

# *Emblica officinalis* seed essential oil exhibits antidiabetic potential by inhibiting the $\alpha$ -amylase, $\alpha$ -glucosidase, and xanthine oxidase

Sri Venu Madhav Tippabhotla<sup>1</sup>, Hemalatha Punati<sup>1</sup>, Kasinadhuni Siva Naga Jyothi<sup>1</sup>, Sudhakar Poda<sup>3</sup>

<sup>1</sup>Ph.D student, Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh

<sup>2</sup>Associate professor, Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh

**Abstract**—The present study extracted essential oil from *Emblica officinalis* seeds using a Clevenger apparatus through steam distillation. The qualitative phytochemical analysis of *Emblica officinalis* seeds essential oil (EOEO) showed that total phenolics and flavonoids were gallic acid equivalents of  $269.58 \pm 3.07$   $\mu\text{g}/\text{mg}$  and the rutin equivalents of  $198.43 \pm 3.24$   $\mu\text{g}/\text{mg}$ , respectively. Under in-vitro conditions, EOEO has dose-dependently inhibited the  $\alpha$ -amylase,  $\alpha$ -glucosidase, and xanthine oxidase. The IC<sub>50</sub> value (50% of inhibition) of EOEO to reduce  $\alpha$ -amylase,  $\alpha$ -glucosidase, and xanthine oxidase was determined as  $63.34 \pm 1.27$ ,  $96.20 \pm 2.84$ , and  $36.05 \pm 0.77$   $\mu\text{g}/\text{mL}$ , respectively. Thus, EOEO proved a potential candidate for diabetes and its related complications.

**Index Terms**—Diabetes, *Emblica officinalis*, phenolics, flavonoids, essential oil.

## I. INTRODUCTION

Diabetes mellitus causes immediate or long-term issues due to insufficient insulin production, resulting in body fat, protein, and glycogen breakdown and ketones production. Hereditary and environmental factors influence the disease's onset and course. Symptoms include thirst, impaired vision, weight loss, and polyuria. Diabetes-induced organ failure can cause disability or death (1).

Insulin-dependent and non-insulin-dependent diabetes are prevalent forms of the disease. Type 1 diabetes is caused by the death of pancreatic islet beta cells, requiring daily insulin. Type 2 diabetes is the most prevalent worldwide, with less sensitive bodily tissue, requiring antidiabetic medications like thiazolidinediones, biguanides,  $\alpha$ -glucosidase inhibitors, sulfonylureas, and non-sulfonylurea secretagogues to regulate blood sugar levels (2).

Plant-based medicines are affordable, easy to obtain, and safer than synthetic ones. They contain bioactive substances like flavonoids, terpenoids, and carotenoids. They're used as an alternative to traditional diabetes treatment, enhancing insulin production, promoting glucose uptake, and blocking absorption (3).

Essential oils are increasingly being used as a supplement to manage diabetes symptoms and complications. These oils are known for their ability to control blood sugar levels, improve cardiovascular health, and insulin sensitivity. Lavender, clove, frankincense, ginger, peppermint, coriander, ylang-ylang, Korean pine, lemon balm, black pepper, black seed, and helichrysum are all effective in managing diabetes. However, they should not replace traditional diabetes treatment and should not be used in place of prescription drugs or evidence-based treatments. It is essential to consult with a healthcare professional before using essential oils (4).

In the present study, essential oil was extracted from *Emblica officinalis* seeds using a Clevenger apparatus through steam distillation. The qualitative phytochemical analysis of *Emblica officinalis* seeds essential oil (EOEO) was estimated by determination of total phenolics and flavonoids. The in-vitro antidiabetic potential of EOEO was evaluated by assessing the inhibition of the  $\alpha$ -amylase,  $\alpha$ -glucosidase, and xanthine oxidase. The study aimed to prove the EOEO as a potential candidate for diabetes and its related complications.

## II. MATERIALS AND METHODS

### A. Chemicals and reagents

The chemicals used in the study were purchased from Sigma-Aldrich (Bengaluru, India). The plasticware of

from Tarsons Products, Kolkata, India. The glassware was received from Borosil, Mumbai, India.

