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Exploring the Antidiabetic Activities of *Murraya Koenigii, Aegle Marmelos* and *Laurus nobilis*: An In Vitro Study

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Abstract: ***Aim and Objective*: This study investigates the antidiabetic properties of Murraya koenigii (curry leaf), Aegle marmelos (bael) and Laurus nobilis (bay leaf) using in vitro assays. The plants have been traditionally used in Indian medicinal systems for the treatment of diabetes and other disorders. *Methodology*: Hydroalcoholic extracts from the leaves of Murraya koenigii, Laurus nobilis and fruit pulp of Aegle marmelos were evaluated for their ability to inhibit key enzymes, α - glucosidase and α - amylase, involved in diabetes management. The studies were performed in vitro using enzyme substrates and plant extracts. *Results & Conclusion*: The results suggest that all the plants exhibit significant antidiabetic activities. But strongest antidiabetic properties were exhibited by Murraya koenigii leaf extracts followed by pulp of Aegle marmelos. The minimum antidiabetic activity was reported by Laurus nobilis leaf extract. These results support their use in traditional medicine and potential development into therapeutic agents. As a result, the study's significant findings demonstrate that it is a step towards evidence - based phytomedicine.***

Keywords: *Murraya koenigii*, *Aegle marmelos*, *Laurus nobilis,* antidiabetic activity, α - glucosidase and α - amylase

1. Introduction

Diabetes mellitus is a chronic disorder of glucose metabolism resulting from dysfunction of pancreatic beta cells and insulin resistance. The incidence of cardiovascular diseases is increased two - to four - fold in people with type 2 diabetes [1]. Although the causes of type 2 diabetes and cardiovascular diseases are multifactorial, diet definitely plays a role in the incidence and severity of these diseases. Diabetes is caused by the body's resistance to insulin (Type - 2 diabetes) or by decreased insulin production (Type - 1 diabetes) [2]. One common therapeutic approach for Type - 2 diabetes is to block the action of enzymes like α - glycosidase and α - amylase, which aid in delaying the absorption of glucose, hence lowering post - meal blood sugar increases and managing hyperglycemia [3, 4]. With almost 60 million cases in India alone, diabetes is one of the top five major health problems in developed countries [5]. Because of the body's insensitivity to insulin, particularly when exposed to high glucose levels, type - 2 diabetes is the most prevalent kind of the disease and results in consistently elevated blood sugar levels [6, 7]. Diabetes cases are on the rise and are associated with dietary practices that vary with social and cultural lifestyle differences and may contribute to obesity, a key risk factor for Type - 2 diabetes [8]. When blood sugar levels cannot be adequately controlled by diet and exercise, oral hypoglycemic medications must be taken. Among these drugs, α - glucosidase and α - amylase inhibitors are used to decrease glucose release and carbohydrate metabolism, therefore reducing the spike in blood sugar following meals. Complex starch molecules are broken down into simpler sugars like dextrin, maltotriose, maltose, and glucose by alpha - amylase, an enzyme that is essential to pancreatic juice and saliva [9, 10]. These enzymes' inhibitors decrease the absorption of carbohydrates and lengthen the small intestine's period of digestion, which lowers blood sugar levels after meals [11]. Treatments for Type - 2 diabetes thus aim to target these enzymes [12]. Recent studies on phytochemistry have investigated the possible antidiabetic effects of several plants; results indicate that chemicals from medicinal plants, such as alkaloids, flavonoids, terpenoids, saponins, polysaccharides, and glycosides, have antidiabetic effects. [13, 14]. Traditional herbal medicines used by ethnic communities are important in the treatment of diabetes, especially in developing and low - income countries [15]. It's believed that 80% of the global population depends on naturally produced medicines, and it's encouraged to maintain this reliance, especially where modern diabetic therapies are limited [16]. The plants used in our study were *Murraya koenigii, Aegle marmelos* and *Laurus nobilis.*

***Murraya koenigii*:** Known by several Indian dialects as curry leaf or curry veppila, it is a member of the Rutaceae family. This little tree or shrub, which grows at a height of 4–6 meters, has a potent scent and lots of shade. Murraya koenigii is a native of tropical Asia, although it can be found in India and other countries. This plant's leaves are utilised in many curries throughout South India as a natural flavour.

Furthermore, the Indian Ayurvedic system has long employed these leaves to treat diabetes. The plant is used to treat body heat, blood disorders, diarrhoea, dysentery, eruptions, inflammation, itching, kidney pain, leukoderma, piles, snakebite, thirst, and vomiting. It also has acrid, analgesic, bitter, cooling, alexiteric, anthelmintic, carminative, purgative, and stimulant qualities. It is used as a blood cleanser, tonic, stomachic, antiemetic, antidiarrheal, dysentery, febrifuge, and flavouring agent in curries and chutneys in traditional medicine [17, 18]. Because plants contain physiologically active substances called phytoconstituents,

the use of plant - based medicine has long been a pillar of traditional societies' approaches to treating health conditions [19, 20].

