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IN VITRO ANTIDIABETIC ACTIVITY OF *Anisomeles malabarica;* (L.) R.Br. ex Sims and *Mirabilis Jalapa;(L).* AND IT'S PHYTOCHEMICAL POTENTIAL

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis by disturbances of carbohydrate, fat and protein metabolism. The world prevalence of diabetes among adults is expected to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030. Considering the importance of this disease the present study is designed to analyze the phytochemical constituents and the anti- diabetic activity of the medicinally important plant species *Anisomeles malabarica*;(L.) R.Br. ex Sims and *Mirabilis jalapa*; (L.) collected from Pachaiyappas college campus in Chennai district of Tamil nadu, India . The fresh leaves of the above plants were used after they dried and powdered for extraction with ethanol solvent. The crude ethanolic leaf extract were screened both qualitatively and quantitative pattern of phytochemical constituents. The results of the study showed the presence of alkaloids, saponins ,protein,tanin and terpenoids in both the species at moderate to higher level . Higher amount of phytoconstituents such as Flavonoids, Alkaloids, Phenols, Terpenoids and Tanins was observed

in *M.jalapa* when compared to *A.malabarica*. The alpha glucosidase activity of ethanolic crude extract of above plants was assayed and observed as 87.19±0.28 (100µg/ml)for *M.jalapa* and 68.16±0.19(100µg/ml) for *A. malabarica* . The alpha amylase activity was found to be 73.28 ±0.24 and 88.07 ±0.34 (100µg/ml) for *A. malabarica* and *M.jalapa* respectively at 100µg/ml. From the present study, it was found that ethanolic leaf extract of *M.jalapa* possessed high anti-diabetic activity and found to have potential phytochemical compounds.

Keywords: *Anisomeles malabarica*, *Mirabilis Jalapa,* alpha-amylase and alpha-glucosidase.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic condition characterized by hyperglycemia and metabolic abnormalities in glucose, protein, and fat. It results in a failure of insulin synthesis or action, or both. According to the International Diabetes Federation, roughly 366 million people worldwide suffer from diabetes, with the number anticipated to double by 2030. Diabetes affects 40.9 million people in India, with the number expected to rise to 60.9 million by 2025 **[1]**. Between the two types of diabetes (T2D), type 2 is more common than type 1, accounting for more than 90% of all diabetic individuals. An imbalance between blood sugar absorption and insulin production causes T2D. Hyperglycemia is a hallmark of T2DM and is involved in the majority of the disease's pathogenic aspects. This syndrome occurs when insulin sensitivity or insulin secretion from pancreatic -cells is reduced, which can further restrict pancreatic insulin secretion and impair insulin-mediated glucose absorption in peripheral tissues **[2]**.

Currently, insulin secretagogues and sensitizers are the most commonly prescribed medications; nevertheless, carbohydrate digesting enzyme inhibitors can help control hyperglycemia by limiting glucose absorption in the intestine **[3]**. Insulin is a hormone generated by the pancreas that plays a key role in the body's glucose and fat metabolism.Patients with type 2 diabetes are frequently insulin resistant and may experien ce a "relative" insulin deficit as a result of this resi stance. If other treatments fail to keep blood glucose levels under control, some people with type 2 diabetes may need insulin. Insulin is required by more than 40% of people with T2D. Inhibition of essential carbohydrates digesting enzymes such as amylase and glucosidase, which also play a key role in preventing diabetic complications, is an important method for controlling hyperglycemia. These enzyme inhibitors slow the digestion of carbohydrates, lowering the rate of glucose absorption from the small intestine and lowering postprandial blood glucose levels (Takahashi *et al*., 2009). Inhibition of alpha - amylase and alpha - glucosidase is thus critical in the treatment and management of hyperglycemia and T2D. The enzymes alpha-amylase and alpha-glucosidase are important in carbohydrates. Intestinal absorption and degradation are two different things. These enzymes can be inhibited, which might cause problems. After a carbohydrate-rich diet, blood glucose levels increased, which could be a useful tactic. Non-insulin-dependent diabetes mellitus (NIDDM) is a kind of diabetes that occurs when the body does not produce enough insulin **[4]**.