#### B. Plant material collection and essential oil extraction

*Emblica officinalis* seeds were collected from rural regions of Vijayawada, Andhra Pradesh, India. The seeds were air-dried for 10 days, ground into powder, and extracted through steam distillation using a Clevenger apparatus. The obtained *E. officinalis* seed essential oil (EOEO) was then dried and stored at 4°C.

#### C. Quantitative phytochemical analysis

With minor adjustments of Singleton & Rossi, the Folin–Ciocalteu reagent—which employs gallic acid as a standard—was used to determine the *E. officinalis* seed essential oil (EOEO) total phenolic content (7). Briefly, 200 µl of Folin–Ciocalteu reagent was combined with 100 µl *E. officinalis* seed essential oil (EOEO) (1 mg/mL to 5 mg/mL). After three minutes of reaction, 1 mL of a 2% (w/v) Na<sub>2</sub>CO<sub>3</sub> aqueous solution was added, and the mixture was gently shaken. The test sample mixture of *E. officinalis* seed essential oil was incubated for one hour at 25 ± 1°C before their absorbance was measured at 764 nm using a spectrophotometer. The standard solutions of gallic acid in concentrations of up to 50 µg/mL underwent the same process. Gallic acid equivalent (GAE) per milligram (mg) was used to express the total phenolic content of the *E. officinalis* seed essential oil (EOEO).

The aluminum chloride method, with some slight adjustments, was utilized in order to determine the total flavonoid content of the *E. officinalis* seed essential oil (EOEO) and carried out as per the methodology of Quettier-Deleu et al. with some modifications (8). To a volume of 2.5% (w/v) AlCl<sub>3</sub> ethanolic solution, 1 mL of sample solution with a concentration of 1 mg/mL was added. Incubation of the samples took place at 25 ± 1°C for a period of one hour. At a wavelength of 418 nm, a spectrophotometer (Shimadzu, Japan) was utilized to determine the absorbance of the substance. In order to determine the calibration line, the same method was performed for the standard quercetin solution at concentrations of up to 50 µg/mL throughout the experiment. The quantification of total flavonoids in *E. officinalis* seed essential oil (EOEO) was given as micrograms of quercetin equivalent (QUE) per milligram of dry weight of *E. officinalis* seed material as the sample was being examined.

#### D. Antidiabetic analysis

The *in-vitro* antidiabetic activity of *E. officinalis* seed essential oil (EOEO) was assessed by α-amylase inhibition, α-glucosidase inhibition, and xanthine oxidase (XO) assays.

The α-amylase inhibition assay was rigorously carried out to investigate the potential antidiabetic effects that are present in the essential oil of *E. officinalis* seed essential oil (EOEO) (9). The EOEO were subjected to a pre-incubation with the α-amylase solution at a concentration of 1 U/mL for 12 minutes at 37 ± 1°C. After adding 30 microliters of soluble starch with a concentration of 0.5 percent in deionized water, the reaction was initiated by incubating it at 37 ± 1°C for 8 minutes. Acarbose is considered a standard for inhibiting α-amylase, which is a common drug for diabetes. After the reaction was precisely stopped, the absorbance at 568 nm was measured with as much precision as possible (Synergy H1, BioTek Instruments, Inc., USA).

At the same time, the α-glucosidase inhibition assay was carried out in a thoroughly meticulous manner to assess the *in-vitro* antidiabetic activity of *E. officinalis* seed essential oil (EOEO) (9). After combining a methanolic stock solution of EOEO with 0.5 U/mL of α-glucosidase enzyme solution and potassium phosphate buffer (0.1 M), the mixture was subjected to an incubation period of 8 minutes at 25 ± 1 °C. Next, a solution of p-nitrophenyl-α-d-glucopyranoside substrate, which had a concentration of 5 mM, was added to the mixture, and it was allowed to incubate for 12 minutes. Acarbose is considered a standard for inhibiting α-glucosidase, which is a common drug for diabetes. A thorough stop was placed on the process, and a precise measurement of the absorbance was taken at a wavelength of 406 nm (Synergy H1, BioTek Instruments, Inc., USA).

