

## INVITRO ANTIDIABETIC STUDY AND PHYTOCHEMICAL SCREENING OF HYDROALCOHOLIC EXTRACT OF *ROTHECA MICROPHYLLA* (BLUME) CALLM. & PHILLIPSON LEAVES

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### ABSTRACT

Diabetes mellitus is a collection of metabolic disorders that are characterized by persistently high blood sugar levels. The study focuses on the invitro anti diabetic study and phytochemical screening of hydroalcoholic extract of *Rothea microphylla* (Blume) Callm. & Phillipson leaves. Herbal medicines have gained increasing attention due to their therapeutic potential, safety profile, affordability and cultural acceptance. The study aims to provide scientific evidence for the traditional use of *Rothea microphylla* and to explore it's potential as a natural Source of anti-diabetic agents. The study includes plant collection, Authentication, shade- drying, and subjected to hydroalcoholic Soxhlet extraction. Pharmacognostic parameters such as stomatal number, stomatal index, vein islet number, and vein termination number were assessed for proper identification and standardization. Preliminary phytochemical screening of the crude extract revealed the presence of bioactive constituents

including flavonoids, phenolics, alkaloids, tannins, saponins, terpenoids, glycosides, and carbohydrates. The antidiabetic activity was confirmed through in-vitro enzyme inhibition assays, specifically  $\alpha$ - amylase and  $\alpha$ -glucosidase inhibition methods, which are key targets in carbohydrate metabolism and post-prandial glucose regulation. Findings from phytochemical and enzyme inhibition analyses suggest that the plant possesses promising bioactive compounds that may contribute to glucose-lowering effects, thereby supporting further

pharmacological and clinical investigations.

**KEYWORDS:** *Rothea microphylla*, Antidiabetic activity, Phytochemical screening, Alpha-amylase inhibition, Alpha-glucosidase inhibition.

## INTRODUCTION

Herbal medicine has a profound influence on many diseases, offering both preventive and therapeutic options that complement conventional treatments. By supplying a diverse array of bioactive compounds- such as alkaloids, flavonoids, terpenes, and polysaccharides-plant based remedies can modulate inflammation, oxidative stress, immune responses, and microbial activity. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety, and lesser side effects. They also offer therapeutics for age related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available.<sup>[1,2]</sup> Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both. It is associated with serious complications including neuropathy, nephropathy, retinopathy, and cardiovascular diseases.

Management of postprandial hyperglycemia is one of the important therapeutic strategies in diabetes treatment. Inhibition of carbohydrate-digesting enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase delays glucose absorption and reduces postprandial blood glucose levels. Medicinal plants are widely used as alternative therapies due to their safety, affordability, and cultural acceptance.

*Rothea microphylla* (Family: Lamiaceae) is traditionally used for treating fever, inflammation, and skin disorders.<sup>[3]</sup> However, scientific validation of its antidiabetic potential is limited. The present study aims to investigate the phytochemical constituents and in vitro antidiabetic activity of the hydroalcoholic leaf extract of *Rothea microphylla*.

## MATERIALS AND METHODS

### MATERIALS

- Dried leaves of *Rothea microphylla*
- Alpha amylase enzyme powder
- Sodium phosphate buffer
- Sodium chloride

- Starch solution
- DNSA colour reagent
- Alpha glucosidase enzyme powder
- Potassium phosphate buffer
- Para-nitrophenyl alpha D-glucopyranoside
- Bovine serum albumin
- Sodium carbonate
- Acarbose powder

## METHODS

### 1. SELECTION AND COLLECTION OF PLANT MATERIALS

*Rothea microphylla* from the family Lamiaceae was selected for the study. Fresh plant of *Rothea microphylla* was collected from Edappal, Malappuram, Kerala, and authenticated by Sree Neelakanta Govt. Sanskrit College, Pattambi, Palakkad, Kerala-679306.

Alpha amylase were purchased from Virat lab and all of the other chemicals utilized in the study were purchased from Prowess Marketing Co. Pvt, Ltd., Bombay Colony Road, Palappuram Post, Ottappalam, Palakkad, Kerala-679103.

### 2. PHARMACOGNOSTIC STUDY

The pharmacognostic evaluation of *Rothea microphylla* was carried out by determining various leaf constants.<sup>[4]</sup>

- Stomatal number
- Stomatal index
- Vein islet number
- Vein termination number

### 3. DRYING AND SIZE REDUCTION

The collected leaves of plant *Rothea microphylla* were thoroughly washed in running water. It is then shade dried under normal environmental conditions and then subjected for size reductions to coarse powder.

