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STUDY OF *IN-VITRO* α -GLUCOSIDASE INHIBITORY ACTIVITY OF *SYZYGIUM AROMATICUM* AND ITS HERB DRUG INTERACTION WITH ACARBOSE AND METFORMIN

S. S. Birje* and N. A. Mehrotra

Department of Biochemistry, Mithibai College, Mumbai - 400056, Maharashtra, India.

ABSTRACT: Background and Aim: Diabetes mellitus is a disorder characterized by the body's inability to produce insulin, leading to an increased level of blood glucose and manifestation into various complications. Diet can be considered an important factor in the prevention or management of diabetes. Spices, apart from their culinary and remedial uses, have been exploited for their pharmacological and physiological activity, e.g., clove. Our study aims to investigate the *in-vitro* α -glucosidase inhibitory activity of *Syzygium aromaticum* and its herb-drug interaction with Acarbose and Metformin. **Materials and Methods:** The α -glucosidase inhibitory activity was measured by glucose oxidase (GOD) method. The clove extract was subjected to phytochemical analysis. The clove extract was microencapsulated in β -cyclodextrin. The release efficiency of the microencapsulated extract was investigated and compared against microencapsulated extract and drugs (Acarbose and Metformin). **Results:** IC₅₀ value of hydrocodone extract of clove was 154.36 μ g/ml while that of microencapsulated spice extract was 89.414 μ g/ml, proving that encapsulation of clove in β -cyclodextrin enhances α -glucosidase inhibition efficiency. IC₅₀ values for herb-drug interaction involving clove and Acarbose was 72.284 μ g/ml and for clove and Metformin was 76.713 μ g/ml, thereby displaying a herb-drug interaction. **Conclusion:** The reduced values of IC₅₀ show that microencapsulated clove extract enhances the α -glucosidase inhibition efficiency. In combination with Acarbose or Metformin, it enhances the inhibition even more. Hence, clove is an inhibitor of enzyme α -glucosidase and showing the anti-diabetic property.

Keywords: *Syzygium aromaticum*, Anti-diabetic, *In-vitro*, α -glucosidase, β -cyclodextrin

Correspondence to Author:

Shantanu Birje

Department of Biochemistry, Mithibai College, Mumbai - 400056, Maharashtra, India.

E-mail: shantanubirje01@gmail.com

INTRODUCTION: Diabetes is a chronic metabolic disease associated with hyperglycemia, resulting from insufficient or inefficient insulin secretion¹.

It is a condition primarily defined by the level of hyperglycemia, giving rise to a risk of microvascular damage (retinopathy, nephropathy, and neuropathy).

It is also associated with reduced life expectancy, significant morbidity due to specific diabetes-related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke, and peripheral vascular disease) and diminished quality of life². Diabetes is fast gaining the status of a potential epidemic in India,

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with more than 68 million diabetic individuals currently diagnosed with the disease³. The prevalence of diabetes in India is expected to increase from 31.7 million in the year 2000 to 79.4 million in 2030, while China (42.3 million) and USA (30.3 million) will also see significant increases in those affected by the disease^{4, 5}. Prevention and control of diabetes is a major challenge and require molding lifestyle towards more physical activity and less calorie intake and avoiding sedentary habits.

However, most people find it difficult to change their lifestyle and look for a less cumbersome alternative⁶. A simple option is consuming an anti-diabetic medication in the form of a tablet. There are many such medicines available in the market; however, they are expensive and have undesirable side effects. Studies in the past have shown that thiazolidinediones (Rosiglitazone, pioglitazone) show a significant increase in myocardial infarction and death from cardiovascular diseases⁷ and sulphonylureas have been associated with congestive heart failure⁸.

Recent trends in the management of diabetes involve regulation/control of postprandial glucose. Carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase help to breakdown complex carbohydrates to facilitate the release of glucose into the blood and their inhibition thereby retards the absorption of glucose⁹. α -glucosidase inhibitors are drugs that delay digestion of complex carbohydrates by acting as competitive inhibitors of the intestinal α -glucosidase enzymes that hydrolyze oligosaccharides into monosaccharides. Some common α -glucosidase inhibitors are Acarbose and Metformin¹⁰. However, natural sources of α -glucosidase inhibitors are of great importance in folk medicine for treatment and management of diabetes¹.

Microencapsulation is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material. The many benefits of microencapsulation include sustained or prolonged release, masking taste or odor of drugs, converting drugs into a free-flowing powder, reduce the sensitivity of drug to oxygen, moisture or light and vaporization of many volatile drugs like

peppermint oil or methyl salicylate¹¹. The use of cyclodextrins for encapsulation is a facile method¹². Cyclodextrin has been used widely to prepare inclusion complexes to improve stability and solubility, modify the drug release, and turn them into free-flowing powder¹³. An effective alternative to anti-diabetic medications can be plants and herbs. Plant-based medicinal products have been known to man since ancient times¹⁴. Traditional herbal medicines are safe and effective with little or no side effects, providing an alternative way to manage diabetes, in comparison to synthetic medications.