The xanthine oxidase assay was intended to examine the potential inhibitory effect of *E. officinalis* seed essential oil (EOEO) on xanthine oxidase (9). This experimental method added a new dimension to the investigation. The assay mixture was preincubated at 25 ± 1°C for fifteen minutes while the experiment was carried out under aerobic conditions. Following the introduction of the substrate solution, which was composed of 150 mM xanthine in a 70 mM phosphate buffer, the reaction was initiated by allowing it to

incubate at a temperature of  $25 \pm 1^\circ\text{C}$  for a period of thirty minutes. Allopurinol is considered a standard for inhibiting  $\alpha$ -xanthine oxidase. Following the intentional cessation of the procedure, an exact measurement of the absorbance at 292 nm was obtained (Synergy H1, BioTek Instruments, Inc., USA).

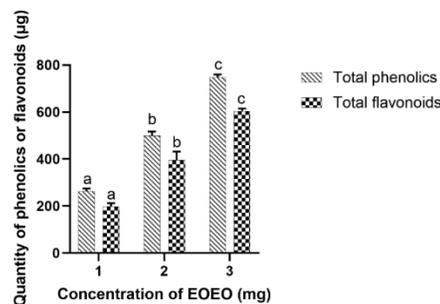
#### E. Statistical analysis

An application, SPSS 25.00 (New York, USA), was utilized to carry out statistical analyses. The data that was obtained was expressed as the mean plus or minus the standard deviation (SD). We carried out statistical studies that were descriptive in nature. The analysis of variance (ANOVA) was used to determine whether or not there were differences between the means, and Dunnett's/Tukey's test was utilized to compare the averages. The level of statistical significance was determined to be in the range of  $P < 0.05$ .

### III. RESULTS AND DISCUSSION

#### A. Phytochemical profile

The essential oil was extracted using the Clevenger type of hydrodistillation from the seeds of *E. officinalis*. After EOEO was obtained, the quantitative phytochemical profile of EOEO was taken into account. To determine the total phenolics and flavonoids content, respectively, the Folin-Ciocalteu and aluminum chloride techniques were applied. The results of the study indicate that the EOEO can include phenolics and flavonoids. Furthermore, the presence of these compounds was demonstrated to be dose-dependent with the quantity of EOEO that was analysed (**Fig. 1**). During the process of determining the quantity of total phenolics and flavonoids, it was discovered that the gallic acid equivalents of  $269.58 \pm 3.07 \mu\text{g}/\text{mg}$  and the rutin equivalents of  $198.43 \pm 3.24 \mu\text{g}/\text{mg}$  were found to be the respective values. The results of the research that was conducted indicate that the EOEO is a source of plant secondary metabolites that have the potential to be beneficial to human health. In instance, it was shown that the EOEO has a considerable amount of phenolics and flavonoids, both of which are beneficial.



**Figure 1:** Total phenolics and flavonoids present in *Emblca officinalis* seed essential oil (EOEO). Statistical analyses were conducted using SPSS 25.00, utilizing the mean  $\pm$  standard deviation as a measure of triplicates ( $n = 3$ ). ANOVA was used to identify statistical differences, with significance set at  $P < 0.05$ . Tukey's test was used to compare averages. The statistical difference between test samples was established by representing different alphabetical letters within the particular study group, like phenolics or flavonoids.

#### B. Anti-diabetic activity

The treatment of Type 2 diabetes involves regulating postprandial glucose levels by inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. These enzymes play a crucial role in converting carbohydrates into glucose. Pancreatic  $\alpha$ -amylase breaks  $\alpha$ -1,4-glycosidic bonds, while  $\alpha$ -glucosidase hydrolyzes short-chain oligosaccharides into glucose. Xanthine oxidase (XO) is involved in purine metabolism. Studies are exploring XO inhibitors' efficacy in treating disorders like gout and oxidative stress-related diseases. Nutraceuticals found in plant foods exert inhibitory actions on these enzymes, similar to conventional glucose-lowering medications.

