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# EVALUATION USING *IN-VITRO* ASSAYS FOR GLUCOSE DIFFUSION AND KINETICS OF AMYLOLYSIS OF *LEEA MACROPHYLLA* STANDARDIZED EXTRACTS

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## ABSTRACT

The standardized aqueous and methanol extracts of *Leea macrophylla* were studied for their effects on assay of diffusion glucose and for kinetics of amylolysis using *in vitro* models.

The results verified the antidiabetic potential of the standardized aqueous and methanol extract of *Leea macrophylla*.

**Keywords: *Leea macrophylla*; glucose diffusion; amylolysis kinetics**

## INTRODUCTION

*Leea macrophylla* (Roxb.), of Leeaceae family, an herbaceous shrub with big sized leaf similar to a elephant ear. Ethnobotanical survey shows some important therapeutic uses in cancer, dysentery, body-ache, and sexual disability **[1]**. It is traditionally used for nephrolithiasis, rheumatism, arthritis, pain, tonsillitis, tetanus, snake bites, sore and blood effusion **[2, 3]**. Leaf juice is used for local anti-inflammatory effects, it is also used to treat

in boils, arthritis, gout, and rheumatism **[4, 5]**.

This study is undertaken to verify the antidiabetic potential of *Leea macrophylla* using various in vitro techniques and also as an attempt to predict its mechanism of action.

**MATERIALS AND METHODS**

**Preparation of *Leea macrophylla* Extracts:** The plant species *Leea macrophylla* was collected from Kalgaon village Taluka. Patan, District- Satara and authenticated at Botanical Department of Yashwantrao Chavan College of Science, Karad. The plant was dried under sunlight and fine powder of the plant was prepared by using hand grinder.

**Preparation of Aqueous Extract [LMAE]**: powder was mixed with 30ml distilled water boiled for 30 minutes in flask attach with reflux condenser. The material was filtered with whatman filter paper no 40 and filtrate was collected.

**Preparation of Alcoholic Extract [LMEE]**: powder was mixed with 30 ml alcohol and 10 ml distilled water boiled for 30 minutes in round bottom flask attach with reflux condenser. The material was filtered whatman filter paper no 40 and filtrate was collected. Filtrate was collected in porcelain dish. Alcohol was evaporated and then adds 4ml distilled water.

**Chemicals**

Glucose oxidase peroxidase (GOP) kit was procured from Pathozyme Diagnostics, Kagal, India. Dialysis bags (12 000 MW cutoff; Himedia laboratories, India) were used. All the chemicals used in the study were of extra pure analytical grade.

**Evaluation of antidiabetic activity of *Leea macrophylla* extracts using various in vitro methods**

**1. Effect of *Leea macrophylla* extracts on in-vitro glucose diffusion**

It was performed according accordingly to the method described by Ahmed *et al* **[5]**. In 25 mL of glucose solution (20 mmol/ L) and the samples LM (1%) were dialyzed using in dialysis bags in 200 ml of distilled water at 37 °C in water bath attached to a shaker. The glucose content in the dialysate measured at duration of 30, 60, 120 and 180 min using GOP kit. A control test carried without any sample for comparison.

**2. Effect of *Leea macrophylla* extracts on in-vitro amylolysis kinetics [6]**

In this test 40 g of potato starch added to 900 mL of 0.05 mol/L phosphate buffer (pH 6.5). The solution is stirred at 65 °C for 30 min duration. Then the final volume is made upto 1000 ml gives 4% (w/v) starch solution. To 25 ml of the prepared starch solution, α-amylase (0.4%) and LM (1%) were dialyzed in a dialysis bags against 200 ml of distilled

water at 37 °C (pH 7.0) in water bath attached to a shaker. The glucose content in the dialysate also measured at duration of at 30, 60, 120 and 180 min. A control test carried without any sample for comparison.

Glucose dialysis retardation index (GDRI) calculated by below formula-

$$GDRI = 100 - \left[\frac{\text{Glucose content + sample / LM (mg/dl)}}{\text{[Glucose content of control (mg/dl)]}} \times 100\right]$$

**Statistical analysis-** All the determinations carried out in triplicates and data was analyzed by ANOVA followed by students T test. Values were considered at $P<0.05$.

## RESULTS

### Effect of *Leea macrophylla* on glucose diffusion

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in **Figures 1 & 2**. The glucose diffusion rate increases with time from 30 to 180 min. In the present study, the glucose movement across the dialysis membrane was monitored once in 30 min until 180 min and it was found that, both the studied extracts of *Leea macrophylla* demonstrate significant inhibition of movement of glucose into the external solution across dialysis membrane in comparison to control.