Many efforts have been done in recent years to uncover effective alpha - amylase and alpha - -glucosidase inhibitors from natural sources in order to build a physiologic functional diet or lead chemicals for diabetes treatment **[5]**. Acarbose is a glucose-lowering medication used to treat diabetes. Acarbose, on the other hand, is best recognised as an alpha -amylase and alphaglucosidase inhibitor, which can produce stomach distension, flatulence, meteorism, and potentially diarrhoea. As a result, finding a chemical that inhibits alpha - amylase and alpha glucosidase strongly seems appealing **[6]**.

Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as "madhumeha‟ and some of them have been experimentally evaluated and the active principles isolated. Many phytoconstituents, including as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, phenolic compounds, and others, have been identified as alpha -amylase and alpha - glycosidase inhibitors **[7]**. Herbal medications, in particular, have been widely utilized for diabetic therapy for thousands of years due to its traditional acceptability and lack of negative effects. As a result, medicinal plant screening for –alpha amylase and alpha glucosidase inhibitors has gotten a lot of attention. Hence in the present study *Anisomeles malabarica* and *Mirabilis Jalapa* were studied. A*nisomeles malabarica* is a medicinal plant which belongs to the family Lamiaceae family, is distributed in southern tropical regions of Asia. *A.malabarica has been* reported to have significant pharmacological therapeutic potential with anti-inflammatory antiepileptic activity, cytotoxic activity, anti-hyperglycemic and anti-hyperlipidemic activities **[8]**. *Mirabilis jalapa* Linn belongs to the family Nyctaginaceae is one of the plants that used for health care and medicinal purposes. *M. jalapa* is possessed various biological activities namely anti-oxidant, antiinflammatory, anti-microbial, anti-diabetic, cytotoxic and antinociceptive **[9]**. Keeping in this view the present study was undertaken to elucidate the anti-diabetic effect of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa.*

2. MATERIALS AND METHODS

2.1. Collection of plant materials

The leaves of *Anisomeles malabarica* and *Mirabilis Jalapa* were collected from Pachaiyappa's college campus, Chennai, Tamilnadu. It was then cleaned and shade dried. The plant material was identified and validated by a botanist, in the Botany Department, Pachaiyappa's College, Chennai, India.

2.2 Preparation of plant extracts

Plant leaves were cleaned, shade dried, and coarsely powdered by using laboratory mixers .Pre-weighed quantity of leaf powder was soaked for in ethanol for 48 hours **[10]** using Whatmann no. 1 filter paper ,the plant extracts have been filtered and concentrated utilizing, EQUITIRON (rotary evaporator). Concentrated leaf samples were dehydrated by utilizing Lyodel Freeze Dryer (freeze drier) to eradicate the final traces of solvents from the extracts of the plant.

2.3. Preliminary Phytochemical studies of different solvent extracts of *Anisomeles malabarica* and *Mirabilis Jalapa*

Qualitative tests were performed to assess the nature of phytochemicals present in ethanol extracts of *Anisomeles malabarica* and *Mirabilis Jalapa*

a) **Liebermann-Burchard Test:** Extract is dissolved in minimum of chloroform. Acetic acid was added and heated. Few drops of acetic anhydride and concentrated H_2SO_4 were added. Green colour shows the presence of Steroid.

b) **Noller's Test:** Extract is treated with tin and thionyl chloride and was heated in a water bath. Purple colour shows the presence of Triterpenoid.

c) **Shinoda Test:** Extract is dissolved in alcohol. Magnesium bits and concentrated hydrochloric acid was added. It was heated in a water bath. Majenta colour shows the presence of Flavonoid.

d) Test for Furan: Extract is dissolved in alcohol. p-dimethylamino benzaldehyde and concentrated hydrochloric acid was added and was heated in a water bath. Pink colour shows the presence of Furanoid compound.

e) Test for Sugar: Extract is treated with anthrone and concentrated H_2SO_4. It was heated in a water bath. Green colour shows the presence of Sugar.

f) Test for Coumarin: Extract is shaken with 10% NaOH. Yellow colour shows the presence of Coumarin. The substance regenerates when concentrated H_2SO_4 is added.