We used  $\alpha$ -amylase inhibition,  $\alpha$ -glucosidase inhibition, and xanthine oxidase inhibition in our study to analyze the antidiabetic activity potential of EOEO under *in-vitro* settings since they are generally accepted as standard methodologies for evaluating antidiabetic activity (**Fig. 2**). By evaluating  $\alpha$ -amylase inhibition at dose-dependent concentrations of up to 90 micrograms per milliliter at various doses, our work investigated the EOEO's antidiabetic activity. The standard antidiabetic for  $\alpha$ -amylase inhibition at dose-dependent concentrations ranging up to 70 micrograms per milliliter at different dosages is

acarbose. We found that the  $\alpha$ -amylase activity had dose-dependent inhibition from EOEO and acarbose. We determined the  $IC_{50}$  value (50% of  $\alpha$ -amylase inhibition) of acarbose and EOEO to reduce  $\alpha$ -amylase activity as  $40.82 \pm 0.60$  and  $63.34 \pm 1.27 \mu\text{g/mL}$ , respectively. In support of our report, researchers demonstrated that essential oils have a promising potential in suppressing  $\alpha$ -amylase, an enzyme that plays a role in the digestion of carbohydrates. As a result, essential oils have been a focal point for study pertaining to diabetes. As an example, research have brought attention to the inhibitory effects of *Nigella sativa* essential oil, which is comprised of bioactive components such as  $\alpha$ -phellandrene and thymol (8).

Furthermore, our studies examined the EOEO's antidiabetic effectiveness by assessing  $\alpha$ -glucosidase inhibition potential at dose-dependent concentrations of up to 140 micrograms per milliliter at different dosages. At dose-dependent concentrations of up to 120 micrograms per milliliter at various dosages, acarbose is the standard antidiabetic for  $\alpha$ -glucosidase inhibition. We discovered that EOEO and acarbose inhibited the  $\alpha$ -glucosidase activity in a dose-dependent manner. The  $IC_{50}$  values of acarbose and EOEO to decrease  $\alpha$ -glucosidase activity were found to be  $58.08 \pm 0.89$  and  $96.20 \pm 2.84 \mu\text{g/mL}$ , respectively, at 50%  $\alpha$ -glucosidase activity inhibition. Researchers provided evidence to back our report by showing that essential oils have the capacity to significantly inhibit  $\alpha$ -glucosidase, an enzyme involved in the digestion of carbohydrates, making them intriguing options for diabetes management. Strong  $\alpha$ -glucosidase inhibition has been shown by essential oils derived from plants like ginger, aloe, fructus cnidii, and chuan-xiong;  $IC_{50}$  values range from 2.88 to  $7.40 \mu\text{g/mL}$  (9).

Furthermore, by evaluating the xanthine oxidase inhibition potential at dose-dependent concentrations of up to 90 micrograms per milliliter at various dosages, our experiments investigated the EOEO's antidiabetic efficacy. The standard antidiabetic for xanthine oxidase inhibition is allopurinol, which can block the enzyme at dose-dependent concentrations of up to 60 micrograms per milliliter at different dosages. We found that the xanthine oxidase inhibition activity was dose-dependently reduced by EOEO and allopurinol. At 50% xanthine oxidase inhibition activity, the  $IC_{50}$

values for allopurinol and EOEO to reduce xanthine oxidase inhibition activity were  $30.43 \pm 1.02$  and  $36.05 \pm 0.77 \mu\text{g/mL}$ , respectively. Researchers supported our findings by demonstrating that essential oils have demonstrated encouraging potential in blocking xanthine oxidase, an enzyme involved in purine metabolism connected to diseases including diabetes and gout. Compounds like limonene and 3-carene, which have a potent inhibitory effect against xanthine oxidase, are found in *Pistacia chinensis* leaf essential oil (10).

