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Evaluation of Antidiabetic Potential of Methanolic Extract of *Myristica fragrans* (Mace) and Cinnamomum Verum- A Comparative in Vitro Study

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Abstract

Myristica fragrans (mace) and Cinnamomum verum are traditional medicinal plants which are used as spices in flavouring of food. They exhibit various medicinal properties. Diabetes is a condition that impairs the body's ability to process blood sugar levels. Anti-diabetic drugs are used to stabilize and control blood glucose levels. From the study, it was evident that the methanolic extract of Myristica fragrans (mace) exhibited significantly increased antioxidant and anti-diabetic potential when compared to that of Cinnamomum verum.

Keywords: *Anti-Diabetic, Antioxidant, Cinnamomum Verum, Innovative Technology, Medicinal Plants, Myristica Fragrans, Novel Method.*

Introduction

Myristica fragrans which are commonly called jaiphal and javitri in India [1]. Myistica belongs to the Myristicae or *Annanceae* family. Myristic seeds are considered important economically and medically. Myristica is an evergreen tree which spreads the aroma and also provides flavours. Myristica consists of two main parts: the nutmeg and mace [2]. Mace is the aril covering of the seed nutmeg. Mace contains essential oil, which possesses the property of anti-inflammatory and hepatoprotective [3]. Nutmeg is mostly used in the tissue culture system and usually helps in heavy leaching. The chemical constituents are myristicin, mace lignin and eugenol [4]. The chemical constituents of *Myristica fragrans* are reported to show antihyperlipidemic, anticholesterol, antidepressant, antimicrobial, memory enhancing, hepatoprotective and antioxidant properties [5].

Cinnamomum verum is a cuisine and it is widely used as a spice in flavouring of food [6]. It is also a traditional folk medicine. It belongs to the family *Lauraceae [7]*. It is found in the tropical and subtropical regions [8]. The chemical constituents present in *Cinnamomum verum* are cinnamaldehyde, copene and eugenol. It is widely used in treating asthma, inflammation, bronchitis and many more [9]. *Cinnamomum verum* shows antioxidant, antimicrobial, antidiabetic and anticancer properties [10]. Cinnamaldehyde acts as a protection against oxidative stress and other chronic diseases [11].

Diabetes mellitus is a metabolic disorder caused by dysregulation in glucose homeostasis. This disease is distinguished by chronic hyperglycemia with disturbances in the macromolecules' metabolism as a result of impairments in insulin secretion, insulin action, or both [12]. The antidiabetic property will help in controlling the diabetics which regulate the level of glucose with the agents such as insulin or by oral hypoglycemic drugs. One class of oral hypoglycemic drugs acts by inhibiting the activities of alpha glucosidase and alpha

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amylase enzymes which help to delay the absorption of glucose and maintain the blood glucose level in hyperglycemic patients [3]. There are about 400 traditional plants that have been reported to possess antidiabetic effects [13, 14].

The research is needed to find out a natural efficient anti-diabetic drug so as to reduce the side effects caused by allopathy medicines [15, 16], The main aim of the study is to evaluate and compare the anti-diabetic potential of *Myristica fragrans* and *Cinnamomum verum*.

Materials and Methods

Chemicals

All chemicals and reagents used for this research work were purchased from Sigma Chemical.

Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd.

Bangalore, India; New England Biolabs (NEB), USA.

Collection of Plant Material

Myristica fragrans(mace) and *Cinnamomum verum* leaves were collected from Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. The bark, leaves and flower parts of the plant were shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with ethanol.

Preparation of Plant Extracts

1kg of dry powders from leaves from both plants was taken in individual aspirator bottles; 3 litres of ethanol were used, and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times, and all extracts were decanted. and pooled. The extracts were filtered before drying using Whatman filter paper no 2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extracts were placed in pre-weighed flasks before drying.

Phytochemical Screening Test

Test For Phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of a red color precipitate indicates the presence of phlorotannins.

Test for Carbohydrates

Three to five drops of Molisch reagent were added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The Development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

A few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids

2ml of the sample was mixed with 2ml of HCl. Then 6 drops of HCN were added and a further 2 drops of picric acid were added which resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2 ml of chloroform and 3 ml of con. H_2SO_4 was added. The red color precipitate obtained indicates the presence of terpenoids.

Test for Proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added to ninhydrin. The formation of a purple colour revealed the presence of protein.