g) Test for Quinone: Extract is treated with concentrated H_2SO_4. Red colour shows the presence of Quinone.

h) Test for Saponin: Extract is shaken with water. Frothing shows the presence of Saponin.

i) Test for Tannin: Extract is shaken with water and lead acetate solution was added. White precipitate shows the presence of Tannin.

j) Test for Acid: Extract is treated with sodium bicarbonate solution. Effervescence shows the presence of Acid.

k) Test for Phenol: Extract is dissolved in alcohol. Ferric chloride is added. Bluish colour shows the presence of Phenol.

l) Test for Alkaloid: Extract is taken in acetic acid and few drops of freshly prepared Dragendorff's reagent are added. A brick red or orange precipitate shows the presence of Alkaloids.

2.4. Quantitative analysis of ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa*

2.4.1. Determination of total phenolic content in the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa*

The amount of total phenolic content in the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* were determined using Folin– Ciocalteau reagent **[11]**. 0.5 ml of the sample, 0.5 ml H2O, 2 ml Folin– Ciocalteau reagent (1:5 H2O) was added, after 3 min, 10 ml of 10% (w/v) Na2CO3 and the contents were mixed and allowed to stand for 30 min. Absorbance at 725 nm was measured in a UV–Vis spectrophotometer. The amount of total phenolics was calculated as gallic acid equivalent (GAE) in mg per g of dry weight (DW).

2.4.2. Determination of total flavonoid content in the ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

The amount of total flavonoids content in the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* were determined by the aluminium chloride colorimetric method **[12 – 13]** (Chelladurai and Chinnachamy, 2018 and Mervat, 2020). 0.5ml of the extracts at a concentration of 1mg/ml were taken and the volume was made up to 3ml. Then 0.1ml A1C13 (10%), 0.1ml of potassium acetate and 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415nm after 30 min of incubation. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of sample.

2.4.3. Determination of total Alkaloids content content in the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa*

Quantitative estimation of alkaloids was carried out following the method of Edeoga *et al*. **[14]** and Okwu Josiah **[15]** 2.5 g of the powder was extracted using 100 ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours and the filtrate was concentrated up to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitated and settled. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and total alkaloids weighed.

2.4.4. Determination of total tannin content in the ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Tannins content in the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* were determined according to the method **[16]**. The aqueous extracts (1 ml) were mixed with Folin-Ciocalteau's reagent (0.5 ml) followed by the addition of saturated sodium carbonate(Na2CO3) solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 min at room temperature. The sample was obtained by centrifugation and Absorbance at 725 nm was measured in a UV–Vis spectrophotometer. Different concentrations of standard tannic acid were prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph.

2.4.5. Determination of total terpenoid content in the ethanol extract of

Anisomeles malabarica* and *Mirabilis Jalapa

Total terpenoid content in ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* were assessed by standard method as described by Ferguson **[17]**. 1g of extracts of were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoid.

2.5. Anti – Diabetic activity

2.5.1. Alpha amylase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Screening of plant extract for α-amylase was carried by following method of Xiao *et al* **[18]** and Sudha *et al.* **[19]**. The total assay mixture composed of 40µl 0.02 Sodium phosphate buffer (pH 6.9, 6mM), 0.02 units of PPA (α-amylase) solution, plant extracts and acarbose (as positive control) of different concentration 0.1-3 mg/ml (w/v), were incubated at 37°C for 10 mins. Then soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 mins. 1M HCl (20 µl) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5mM iodine and 5mM KI). The absorbance was read at 620 nm on a micro plate reader. A dark blue colour indicates the presence of starch; yellow colour indicates the absence of starch; brown colour indicates partially degraded starch in the reaction mixture.

