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EVALUATION OF *IN VITRO* ANTIDIABETIC ACTIVITY OF TOTAL AQUEOUS LEAF EXTRACTS *MORINDA CITRIFOLIA* Linn.

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ABSTRACT

Morinda citrifolia, L. popularly known as Indian Noni or Indian mulberry is an ever green small tree bearing flowers and fruits throughout the year. It belongs to family Rubiaceae. Noni is one of the important traditional folk medicinal plants that have been used for over 2000 years in Polynesia. It has been reported to have a broad range of therapeutic and nutritional value.. All the plant parts are used in the treatment of various diseases and disorders. The fruit is important because of its wide range of therapeutic potentials such as anti-bacterial, anti-viral, anti-tumor, anti-helminthes, analgesic, hypertensive, anti-inflammatory and immune enhancing effects. The total aqueous extract of *Morinda citrifolia* leaves show significant invitro antidiabetic activity when compared with the standard acarbose. The *in vitro* antidiabetic activity was carried out by using α-amylase inhibition method.

Key Words: *Morinda citrifolia*, α-amylase inhibition.

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INTRODUCTION

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences. Iran is home to several indigenous tribes with a rich heritage of knowledge on the uses of medicinal plants. Iran has varied climates and geographical regions that have caused a wide distribution of individual medicinal plant species such that each tribe has its own plants and customs. Alamut is one of the most important geographic regions in Iran because of its ancient history of cultivating traditional medicinal plants. Alamut region and the several villages it encompasses are secluded from other cities in Iran, which is why the people living in this region have relied on indigenous medical knowledge and medicinal plants. In this study, we analyzed the medicinal plants with most therapeutic usage in the region (1-3). *Morinda citrifolia* Linn (family: Rubiaceae) is also known as "noni", is distributed throughout the tropical and sub-tropical regions of the world such as French Polynesia and Hawaii. This

plant have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelminths, analgesic, cardio-protective, hypotensive, immune enhancing, antiinflammatory, antioxidant, antiosteoporotic, insulinotropic, antidyslipidemic effects and hepatoprotective. The fruit contains hydrophilic compounds like carbohydrates, proteins, minerals, vitamins and small amount of fat. The products obtained from the different parts of *M. citrifolia* plant viz. leaves, fruits, roots and barks are available in the market as Noni juice, capsule, powder, Noni concentrates, and also tea in the market. Among them Noni juice is most popular for its nutraceuticals and high therapeutic values around the globe. Noni juice has been recently established in European Union as a novel food (4, 5).

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as Allium sativum (Garlic), Azadirachta indica(Neem), Vinca rosea (Nayantara), Trigonella foenum (Fenugreek), Momordica charantia (Bitter ground), Ocimum sanctum (Tulsi). Many of these are less effective in lowering glucose levels in severe diabetes (6).

The present study focuses the antidiabetic activity of the total aqueous leaf extracts of *Morinda citrifolia*.

MATERIALS AND METHODS

Plant Collection and Drying

The plant *Morinda citrifolia* was collected from Kasaragod and Kannur. The plant material was taxonomically identified by the botanist Mr. Biju, Assistant Professor, Botany Department, Government College, Kasaragod. The plant dried under shade for about 7 days and then powdered with mechanical grinder and stored in an air tight container.

Preliminary Phytochemical Screening (7, 8)

Extraction of the dried powder of the leaves *Morinda citrifolia* was carried out by successive solvent extraction using solvents of increasing polarity viz. petroleum ether, n-hexane, chloroform, acetone, ethanol and water. Around 25 g of dried powder was weighed, moistened with the respective solvent and packed in the soxhlet extractor and was then extracted with 500 ml each of the petroleum ether, n-hexane, chloroform, acetone, ethanol and water. After each extraction the same dried marc was used for the subsequent extraction. Each extract was then filtered, the solvent distilled off and finally the dried extract was obtained. The percentage yield of each extracts was calculated. These extracts were used for preliminary phytochemical screening.

Extraction of Total Aqueous Extract of Plant

Total aqueous extract was prepared by Maceration method. 100 gm of the air dried powder was weigh and macerated by using chloroform water for 24 hours to obtain the aqueous extract. The percentage yield of both the extracts was calculated.

In Vitro Antidiabetic Studies of Total Aqueous Leaf Extract of *Morinda citrifoli* (9, 10)

Assay of α- amylase inhibition

Determination of α- amylase inhibition carried out by quantifying the reducing sugar(maltose equivalent) liberated under assay condition.Enzyme inhibitory activity expressed as a decrease in unit of maltose liberated.Modified dinitrosalicylic acid(DNS) method adopted to estimate the maltose equivalent.