Clove (*Syzygium aromaticum*, *Eugenia caryophyllata*, *Eugenia aromaticum*) is an unopened flower bud belonging to family Myrtaceae. Commonly called laung, it has been designated 'A Champion Spice' due to its antioxidant, anti-inflammatory, antiviral, chemoprotective, hepatoprotective, antimicrobial and antistress activity. It has also been exploited in culinary uses and home remedies¹⁵. Several attempts have been made in the past by researchers to highlight the effectiveness of clove in treating diabetes. Oleanolic acid derived from clove showed hypoglycemic activity in streptozotocin-induced diabetic rats with the concomitant restoration of glycogen concentration to near normalcy¹⁶.

Studies have shown that free and bound phenolic extracts of clove bud displayed α -amylase and α -glucosidase inhibitory activity in a dose-dependent manner and Fe^{2+} lipid peroxidation in rat pancreas *in-vitro*. α -glucosidase inhibitory activity was slightly higher than α -amylase inhibitory activity, thereby providing a biochemical rationale by which clove elicits a therapeutic effect on type 2 diabetes¹⁷. Clove extract acts like insulin in hepatocytes and hepatoma cells by reducing phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) gene expression. Much like insulin, clove mediated repression is reversed by PI3K inhibitors and N-acetylcysteine (NAC). A more global analysis of gene expression by DNA microarray analysis revealed that clove and insulin-regulated the expression of many same genes in similar manner^{15, 18}.

In the present study, we have used clove and have tried to study the *in-vitro* α -glucosidase inhibitory

activity of its microencapsulated extract and its herb drug interaction with Acarbose and Metformin.

MATERIALS AND METHODS:

Procurement of Herb Sample: Clove (*Syzygium aromaticum*) buds were procured from the local market of Mumbai. The plant was authenticated by Dr. Bindu Gopalakrishnan, Department of Botany, Mithibai College, Mumbai.

Preparation of Herb Extract: Clove buds were cleaned and oven dried to remove any moisture. The dried buds were finely powdered using an electric grinder and sieved. 0.2 gm of this powder was extracted with 10 ml hydroacetone extract (5ml water + 5 ml acetone) by keeping it on a rotary shaker for 24 h. After 24 h, the extract was filtered using muslin cloth initially and then with filter paper. The residue was discarded, and the filtrate was taken in a pre-weighed evaporating dish and dried over a water bath at 60 °C. The dried extract was dissolved in a minimum amount of dimethyl sulphoxide (DMSO) and reconstituted into 5 concentrations: 10 µg/ml, 20 µg/ml, 40 µg/ml, 80µg/ml, and 160 µg/ml.

Chemicals: The following chemicals and drugs were used for carrying out the study: Acetone (AR grade), dimethylsulphoxide, phosphate buffer, ethanol, hexane, sodium sulphate (Fischer Scientific), maltose monohydrate (Otto Laboratories Pvt. Ltd.), GOD kit (Bio Lab Diagnostics), β-cyclodextrin (Loba Chemie), Acarbose (Bayer), Metformin (USV Limited).

Instruments: The following instruments were used to carry out the study: rotary shaker, incubator, hot air oven, hot plate magnetic stirrer and water bath (Remi Pvt. Ltd), weighing machine (Con-Tech Pvt. Ltd.), pH meter and colorimeter (Zeal Tech), micropipettes (Superfit XL), UV-Visible spectrophotometer (Systronics), photographic microscope (Motic) and refrigerator (LG).

Methods:

α-Glucosidase Inhibition Assay:¹⁹ To assess the α-glucosidase inhibitory assay of clove extract, 3 test tubes were taken consisting of a mixture of 1% yeast (200 µl), 0.02 M phosphate buffer (100 µl), plant extract (50 µl) and distilled water (50 µl in control and 100 µl in blank). The tubes were incubated for 10 min. After incubation 200 µl of

maltose was added, and the tubes were again incubated at 37 °C for 30 min. The tubes were kept in a boiling water bath for 5 min and then cooled in an ice bath (tubes without plant extract were used as a control, without 1% yeast was used as blank and without distilled water was used as a test).

For glucose estimation, 100 µl of the solution from the above tubes was transferred to another set of tubes along with 3 ml of glucose reagent. Tube for glucose standard was prepared by taking 20 µl of glucose standard, 3 ml of glucose reagent, and 100 µl of the denatured enzyme. Tube for glucose blank was prepared with 100 µl distilled water, 3 ml glucose reagent, and 100 µl of the denatured enzyme. The tubes were shaken and incubated at 37 °C for 15 min.