### 4. EXTRACTION OF LEAVES

Hydroalcoholic extract of *Rothea Microphylla* were prepared by soxhlation. About 8.65g of coarse powder material was added and subjected to successive extraction with 80% ethanol

and 20% water (hydroalcoholic) extraction in Soxhlet apparatus. The extraction was continued until solvent in the thimble becomes clear indicating the completion of the extraction. After extraction, the solvent was distilled off, and the extract was poured in petri plate and concentrated at room temperature.<sup>[5]</sup>

Alcohol soluble extractive value (%)

$$= [\text{Weight of extract (g)}/\text{Weight of sample(g)}] \times 100$$

## 5. PHYTOCHEMICAL SCREENING

Qualitative analysis Standard tests were performed to detect.

- Carbohydrates (Molischs test)
- Alkaloids (Mayers, Dragondroffs test)
- Glycosides (Trim hill test)
- Saponin (Foam test)
- Phytosterol and Triterpinoid (Salkowaski)
- Phenolic compound and Tannins (Ferric Chloride)
- Flavanoid (Alkaline reagent test)

## 6, INVITRO ANTIDIABETIC ACTIVITY

### 1.1 Alpha amylase inhibition

Different concentrations of plant extract were prepared in distilled water. A total of 500 µl of plant extract and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing alpha amylase solution (0.5 mg/ ml) were incubated for 10 minutes at 37°C. After pre incubation 500 µl of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube. This reaction mixture was the incubated for 10 minutes at 37°C. 1 ml of DNSA colour reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10 ml distilled water following which absorbance was measured at 540 nm. This procedure was repeated for the standard drug Acarbose. The percentage alpha amylase inhibition of both extract and standard drug was calculated by the following formula.<sup>[6]</sup>

$$\% \text{ inhibition} = (\text{Abs of control} - \text{Abs of test}) / \text{Abs of control} \times 100$$

### 1.2 Alpha glucosidase inhibition assay

The enzyme solution is prepared by dissolving 0.5 mg  $\alpha$ -glucosidase in 10ml phosphate buffer (pH 7) containing 20 mg BSA. It is diluted further to 1:10 with phosphate buffer. Different concentrations of samples are prepared and 5 $\mu$ l each of the solution or blank is then added to 250  $\mu$ l of 20 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside and 490  $\mu$ l of 100 mM phosphate buffer. It is then pre-incubated at 37°C for 5 minute and the reaction started by addition of 250  $\mu$ l of enzyme solution and incubated at 37°C for 15 minutes 250  $\mu$ l of phosphate buffer is added instead of enzyme for blank. The reaction is then stopped by addition of 1000  $\mu$ l of 200 mM sodium carbonate solution and the amount of p-nitrophenol released is measured by reading the absorbance of sample against a blank at 400 nm. This procedure was repeated for the standard drug Acarbose. The percentage inhibition of both extract and standard drug was calculated by the following formula.<sup>[7]</sup>

$$\% \text{ inhibition} = (\text{Abs of control} - \text{Abs of test}) / \text{Abs of control} \times 100$$

## RESULTS AND DISCUSSION RESULTS

The study involved Pharmacognostic study, Phytochemical screening and Invitro antidiabetic study of *Rothea microphylla* (Blume) Callm. & Phillipson.

### 1. PHARMACOGNOSTIC STUDY

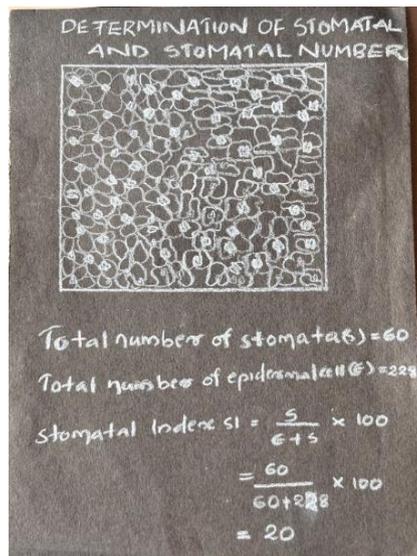
Determination of Leaf constants

- The plant *Rothea microphylla* possess Anomocytic stomata, where the guard cells are surrounded by a limited number of epidermal cells that are not distinct in shape or size from the rest of the leaf epidermis.
- *Rothea* species are typically hypostomatic.