2.5.2. Alpha glucosidase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Enzyme inhibition assay was measured based on solving the substrate to produce colored products **[20]**. Enzyme alpha glucosidase (Sigma) with a concentration 0.75 units / ml was dissolved in 0.1 M phosphate buffer pH 7. p-nitrophenyl-alpha-D-glucopyranoside 20 mM dissolved in 0.1 M phosphate buffer pH 7 was used as substrate. The reaction mixture contains 125 µl substrate, 240 µl 0.1 M phospate buffer pH 7 and 10 µl sample at various concentrations. After the reaction mixture was incubated at 37°C for 5 mins, 125 µl of enzyme was added and incubated for 15 mins at 37°C. The reaction was stopped by adding 500 µl of sodium carbonate and p-nitrophenol produced was measured at 400nm. As a comparison, we used 1 mg/ml solution of acarbose (Sigma). Inhibition of alpha glucosidase activity was determined by the formula: Inhibition (%) = (Ac-(As-Ab) / Ac x 100 % (Ac: absorbance of control, Ab: absorbance of background, As: absorbance of sample) The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

2.6. Statistical analysis

All the data obtained in the present study were statistically analysed using the statistical software SPSS version 16.0. One-way Anova using Bonferroni test was applied to find out the significant difference between the different concentrations of plant extracts.

3. RESULTS AND DISCUSSION

Natural compounds screening is the process of evaluating all pharmaceuticals using phytochemical and pharmacological methodologies that lead to drug discovery. Chemicals produced by various portions of plants are known as phytochemicals. Steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, and glycosides are some of the bioactive elements of plants. Qualitative phytochemical assessment might very well aid in understanding a number of chemical complexes obtained from plants, and qualitative phytochemical screening will help to understand a range of chemical compounds obtained from plants.

3.1. Qualitative and quantitative analysis of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Preliminary screening of the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* were found to contain various phytochemical constituents which are shown in **Table 1**. Ethanol extract of *Mirabilis Jalapa* showed the presence of alkaloids, saponins, protein, phenols, flavonoids, terpenoids and steroids. Whereas in ethanol extract of *Anisomeles malabarica* showed the maximum phytoconstituents namely alkaloids, glycosides, saponins, protein, coumarin, quinone, furan, tannin, phenols, flavonoids, terpenoids and steroids. Both the ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* showed the alkaloids, saponins, protein, tannin and terpenoids respectively.

Quantitative analysis of ethanol extract of *Anisomeles malabarica and Mirabilis Jalapa* were studied. Ethanol extract of *Anisomeles malabarica* of Flavonoids, alklaloids, phenols, tannins and terpenoids showed the 16.10 ± 0.02, 17.19 ± 0.05, 12.36 ± 0.03, 23.04 ± 0.02 and 15.12 ± 0.03; whereas in ethanol extract of *Mirabilis Jalapa* possessed 35.16 ± 0.07, 29.16 ± 0.05, 19.12 ± 0.04, 38.06 ±0.10 and 32.12 ± 0.05 respectively (Table 2) . Maximum percentage of phytoconstituents was observed in ethanol extract of *Mirabilis Jalapa* when compared to ethanol extract of *Anisomeles malabarica*.

Normally, phytochemicals are found to be present in parts of the plants such as roots, stems, leaves, bark, flowers, fruits or seeds **[21- 23]**. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The most important of these bioactive constituents in plants are tannins, flavonoids, phenolic compounds, terpenoids, alkaloids, steroids and saponins **[24]**. The medicinal value of plants lies in the phytochemicals that have a definite physiological action on the human body. Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenolics and triterpenoids such as oleanane, ursane, lupane, and flavonoids have a positive correlation as antidiabetic agents **[25- 26]**.

Alkaloids comprise one of the major groups of plant constituents. They are found in most of the medicinal plants and they are commonly used as anaesthetic agents **[27]**. Several of the alkaloids were in clinical use, including reserpine (the first tranquilizer) and the dimeric indole alkaloids, vinblastine and vincristine (anticancer agents) reported **[28-29]**. Flavonoids are the most important natural phenolic compound and they possess a broad spectrum of chemical and biological activities **[30]**. Patel *et al*. **[31]** illustrated that flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic activities etc.

Polyphenols possess ideal structural chemistry for free radical scavenging activity and they have been shown to be more effective antioxidants in vitro than tocopherols and ascorbate. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol derived radical to stabilize, delocalize the unpaired electron and chelate transition metal ions. Phenolic compounds and flavonoids have also been ascribed to various properties like anticancer, antidiabetic, antiaging and prevention of cardiovascular diseases **[32]**.