***Aegle marmelos*:** A member of the Rutaceae family, commonly called Bael in Bangla, is a tree that grows in Bangladesh, India, and Southeast Asia. Many bioactive substances have been extracted from the plant, including tannin, aegeline, lupeol, cineole, citral, citronella, cuminaldehyde, eugenol, marmesinin, marmelosin, luvangetin, aurapten, psoralen, marmelide, fagarine, and tannin. Various parts of A. marmelos have been found to have therapeutic uses in the past. These include the treatment of intestinal ailments, asthma, anaemia, fractures, wound healing, swollen joints, high blood pressure, jaundice, diarrhoea, intermittent fever, and fish poisoning [21 - 23]. *A. marmelos* is also used in Indian, Bangladeshi, and Sri Lankan traditional medical systems as a traditional herbal remedy for diabetes mellitus. The unripe dried fruit has stomachic, digestive and astringent qualities that make it useful in treating diarrhoea and dysentery. After recovering from bacillary dysentery, patients can benefit from a sweet drink made from fruit pulp [24, 25].

***Laurus nobilis*:** Bay leaf, also known as laurel leaf, is produced by the sweet bay tree (*Laurus nobilis*), an evergreen member of the Lauraceae family that is indigenous to areas bordering the Mediterranean. Apart from their significant health benefits, Indian bay leaves also serve as a rich source of vitamins, minerals, and other essential nutrients. These leaves contain a variety of essential nutrients, including ferrous, manganous, calciferous, cellulose, saccharides, ascorbic acid, cupric, pyridoxine, folic acid, and zinc [26]. Bay leaves have numerous biological properties that make them useful for various purposes. They have wound - healing abilities [27], act as antioxidants, and have antibacterial, [28, 29] antiviral, and immunostimulant properties. [30] Additionally, they possess anticholinergic, antifungal [31] and insect - repellent properties, making them versatile for different applications. They also have anticonvulsant and antimutagenic effects and can act as analgesics and anti - inflammatory agents [32]. It can be applied to alleviate paralysis, convulsions, neuropathic pain, nerve entrapment, swelling and bruises, migraines, earaches, and joint inflammation [33].

Our research aims to systematically validate the antidiabetic properties of *Murraya koenigii, Aegle marmelos* and *Laurus nobilis* using *in - vitro* α - glucosidase and α - amylase inhibition assays to ensure their efficacy and safety in traditional healthcare applications.

2. Materials and Methods

Collection of Plant Material: The fresh leaves of *Murraya koenigii* and pulp of *Aegle marmelos* and dried leaves of *Laurus nobilis* were collected from the herbal garden.

Preparation of Plant Extracts: The preparation of plant extracts for each assay were carried out in compliance with the published protocol [34]. In short, 3.0 g of dried and ground plant material was weighed and added to a powerful waring blender. After blending the combination for 8.0 minutes with 80 ml of 80% (v/v) ethanol, it was vacuum filtered through No.1 Whatman filter paper. The filtrates were mixed and brought to a final volume of 250 ml after the solids were extracted again using 60 ml of 80% ethanol.

***In - vitro* Antidiabetic Assay:**

α - Amylase Inhibitory Assay: The α - amylase inhibitory assay was conducted with minor adjustments to the standard methodology [35, 36]. A 10 - by - 60 - mm glass test tube with 200 µl of 0.1% starch was pre - incubated for five minutes at 37°C. Following the addition of 25 µl of the sample (1 mg/ml) and 5 µl of the α - amylase enzyme (0.5U), 500 µl of assay buffer (Tris - HCl buffer, pH 6.9) was created. After that, the reaction mixture was incubated for 15 minutes at 37°C.500 µl of 50% acetic acid was added to halt the reaction after incubation. Subsequently, each test tube received 1000 µl of iodine solution (254 mg Iodine crystals + 4 g KI in 1000 ml sterile water). A UV - visible spectrophotometer (Multiskan GO UV - Vis spectrophotometer, Thermo - Scientific, Finland) was used to measure the absorbance of the top supernatant after the tubes were vortexed for 30 seconds at a wavelength of 565 nm.

An enzyme - free control reaction was also provided. Additionally, as a typical inhibitor medication for comparison, acarbose was provided. Appropriate blanks were included for each sample treatment. For each example experiment, at least three replicate reactions were conducted. The formula was utilised to determine the percentage of inhibition of α - amylase enzyme activity. Inhibition of α - amylase (%) = (Test OD/Control OD) x 100 [Test OD= Blank OD - Sample OD; Control OD= Positive Control OD - Blank OD].