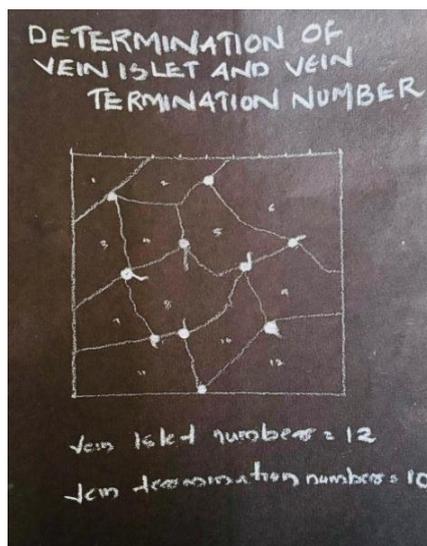
Microscopical view of *R. microphylla* leaf

Stomatal number & stomatal index.



- Stomatal number was found to be 60.
- Stomatal index was found to be 20.

Vein islet number & vein termination number:



- Vein islet number was found to be 12.
- Vein termination number was found to be 10.

## 2. EXTRACTION

- The alcohol soluble extractive value of *Rotheca microphylla* was found to be 26.48%

### 3. PHYTOCHEMICAL SCREENING

**Table 1: Phytochemical constituents of Hydroalcoholic extract of *R.microphylla*.**

SI.NO	CHEMICAL TEST	RESULT OBTAINED
1	Carbohydrate	+
2	Alkaloid	+
3	Anthraquinone glycoside	-
4	Cardiac glycoside	-
5	Iridoid glycoside	+
6	Phytosterols & Triterpenoid	+
7	Phenolic compound & Tannin	+

### 4. INVITRO ANTIDIABETIC ACTIVITY

#### 4.1 Alpha amylase inhibition assay

The alpha amylase inhibition activity of *Rothea microphylla* was performed. The absorbance of various concentration of plant extract and standard acarbose was measured at 540nm. The results from the study showed that *Rothea microphylla* has significant alpha amylase inhibitory assay.

**Table 2: Results of Alpha amylase inhibition assay.**

SI.NO.	CONCENTRATION (%w/w)	ABSORBANCE	PERCENTAGE INHIBITION (%)
1	10	0.338	28.84
2	20	0.249	47.57
3	30	0.234	50.73
4	40	0.208	56.21
5	50	0.172	63.78
6	Acarbose	0.079	83.36

#### 4.2 Alpha glucosidase inhibition assay

The alpha glucosidase inhibition activity of *Rothea microphylla* was performed. The absorbance of various concentration of plant extract and standard acarbose was measured at 400nm. The results from the study showed that *Rothea microphylla* has significant alpha glucosidase inhibitory assay.

**Table 3: Results of Alpha glucosidase inhibition assay.**

SI.NO.	CONCENTRATION (%w/w)	ABSORBANCE	PERCENTAGE INHIBITION (%)
1	10	0.380	22.44
2	20	0.354	27.75
3	30	0.301	38.57
4	40	0.268	45.30
5	50	0.224	54.28
6	Acarbose	0.083	83.06

## DISCUSSION

The present study evaluated the *in vitro* antidiabetic potential of the hydroalcoholic leaf extract of *Rothea microphylla*. Phytochemical screening revealed the presence of flavonoids, phenolics, alkaloids, tannins, saponins, terpenoids, and iridoid glycosides, which are associated with antioxidant and hypoglycemic activities. The extract showed significant, dose-dependent inhibition of  $\alpha$ -amylase (28.84% to 63.78%) and  $\alpha$ -glucosidase (22.44% to 54.28%), though lower than the standard drug acarbose. These findings indicate that the extract can delay carbohydrate digestion and glucose absorption, thereby reduce post-prandial hyperglycemia and support its potential as a natural antidiabetic agent.

## CONCLUSION

The hydroalcoholic leaf extract of *Rothea microphylla* exhibited significant *in vitro* antidiabetic activity through  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition. The presence of bioactive phytoconstituents such as flavonoids and phenolics may contribute to this activity. Although less potent than acarbose, the extract demonstrated promising enzyme inhibitory effects, indicating its potential as a natural antidiabetic agent. Further *in vivo* studies and clinical evaluation are required to confirm its therapeutic efficacy and safety.

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