract of *Macaranga peltata* in alpha amylase inhibition assay.

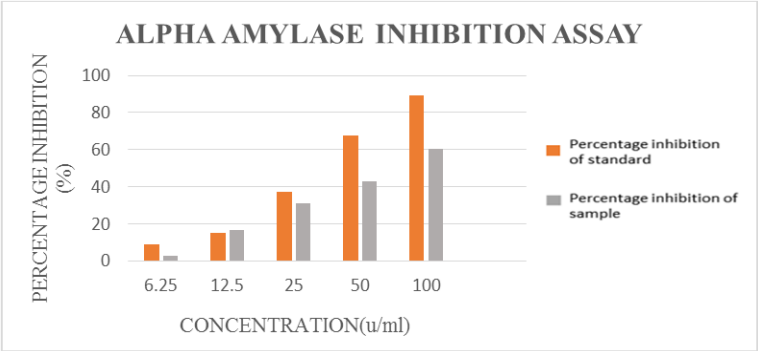

Figure no.7: Comparison of percentage inhibition of acarbose and EEMP.

Table no. 7: Percentage inhibition of standard drug.

Standard	Concentration (µg/ml)	Absorbance at 540 nm	Percentage of Inhibition (%)
Blank	-	0.998	
Control	-	0.034	
Acarbose	6.25	0.911	9.02
	12.5	0.852	15.15
	25	0.639	37.24
	50	0.346	67.63
	100	0.139	89.11
	IC 50		**36.44 µg/ml**

Table no. 8: Percentage inhibition of EEMP.

Sample code	Concentration (µg/ml)	Absorbance at 540 nm	Percentage of Inhibition (%)
Blank	-	1.437	-
Control	-	0.032	-
EEMP	6.25	1.402	2.49

	12.5	1.201	16.80
	25	0.998	31.25
	50	0.831	43.13
	100	0.592	60.14
	IC 50		**71.95 µg/ml**

DISCUSSION

The study investigates the anti-diabetic potential of phytoconstituents from *Macaranga peltata* through *in vitro* molecular docking studies. Diabetes mellitus, a chronic endocrine disorder, is associated with severe complications, necessitating the search for alternative therapeutic agents.

The plant material was collected, authenticated, and extracted using ethanol. Preliminary phytochemical screening confirmed the presence of flavonoids, tannins, saponins, alkaloids, proteins, and carbohydrates, indicating potential bioactivity.

The anti-diabetic activity was evaluated using α-glucosidase and α-amylase inhibition assays. The EEMP showed significant enzyme inhibition, with an IC_{50} of 10.29 µg/mL for α-glucosidase (comparable to acarbose: 16.12 µg/mL) and 11.92 µg/mL for α-amylase (compared to acarbose: 36.44 µg/mL). These results suggest that *Macaranga peltata* exhibits promising anti-diabetic properties.

The study concludes that *Macaranga peltata* could be a potential natural source for anti-diabetic drug development, warranting further research, including molecular docking studies and *in vivo* evaluations.

CONCLUSION

The present study highlights the anti-diabetic potential of *Macaranga peltata* through in vitro α-glucosidase and α-amylase inhibition assays. The EEMP demonstrated significant enzyme inhibitory activity, with an IC_{50} of 10.29 µg/mL for α-glucosidase and 11.92 µg/mL for α-amylase, comparable to the standard drug acarbose.

The preliminary phytochemical screening confirmed the presence of bioactive compounds, including flavonoids, tannins, alkaloids, and saponins, which may contribute to the observed anti-diabetic effects. These findings suggest that *Macaranga peltata* could serve as a natural alternative for diabetes management, warranting further molecular docking, *in vivo* studies, and clinical evaluations to validate its therapeutic efficacy and safety.

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