Tannins are one of the major phytochemicals found in many higher plants. Tannins have a characteristic strange smell and astringent taste and could bind to proteins (and consequently precipitate proteins) through the effective formation of strong complexes with proteins and other macromolecules. Thus, they could have a major impact on animal nutrition, including inhibition of growth rate digestive enzymes **[33]**. They are useful as an anti-inflammatory agent and in the treatment of burns and other wounds based on their anti hemorrhagic and antiseptic potentials. In particular, tannins-rich remedies are used as antihelmintics **[34]**, antioxidants **[35]**, antimicrobials and antivirals **[36]** in cancer chemotherapy [37] and to chelate dietary iron [38].

Terpenoids are one of the most important classes of bioactive compounds in higher plants, hence the foremost task is the screening of these compounds in the plants. The terpenoids are a class of natural products which have been derived from five-carbon isoprene units. A large number of triterpenoids suppress the growth of a variety of cancer cells without exerting any toxicity in normal cells. Numerous preclinical efficacy studies have provided extensive evidence that both naturally occurring and synthetic derivatives of triterpenoids possess chemopreventive as well as therapeutic effects against colon, breast, prostate and skin cancer **[39]**. Several researchers have been investigated the phytochemicals from medicinal plants which possessed the biological activities **[40- 45]**.

3.2. Alpha amylase and Alpha glucosidase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Hyperglycemia has been proposed as an independent risk factor for diabetes. Therefore, control of postprandial hyperglycemia is suggested to be important in the treatment of diabetes. Inhibitors of alpha-amylase and alpha glucosidase, which are particularly useful in postprandial alpha-glycemic control, are good candidates for prevention of diabetic complications. In the present study ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* were evaluated for their inhibitory effect on α-amylase and α-glucosidase by *in-vitro* method. The percentage inhibition at 20, 40, 60, 80 and 100 µg/ml concentrations of plant extracts showed a concentration-dependent reduction in percentage inhibition. The ethanol extract of *Anisomeles malabarica* (20, 40, 60, 80 and 100 µg/ml) exhibited 39.14 ± 0.10, 46.08 ± 0.12, 55.35 ± 0.14, 68.14 ± 0.16 and 73.28 ± 0.24 and ethanol extract of *Mirabilis Jalapa* (20, 40, 60, 80 and 100 µg/ml) exhibited 43.05 ± 0.12, 56.17 ± 0.18, 65.17 ± 0.26,76.15 ± 0.30 and 88.07 ± 0.34 **(Table 3; Figure 1)** respectively. Acarbose was used as a standard reference drug, which showed a maximum inhibition when compared to ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*.

Alpha amylase inhibition activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* were significantly different (P<0.05). Values are expressed as mean ± sd of triplicates

Alpha glucosidase inhibition activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* were also investigated. Our results demonstrated that the highest potential

α-glucosidase inhibitory activity was shown in ethanol extract of *Mirabilis Jalapa* at 100 µg/ml and it was found to be 87.19 ± 0.28 whereas in ethanol extract of *Anisomeles malabarica* 68.16 ± 0.19 respectively **(Table 4 and Figure 2)**. Alpha glucosidase inhibition activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* were significantly different (P<0.05).