Procedure

Incubate 250µl of plant extracts with varying concentrations (50,100,150 and 200µg/ml) and 250µl of 0.02M sodium phosphate buffer (p^H 6.9 with 0.006M sodium chloride) containing alpha amylase solution (0.5µg/ml) at 25^{o}C for 10 minutes. Add 250µl of 1% starch solution in 0.02M sodium phosphate buffer p^H 6.9 to each tube at timed intervals. Incubate the reaction mixture at 25^{o}C for 10 minutes. The reaction will stop with 500µl of dinitrosalicylic acid colour reagent. The test tubes are then incubate in a boiling water bath for 5 minutes, then cool to room temperature. Dilute the reaction mixture by adding 5ml distilled water and measure the absorbence at 540 nm. Acarbose at various concentrations (50,100,150,200µg/ml) was included as a standard.

Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula

Inhibitory activity (%) = (Ac – As/Ac) x 100

Where,

As- absorbance of test substance

Ac- absorbance of control

Calculation of IC_{50} (50% inhibitory concentration)

The concentration (µg/ml) of the drug required to inhibit 50% α-amylase was calculated from the graph. The IC_{50} value was calculated for inhibitory concentration of both the sample and standard solution.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the plant shows the presence of different chemical constituents such as alkaloids, glycosides, saponins, phenolic compounds, flavanoids, steroids and carbohydrates. Table-1 shows the preliminary phytochemical screening of the plant. The invitro antidiabetic activity was carried out by using α-amylase inhibition method. Total aqueous extract and standard exhibited dose dependent activity. The results are shown in table-2 and fig-1.

Table-1 Results showing qualitative phytochemical screening of *Morinda citrifolia*

S.No	Phyto constituents	Pet.ether	Chloroform	Acetone	Methanol	Distilled water
1	Alkaloid	-	+	+	+	+
2	Glycoside	-	-	-	+	-
3	Flavanoid	-	-	+	+	+
4	Tannins	-	-	-	+	-
5	Phenolic compounds	-	-	+	+	+
6	Carbohydrates	-	-	-	-	+
7	Saponins	-	-	-	+	+
8	Sterols	+	-	-	-	-

Table-2 Results showing in vitro antidiabetic activity of total aqueous leaf extract of *Morinda citrifolia* by α-amylase inhibition method

Sl No	Sample	Concentration (µg/ml)	Absorbance 540nm	Percentage Inhibiton (%)
1	Standard	50	0.055	23.61
		100	0.045	37.50
		200	0.041	43.05
		400	0.039	45.83
2	Total Aqueous	50	0.056	22.2
		100	0.039	45.83
		200	0.038	47.22
		400	0.033	54.16

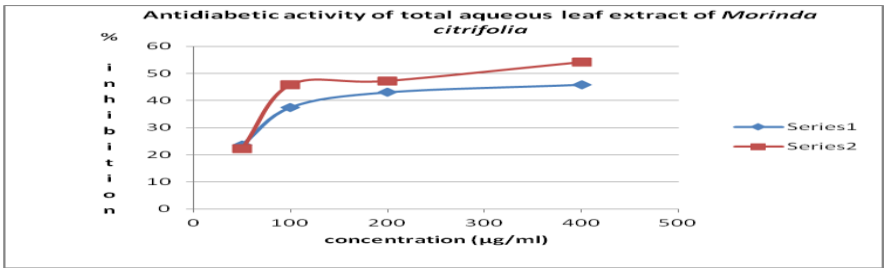

Fig-1 Result showing antidiabetic activity of total aqueous leaf extract of *Morinda citrifolia* by α-amylase inhibition method

IC_{50} value was calculated for total aqueous extract and standard from the graph. The minimum concentration for 50% inhibition was shown by total aqueous extract. This was highly significant when compared with the concentration of the standard drug. The values are shown in table-3.

Table-3 Results showing IC_{50} values of total aqueous leaf extract of *Morinda citrifolia* by α-amylase inhibition method

S No	Sample	IC $_{50}$
1	Standard	325 µg/ml
2	Total Aqueous	360 µg/ml

CONCLUSION

The air dried powder of *Morinda citrifolia* leaves subjected to successive solvent extraction using petroleum ether, chloroform, acetone, ethanol and water as per standard procedure. The various extracts obtained from successive solvent extraction were used for preliminary phytochemical analysis it reveals the presence of alkaloids, glycosides, flavanoids, sterols, tannins, carbohydrates, a, saponins. The *In vitro* Antidiabetic activity was performed by α-amylase inhibition method. The total aqueous extract of plant leaves showed more significant activity when compared with standard acarbose.

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