Detection of Saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for Steroids

One ml of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride. and five drops of concentrated sulphuric acid were added and mixed. The formation of a dark red colour or dark pink colour indicates the presence of steroids.

In Vitro Antioxidant Activity (Dpph Free Radical Scavenging Activity)

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method [17]. DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard.

Alpha Amylase Inhibitory Activity of Methanolic Extract of Myristica Fragrans (Mace) and *Cinnamomum Verum*

Alpha amylase inhibitory activity of the extract was carried out according to the standard method of Ademiluyi et al [2013], In a test tube a reaction mixture containing 500 mu/l phosphate buffer (100mM; pH=6.8). 100 mu alpha-amylase (2 mu/l) and varying concentrations of extract (0.1 - 0.5 mg/ml) were Incubated at 37 °C for 20 minutes. Then 200 mu/l of 1% soluble starch (100 MM phosphate buffer 6.8) was added as a substrate and incubated further at 37 degrees Celsius for 30 minutes, 1000 mu/l of the 3,5 Dinitrosalicylic acid [DNS], DNS colour reagent was then added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nm using a multi-plate reader. Acarbose at various concentrations (0.1-0.5 mg/ml) was used as a standard.

Alpha Glucosidase Inhibitory Activity of Methanolic Extract of Myristica Fragrans (Mace) and *Cinnamomum Verum*

Alpha-glucosidase inhibitory activity of extract was carried out according to the method by [18] Reaction mixture containing 500 mu/l phosphate buffer(100 mM pH 6.8), 100mu/l glucosidase (10 ml) and varying concentration of extract (0.1 to 0.5 mg /ml) was pre-incubated at 37 degree Celsius for 15 minutes. Then 200 mu/l p-NPG(5mM) was added as a substrate and incubated further at 37 °C for 30 minutes. The reaction was stopped by adding 50 mu/l sodium carbonate (0.1M). The absorbance of the released p-nitrophenol was measured at 405 nm using multiple readers. Acarbose at various concentrations (0.1-0.5mg/ml) was used as a standard.

Statistical Analysis

The data were subjected to statistical analysis using Two-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups. In Tukey's test, significance was considered at the level of $p < 0.05$.

Results

Table 1. Phytochemical Analysis of Methanolic Extract of *Myristica fragrans (Mace)* and *Cinnamomum Verum*

PHYTOCHEMICALS	*MYRISTICA FRAGRANS*	*CINNAMOMUM VERUM*
ALKALOIDS	++	++
FLAVONOIDS	++	+

TERPENOIDS	++	++
SAPONINS	++	+
CARBOHYDRATE	+	+
PROTEINS	-	-
STEROIDS	+	+
TANNINS	+++	-
PHENOLS	+	-
AMINO ACIDS	-	-

The methanolic extract of Myristica fragrans was found to be rich in alkaloids, flavonoid, terpenoids, carbohydrates, saponins, phenols, tannins and steroids and that of Cinnamomum verum was found to be alkaloids, flavonoids, terpenoids, saponins, carbohydrates and steroids.

From the study of phytochemical analysis, it was evident that the methanolic extract of Myristica fragrans was found to be rich in alkaloids, flavonoids, terpenoids, carbohydrates, saponins, phenols, tannins and steroids and that of Cinnamomum verum was found be alkaloids, flavonoids, terpenoids, saponins, carbohydrates and steroids. The presence of these phytochemicals helps the extract to act as a good antioxidant (Table 1). Antioxidant analysis of *Myristica fragrans and Cinnamomum verum* was analysed and compared with the standard vitamin C. The methanolic extracts of *Myristica fragrans and Cinnamomum verum* exhibited a significant antioxidant potential IC_{50} at 280µg/ml and 400µg/ml respectively with significance at $p<0.05$ (Figure 1). The antioxidant potential of the extract increased in a dose-dependent manner as compared to the standard (Vitamin C). From the invitro antidiabetic activity, it was evident that the methanolic extract of Myristica fragrans and Cinnamomum verum was analysed by estimating the extract's alpha amylase and alpha-glucosidase inhibitory potential and compared with the standard Acarbose. The enzymes amylase and glucosidase act on starch and release free glucose molecules. If the extract has significant inhibition of these enzymes, it is proportional to its antidiabetic potential. The extract exhibited a significant alpha-amylase of IC_{50} at 330µg/ml and 380µg/ml respectively with significance at $p<0.05$ (Figure 2) and alpha-glucosidase inhibitory potential of IC_{50}at 390µg/ml and 430µg/ml with significance at $p<0.05$ (Figure 3). The antidiabetic potential of the extract increased in a dose-dependent manner as compared to the standard - Acarbose. In comparison, methanolic extracts of *Myristica fragrans* exhibited significantly more anti-diabetic potential than *Cinnamomum verum.*