### Effect of *Leea macrophylla* extracts on kinetic amylolysis

The effects of *Leea macrophylla* on the amylolysis kinetics are shown in the **Figures 3 & 4**. The GDRI for LM is calculated as 55.67 % and 44.32 % in LMAE and LMEE respectively at duration of 60 min that gradually reduced to 12.34 % and 7.57% respectively at 120 min.

**Table 1: Preliminary phytochemical screening of extracts**

| Test | Extracts | |
|---|---|---|
| | Aqueous [LMAE] | Ethanolic [LMEE] |
| Flavonoids | ++ | ++ |
| Steroids | ++ | ++ |
| Terpenoids | ++ | ++ |
| Alkaloids | -- | -- |
| Tannins | -- | -- |
| Glycosides | - | - |
| Saponins | + | -- |

**Interpretation of results: (−) absent; (+) low; (++) good**

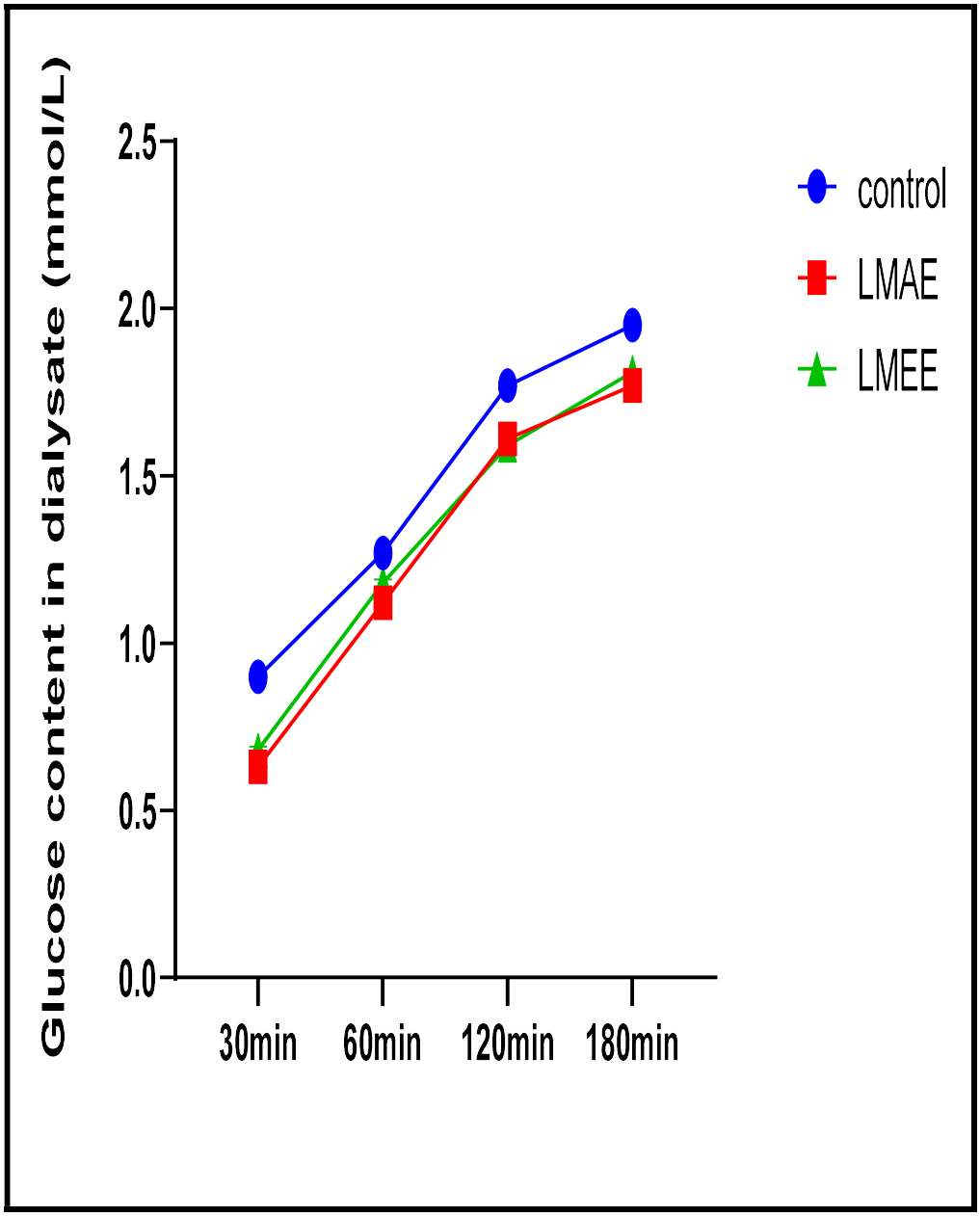


**Figure 1: Effect of *Leea macrophylla* extracts on glucose diffusion**

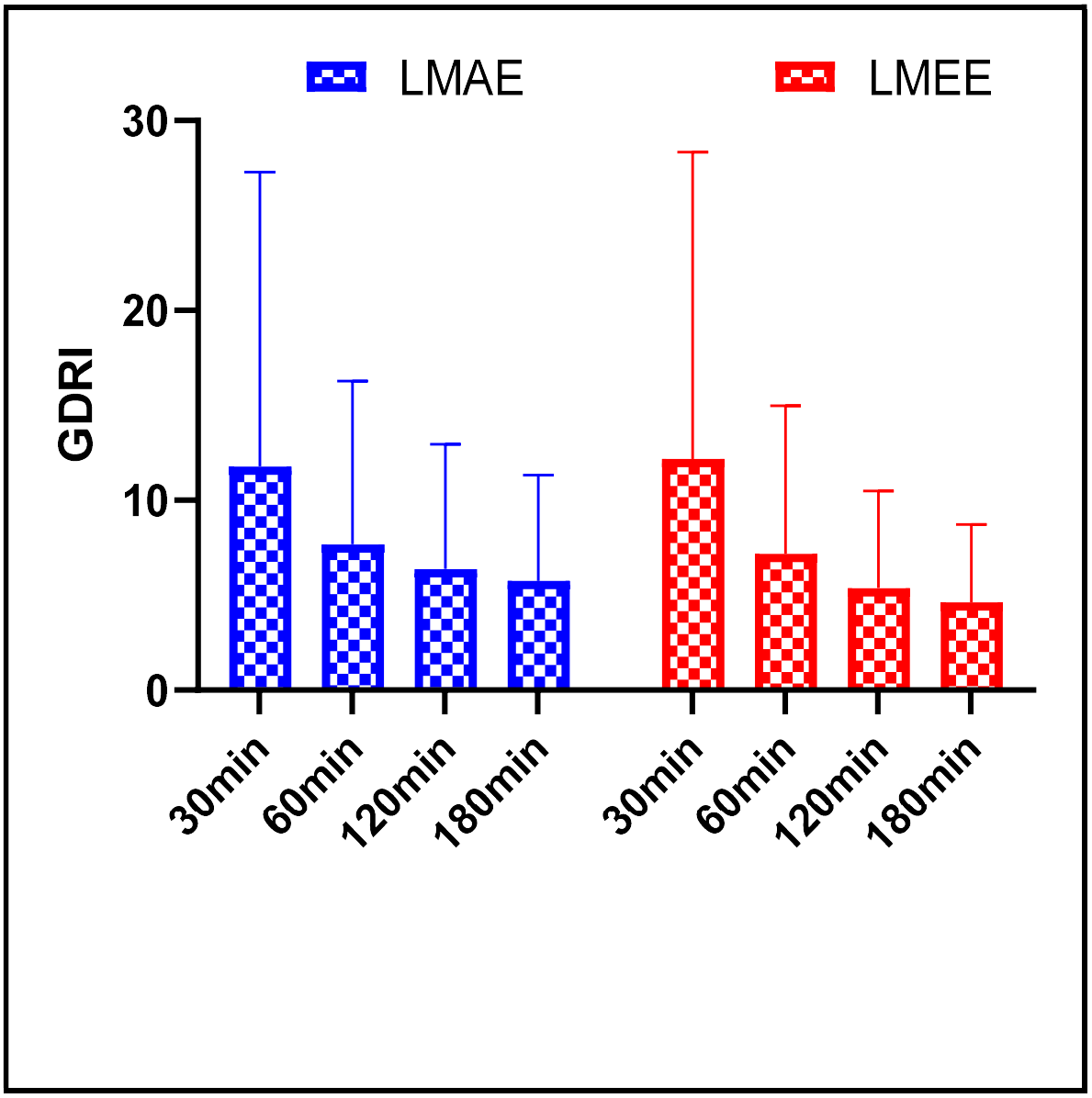


**Figure 2: Effect of Leea macrophylla extracts on glucose GDRI**

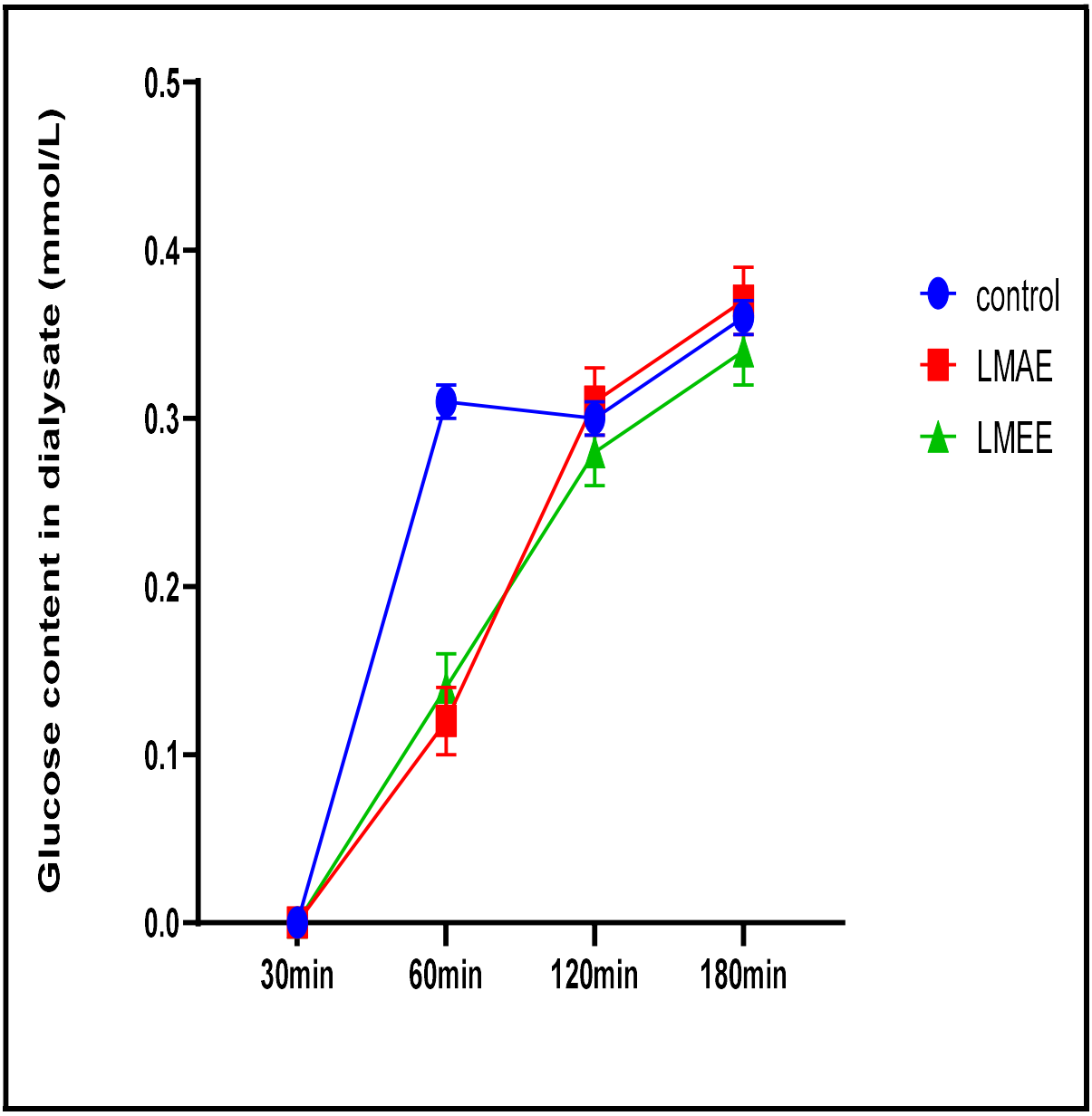


**Figure 3: Effect of selected samples on starch digestibility**

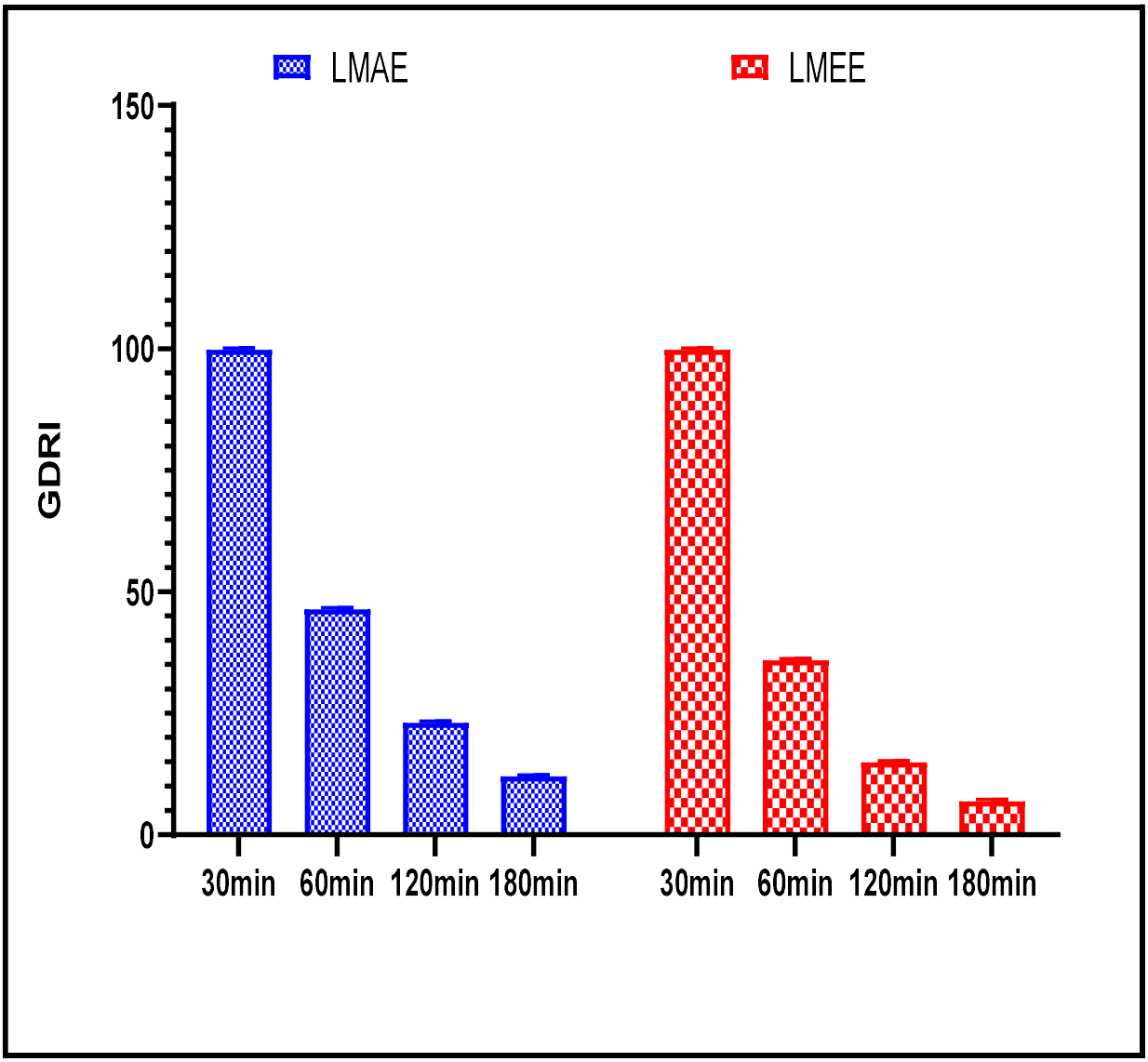


**Figure 4: Effect of selected samples on starch GDRI**

## DISCUSSION

The glucose diffusion retardation can be due to the inhibiting α-amylase by the plant extracts thereby limiting the release of glucose from the starch. Ou *et al*. mentions possible facts are responsible in α-amylase inhibition like fiber concentration, presence of fiber inhibitors, starch encapsulation and fiber enzymes, which may reduce accessibility of starch to enzyme and direct adsorption of enzyme on fibers ultimately resulting in decrease in amylase activity **[7]**. In-vitro index such as GDRI used to predict the effectiveness of fiber for delay in glucose absorption in the gastrointestinal tract. A high GDRI indicate high retardation index of glucose by the LM **[5, 7]**. The GDRI

calculated to 30.22% and 31.63% in LMAE and LMEE respectively at 30 min.

Glucose diffusion rate in amylolysis kinetic experiments increases with the time duration such as from 30 to 180 minutes and both the LM extracts show significant inhibition of movement of glucose across dialysis membrane in to the external solution as compared with control.

The summarized preliminary phytochemical screening report of LM extracts summarized in **Table 1**. It was clear that the Flavonoids and Terpenoids present in LM could be responsible for the observed activity **[6]**. In concluding, the results of this study suggest possible hypoglycemic activity of *Leea macrophylla* extracts, that could be mediated by decreased rate of glucose diffusion rate.

**Conflicts of interest**

We all the authors declare no conflict of interest.

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