This result is in agreement with previous reports which indicated that hypoglycaemic activity through either increased secretion of the insulin from pancreas or similar action to the insulin. Several plant species have been described as hypoglycaemic such as *Opuntia streptacantha*, *Trigonella foenum graecum*, *Momordica charantia*, *Ficus bengalensis*, *Polygala senega*, *Gymnema sylvestre*, *Allium sativum*, *Citrullus colocynthis* and *Aloe vera* [46]. Similar dose dependent increasing results were obtained on the plants like Syzygium cumini, Psidium guajava **[47 – 48]**. Most of the herbal plants and its parts have the tendency to reduce the blood glucose level. This is due to the availability of tannins, terpenoids and flavonoids **[49]**. According to Kumar *et al*. **[50]** medicinal plants have been demonstrated to have a substantial inhibitory activity against alpha – amylase could be employed as an effective therapeutic for postprandial hyperglycemia. Bhatia *et al*. **[51]** profounded that the alpha-glucosidase is a membrane-bound enzyme found in the small intestine epithelium that catalyses the conversion of disaccharides to glucose. Inhibitors can prevent dietary carbohydrates from being absorbed and control postprandial hyperglycemia. Glucosidases are required for the digestion of carbohydrates as well as the processing of glycoproteins and glycolipids. Several studies have revealed that plant extracts can serve as -glucosidase inhibitors, implying that they can be used to treat hyperglycemia **[51 -54; 12]**. The ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* inhibit the α-amylase and α-glucosidase might be due to the presence of several phytochemicals such as flavonoid, steroid, alkaloid, saponin, triterpenoid, quinone, tannins, coumarin, and phenols. The active components in the ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* do not compete with the substrate for binding to the active site; instead, the inhibitors attach to a different spot on the enzyme to slow down the conversion of disaccharides to monosaccharides in the mixed noncompetitive method of inhibition. This study also suggests that one of the mechanisms by which this plant displayed its antidiabetic potential is by the inhibition of alpha amylase and alpha glucosidase. However, further study is needed to isolate the active principle(s) in this plant which is responsible for this activity.

Table 1: Phytochemical screening of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Phytochemical tests	*Am*EE	*Mj*EE
Alkaloid test	+	+
Shinoda test (Flavonoids)	+	+
Phenol test	-	+
Tannin test	-	+
Liebermann– Burchad test (Steroid)	+	+
Noller's test (Triterpenoid)	+	+
Coumarin test	–	+
Saponin test	–	+
Quinones test	–	–
Sugar test	–	+
Furan's test (Furanoid)	+	-
Acid test	-	-

Table 2: Quantitative analysis of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

S. No.	Phytoconstituents	*Am*EE	*Mj*EE
1	Flavonoids	16.10 ± 0.02	35.16 ± 0.07
2	Alklaloids	17.19 ± 0.05	29.16 ± 0.05
3	Phenols	12.36 ± 0.03	19.12 ± 0.04
4	Tannins	23.04 ± 0.02	38.06 ±0.10
5	Terpenoids	15.12 ± 0.03	32.12 ± 0.05

Am*EE - Ethanol extract of *Anisomeles malabarica; Mj*EE - Ethanol extract of *Mirabilis Jalapa

Table 3: Alpha amylase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Conc. µg/ml	Acarbose	*Am*EE	*Mj*EE
20	60.12 ± 0.12	39.14 ± 0.10	43.05 ± 0.12
40	68.08 ± 0.17	46.08 ± 0.12	56.17 ± 0.18
60	75.10 ± 0.21	55.35 ± 0.14	65.17 ± 0.26
80	81.12 ± 0.25	68.14 ± 0.16	76.15 ± 0.30
100	98.12 ± 0.31	73.28 ± 0.24	88.07 ± 0.34

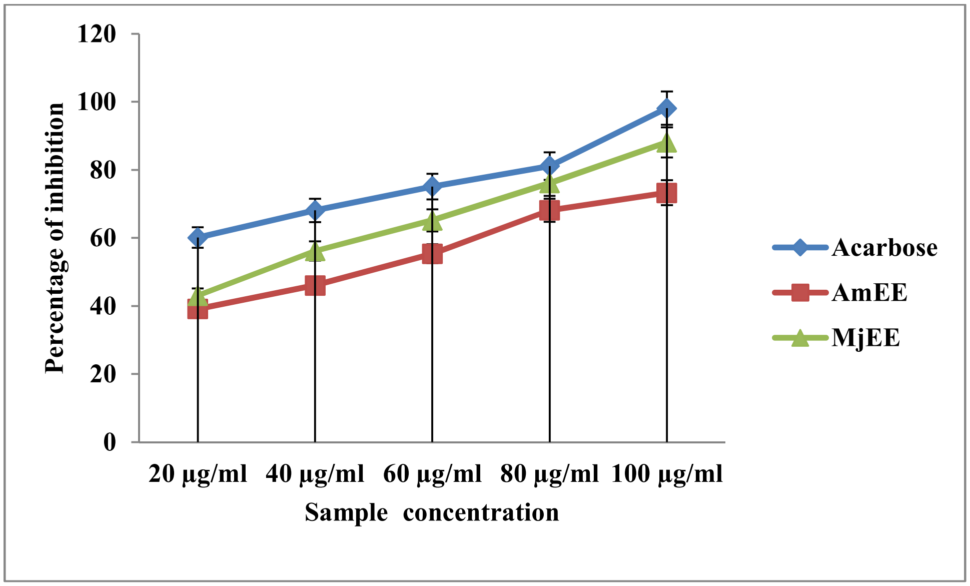

Figure 1: Alpha amylase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Table 4: Alpha glucosidase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