α - Glucosidase Inhibitory Assay: The anti - diabetic characteristics of particular plants were screened using the 96 - well microplate - based α - glucosidase inhibitory assay, which was previously reported by Kumar et al. [37]. In order to prepare test samples, 1 mg of the dried extract was dissolved in 20 microlitres of solvent (which the extract was soluble in), and the volume was then adjusted to 1000 microlitres using sterile water (MilliQ) in a 1.5 or 2 millilitre centrifuge tube. Using a 96 - well microplate, the α - glucosidase test was carried out in a reaction volume of 75µl. After mixing 25µl of the α - glucosidase enzyme (0.5U) with a sample solution, the mixture was pre - incubated for 10 minutes at 37°C ± 1°C. Following the pre - incubation period, the reaction mixture was incubated at 37°C ± 1°C for 30 minutes, with 25µl of the substrate (0.5mM, p - nitrophenyl α - D glucopyronoside) added.100 µl of a 0.2M sodium carbonate solution was added to stop the reaction. Using a UV visible spectrophotometer (Mustikan GO, Thermo - Scientific, Finland) and microplate reader, the quantity of p - nitrophenol (yellow colour) emitted from PNPG was measured on a 96 - well microplate at 405 nm. Appropriate sample blanks and controls were included for each sample treatment. All reactions were performed in three replicates. The percentage of α - glucosidase inhibition activity was calculated by using the formula. α - glucosidase inhibition (%) = [(Control OD - Sample OD) / Control OD] ×100 [Control OD = OD of the control reaction without inhibitor - Blank OD; Sample OD = Sample OD - Sample blank OD].

3. Results

***In - vitro* Antidiabetic Assay:** α - amylase inhibition properties were exhibited by all the plant extracts, but hydroalcoholic extract of *Murraya koenigii* leaves, showed highest enzyme inhibition properties (71%) followed by *Aegle marmelos* (46%). The lowest enzyme inhibition property was exhibited by *Laurus nobilis* (26%).

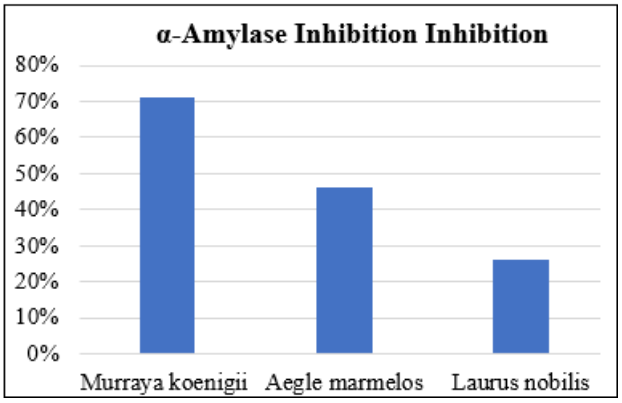

Figure 1: α - Amylase inhibitory properties of hydroalcoholic extracts of *Murraya koenigii* leaves, pulp of *Aegle marmelos* and leaves of *Laurus nobilis.*

Similarly, highest α - glucosidase inhibition properties were exhibited by hydroalcoholic extract of *Murraya koenigii* (84%) as compared to that of *Aegle marmelos* extract (39%). The lowest enzyme inhibition activity was shown by *Laurus nobilis* (15%).

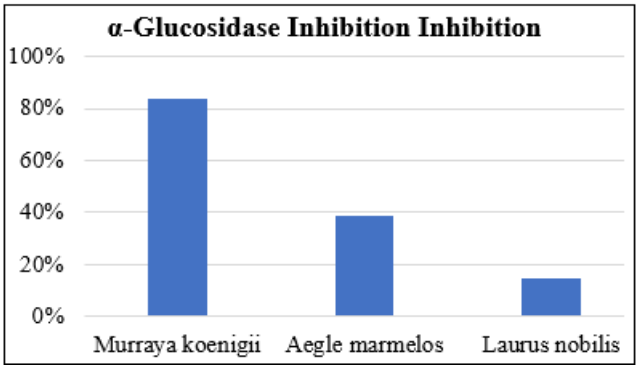

Figure 2: α - Glucosidase inhibitory properties of hydroalcoholic extracts of *Murraya koenigii* leaves, pulp of *Aegle marmelos* and leaves of *Laurus nobilis.*

Statistical Analysis
All data presented were analyzed in triplicate or quadruplet and mean values were presented in the table and text with respective standard deviations (SD).

4. Conclusion

We investigated the antidiabetic efficacy of traditional medicinal plants like *Murraya koenigii*, *Aegle marmelos* and *Laurus nobilis*. The present study demonstrates that among the tested plant extracts, *Murraya koenigii* exhibits the highest enzyme inhibition potential, indicating its strong bioactivity and potential for therapeutic applications. *Aegle marmelos* follows with moderate enzyme inhibition activity, suggesting its efficacy, albeit to a lesser extent. In contrast, *Laurus nobilis* showed the lowest enzyme inhibition activity, reflecting its comparatively weaker bioactive properties. These findings highlight the significant variance in enzyme inhibition potential among these plant species, paving the way for further exploration of *Murraya koenigii* as a potent candidate for enzyme - targeted therapies. The findings pave the way for future research to isolate active compounds from these ethnomedicinal plants, which could lead to the creation of new herbal remedies or pharmaceuticals for diabetes care.

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