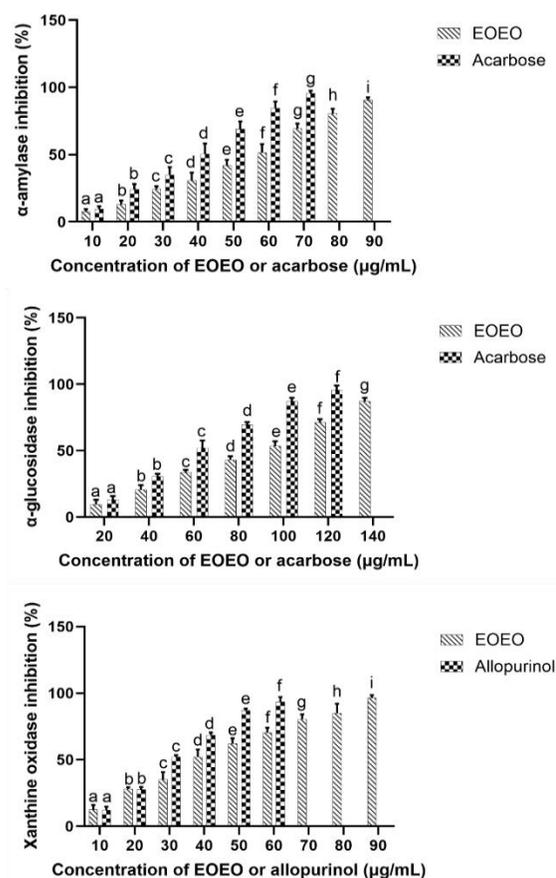


Figure 2: Dose-dependent inhibitory action of EOEO on  $\alpha$ -amylase,  $\alpha$ -glucosidase, and xanthine oxidase activity. Statistical analyses were conducted using SPSS 25.00, utilizing the mean  $\pm$  standard deviation as a measure of triplicates ( $n = 3$ ). ANOVA was used to identify statistical differences, with significance set at  $P < 0.05$ . Tukey's test was used to compare averages. The statistical difference between test samples was established by representing different alphabetical letters within the particular study group.

#### IV. CONCLUSION

Essential oil was successfully extracted from *Embllica officinalis* seeds by steam distillation using Clevenger equipment. *Embllica officinalis* seeds essential oil (EOEO) is found to be rich in total phenolics and flavonoids and could be useful as an antioxidant in the biomedical and food industries. In vitro, EOEO has inhibited  $\alpha$ -amylase,  $\alpha$ -glucosidase, and xanthine oxidase in a dose-dependent manner. As a result, EOEO showed promise as a treatment for diabetes and its associated consequences. However, in-detailed studies need to be carried out at the molecular level to confirm the antidiabetic potential of EOEO and for further usage.

#### ACKNOWLEDGMENT

The authors are thankful to Acharya Nagarjuna University for their support and encouragement.

#### REFERENCES

- [1]. Alam, U., Asghar, O., Azmi, S., & Malik, R. A. (2014). General aspects of diabetes mellitus. *Handbook of clinical neurology*, 126, 211-222.
- [2]. DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., ... & Weiss, R. (2015). Type 2 diabetes mellitus. *Nature reviews Disease primers*, 1(1), 1-22.
- [3]. Jacob, B., & Narendhirakannan, R. T. (2019). Role of medicinal plants in the management of diabetes mellitus: a review. *3 Biotech*, 9(1), 4.
- [4]. Bungau, S. G., Vesa, C. M., Bustea, C., Purza, A. L., Tit, D. M., Brisc, M. C., & Radu, A. F. (2023). Antioxidant and hypoglycemic potential of essential oils in diabetes mellitus and its complications. *International Journal of Molecular Sciences*, 24(22), 16501.
- [5]. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158.
- [6]. Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., ... & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum*

- Moench) hulls and flour. *Journal of ethnopharmacology*, 72(1-2), 35-42.
- [7]. Minh, T. N., Van, T. M., Andriana, Y., Vinh, L. T., Hau, D. V., Duyen, D. H., & Guzman-Gelani, C. D. (2019). Antioxidant, xanthine oxidase,  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities of bioactive compounds from *Rumex crispus* L. root. *Molecules*, 24(21), 3899.
- [8]. Dalli, M., Daoudi, N. E., Abridach, F., Azizi, S. E., Bnouham, M., Kim, B., & Gseyra, N. (2022). In vitro  $\alpha$ -amylase and hemoglobin glycation inhibitory potential of *Nigella sativa* essential oil, and molecular docking studies of its principal components. *Frontiers in Pharmacology*, 13, 1036129.
- [9]. You, G., Liu, X. L., & Zhao, M. M. (2018). Preparation and characterization of hsian-tsoo gum and chitosan complex coacervates. *Food hydrocolloids*, 74, 255-266.
- [10]. Huang, C. Y., Chang, Y. Y., Chang, S. T., & Chang, H. T. (2022). Xanthine oxidase inhibitory activity and chemical composition of *Pistacia chinensis* leaf essential oil. *Pharmaceutics*, 14(10), 1982.