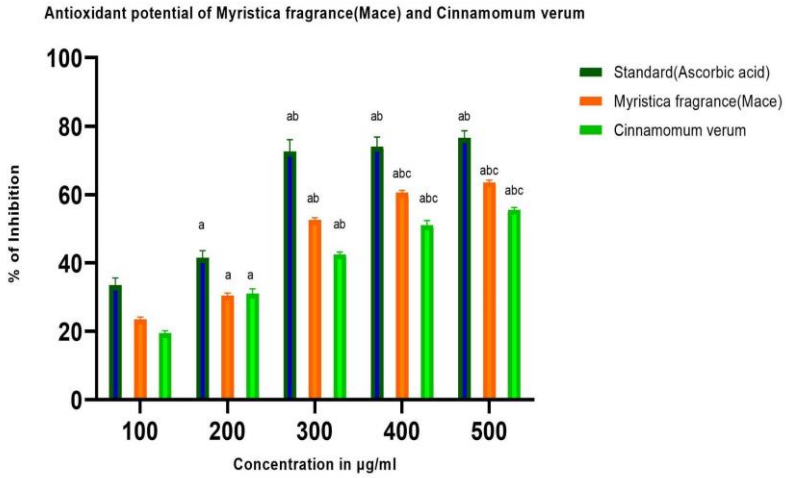

Figure 1. Antioxidant Potential of Methanolic Extract of *Myristica fragrance and Cinnamomum verum* Compared with the Standard (Vitamin C)- DPPH Assay

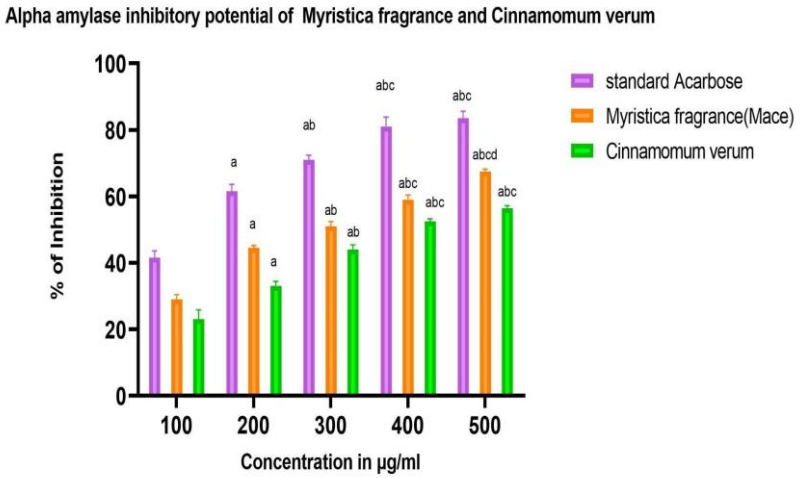

Figure 2. Alpha Amylase Inhibitory Potential of Methanolic Extract of *Myristica fragrance and Cinnamomum Verum* Compared with the Standard (Acarbose)