Conc. µg/ml	Acarbose	*Am*EE	*Mj*EE
20	57.08 ± 0.10	37.02 ± 0.10	49.12 ± 0.12
40	64.10 ± 0.12	44.19 ± 0.12	56.28 ± 0.18
60	75.26 ± 0.20	53.02 ± 0.17	67.15 ± 0.21
80	80.32 ± 0.22	60.18 ± 0.15	74.25 ± 0.25
100	93.20 ± 0.10	68.16 ± 0.19	87.19 ± 0.28

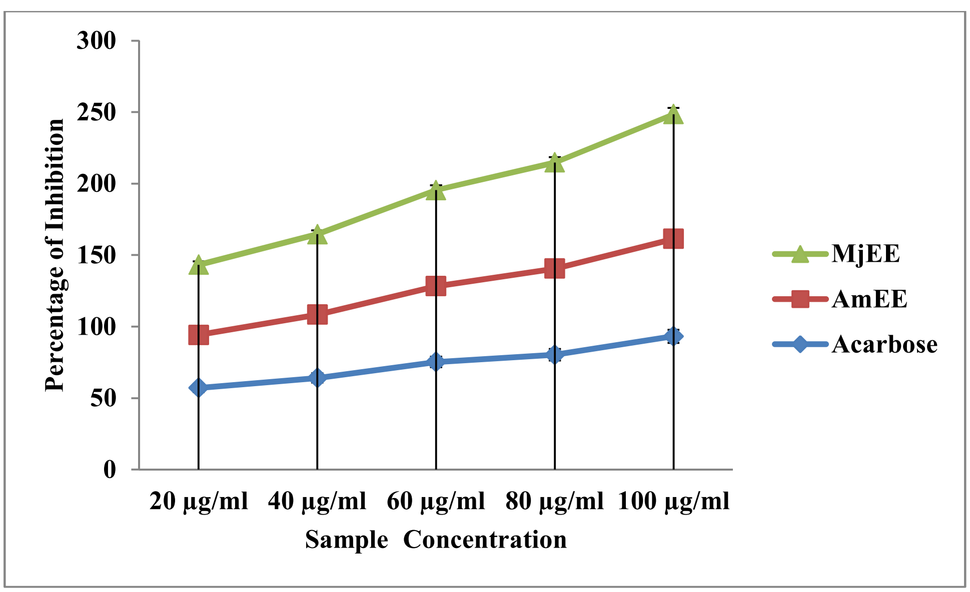

Figure 2: Alpha glucosidase activity of ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa*

4. CONCLUSION

Diabetes mellitus (DM) is the most common form of diabetes and is usually caused by life-style factors and also related to insufficient insulin production and resistance of target tissues to insulin. Hyperglycemia can be handled initially with oral synthetic agents and insulin therapy. However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries. One of the current trends in the management of diabetes is to decrease post-prandial hyperglycemia. This can be achieved through the inhibition of carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase. Alpha-amylase and glucosidase inhibitors from medicinal plants can be used for developing new target drugs for the treatment of diabetes, obesity and hyperlipidemia. The ethanol extract of *Anisomeles malabarica* and *Mirabilis Jalapa* inhibit the α-amylase and α-glucosidase might be due to the presence of several phytochemicals such as flavonoid, steroid, alkaloid, saponin, triterpenoid, quinone, tannins, coumarin, and phenols. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents. This study also suggests that one of the mechanisms by which this plant displayed its antidiabetic potential is by the inhibition of alpha amylase and alpha glucosidase. However, further study is needed to isolate the active principle(s) in this plant which is responsible for this activity.

Conflict of Interest: Nil

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