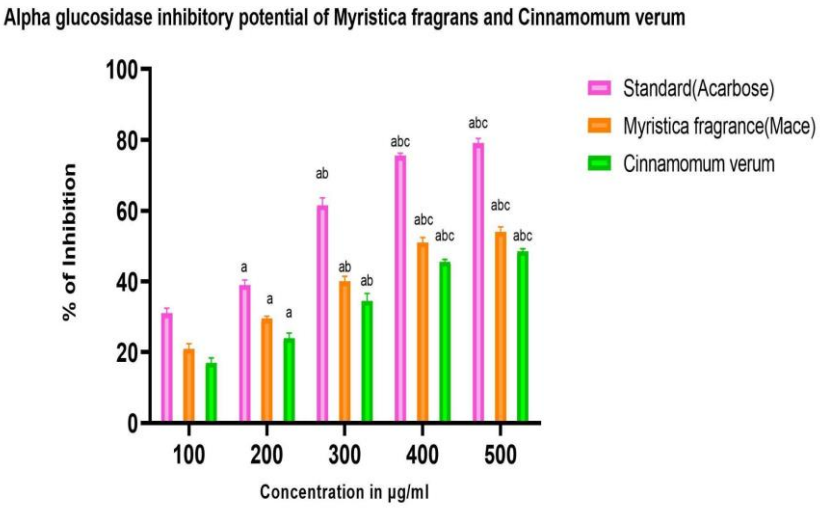

Figure 3. Alpha Glucosidase Inhibitory Potential of Methanolic Extract of *Myristica fragrance and Cinnamomum Verum* as Comparas to the Standard (Acarbose)

Discussion

Myristica fragrans showed the existence of phytochemicals like alkaloids, flavonoids, terpenoids, carbohydrates, phenols, tannins and steroids. *Cinnamomum verum* showed the presence of screening in medicinal plants is used to identify active compounds that are beneficial to phytochemicals like alkaloids, flavonoids, terpenoids, carbohydrates, saponins and steroids. Phytochemical the body's health [19]. Phytochemicals are the compounds produced by plants, which exhibit pharmacological properties applicable in the treatment of infections, and many chronic degenerative diseases such as diabetes and cancer [20]. Hence the presence of the phytochemicals in both the plant extract might have contributed to their antioxidant and antidiabetic potential.

The extracts were prepared and analyzed for antioxidant activity by DPPH free radical scavenging assay. Both the extracts showed a significant dose-dependent increase in the radical scavenging activity, which was compared with the standard vitamin C. The effect of anti with the standard vitamin C. This indicates the in vitro antioxidant activity of both extracts. Phytochemicals are compounds produced in plants that have great importance in free radical scavenging activity. Free radicals and molecules possessing an unpaired electron emerge in oxidative stress. Reactive oxygen species including superoxide radicals, hydroxyl radicals and hydrogen peroxide are the by-products of biological reactions [21] and are associated with various pathological effects like damage of the DNA, carcinogenesis and degenerative disorders [22]. Free radical scavenging of antioxidants was considered due to their hydrogen-donating ability. The IC 50 values of *Myristica fragrans*, and *Cinnamomum verum* were found to be 280µ g/ ml, and 400µ g/ ml respectively, which indicates that *Myristica fragrans* showed higher antioxidant activity than *Cinnamomum verum.*

Management of diabetes without side effects is still a challenging fact for the medical society. Several recent studies have contributed valuable insights to research [23,24,25]. It proposed that inhibition of the activity of the digestive enzymes alpha-amylase and alpha-glucosidase is one of the mechanisms used in the management of diabetes which would delay the degradation of carbohydrates. This, in turn, causes a decrease in the absorption of glucose and a reduction in the postprandial elevation of blood glucose [26]. Acarbose and miglitol are drugs that can inhibit these enzymes and are used in diabetes management but are associated with many side effects like flatulence and discomfort [27]. Hence interest in identifying pharmacologically active phytoconstituents that can inhibit α-amylase and α-glucosidase is increasing as they have fewer side effects and are less expensive compared to synthetic drugs. Recent studies have delved into diverse aspects of research [28,29,30]

Both the extracts evaluated in this study showed potent inhibition towards alpha-amylase (with an IC50 value of 330 µg/ ml *Myristica fragrans* and 380 µg/ ml for Cinnamomum verum) and alpha glucosidase (with an IC50value of 390 µg/ ml *Myristica fragrans* and 430 µg/ ml for Cinnamomum verum) enzymes. Thus, these extracts can be used for the formulation of antidiabetic drugs if detailed studies are done on these plants. However, *Myristica fragrans* showed more activity than *Cinnamomum verum.*

Conclusion

Within the limits of the study, it is evident that methanolic extract of *Myristica fragrans* and *Cinnamomum verum* possess antidiabetic and antioxidant potentials. Methanolic extract of *Myristica fragrans* showed greater activity compared to *Cinnamomum verum*. More research has to be done to explore the mechanism of the anti-diabetic potential of these plant extracts in detail. Further downstream processing can be done to isolate

these principal components which will improve the pharmacokinetics of the herbal extract.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest in this stud

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