After incubation, the tubes of control, blank and test solutions were diluted with 900 µl of distilled water, and glucose standard and blank glucose tubes were diluted with 800 µl and 880 µl of distilled water respectively. The tubes were shaken, and absorbance was read colorimetrically at 530 nm. Calculations for α-glucosidase inhibitory activity:

Amount of glucose in control: $[O.D \text{ (control)}] / [O.D \text{ (standard)}] \times 100 = A \text{ } \mu\text{g/ml}$

Amount of glucose present in test: $[O.D \text{ (test)}] / [O.D \text{ (standard)}] \times 100 = B \text{ } \mu\text{g/ml}$

In this, O. D (test) = O.D (test) – O.D (blank). O. D (test) was subtracted by O.D (blank) to avoid the interference of glucose present in the plant extract.

Enhancement between lowest and highest concentration studied: $(A - B) / A \times 100$

Where, A = Amount of glucose in control, B = Amount of glucose in the test.

Phytochemical Analysis:²⁰ The clove extract was tested for its phytochemical constituents. The extract was tested for detection of carbohydrates (Molisch test), reducing sugars (Barford's test), free sugars (Fehling's test), combined reducing sugars (Fehling's test), tannins (ferric chloride test), sterols (Liebermann-Burchard test), terpenoids (Chloroform test and Liebermann-Burchard test), phenols (ferric chloride test), flavonoids, saponins (foam test), alkaloids (Mayer's test and Hager's test), proteins (Biuret test), and soluble starch.

Microencapsulation: ²¹ For microencapsulating clove extract in β -cyclodextrin, 50 ml of 1: 2 ethanol: water mixture was heated on a hot-plate magnetic stirrer for 30 min at 55 °C. To this mixture, β -cyclodextrin was slowly added, to dissolve it completely. When the solution was clear in appearance, the spice extract was added to it, the heat plate was turned off, and the solution was stirred for 4 h.

Then the solution was cooled in a refrigerator overnight. After refrigeration, there was the formation of precipitate in solution, which was obtained by vacuum filtration. The precipitate was dried in a hot air oven, weighed and stored in an airtight container.

a) Encapsulated Particle Size: The micro-encapsulated extract was taken on a clean glass slide. A drop of water was added on the powder, and it was covered with a coverslip. The particle size of the encapsulated powder was detected by a photographic microscope using the software Motic Live Imaging and Motic Image Plus 2.0.

b) Total Oil Extraction: ²¹ The total oil present in the complex powder was determined by using a solvent extraction method. The solvent used was hexane. Distilled water (10 ml), hexane (20 ml), and 0.5 g of encapsulated powder were taken in a conical flask, and this flask was kept on a rotary shaker for 24 h. After 24 h, using a separating funnel, the organic and aqueous layers were separated. The aqueous layer was discarded. The minimum amount of sodium sulfate was added to the separating funnel to absorb any remaining aqueous content in the solution. The organic layer was collected in a pre-weighed evaporating dish and kept on a hot water bath at 60 °C to evaporate hexane. The weight difference was calculated, and the amount of total oil was determined using the formula:

Total oil: (weight of evaporating dish and residue - the weight of evaporating dish) \times 2 mg/g.

c) Surface Oil Extraction: ²¹ To determine surface oil, 0.5 gm of encapsulated powder was taken in a beaker. 10 ml of hexane was added to it, and this mixture was magnetically stirred for 30 min. After completion of 30 min, the mixture was transferred to a pre-weighed evaporating dish, and hexane was

evaporated until only oil was left behind. The weight difference was noted, and surface oil present was determined by using the formula:

Surface oil: (Weight of evaporating dish and residue - Weight of evaporating dish) \times 2 mg/g.

d) Total Encapsulated Oil: Total encapsulated oil was estimated by obtaining the difference between total oil and surface oil.

Total encapsulated oil = Total oil - Surface oil

Anti-diabetic Potential of Acarbose and Metformin: Acarbose concentrations were prepared by dissolving 0.013 g Acarbose tablet in 3 ml of distilled water and then centrifuging for 5 min. From this stock of 5 mg/ml, different concentrations were prepared like 2500 μ g/ml, 1250 μ g/ml, 625 μ g/ml, 312.5 μ g/ml and 156.25 μ g/ml. Metformin concentrations were prepared by dissolving a tablet of Metformin in 1 ml of methanol diluted to 10 ml with distilled water. This solution was then centrifuged for 10 min, and the supernatant was transferred to another tube. 32 μ l of this supernatant was diluted to 10 ml with distilled water. This was used to make the following concentrations: 10 μ g/ml, 20 μ g/ml, 40 μ g/ml, 80 μ g/ml and 160 μ g/ml.

α -Glucosidase Inhibitory Activity of Acarbose And Metformin: ¹⁹ Test tubes contained reaction mixture consisting of 4% yeast (50 μ l), phosphate buffer (100 μ l), distilled water (50 μ l for control and 100 μ l for blank) and 50 μ l of drug concentrations (*i.e.*, Acarbose or Metformin). The tubes were incubated for 10 minutes, and then 50 μ l of maltose was added to test and control tubes. The tubes were again incubated for 30 min and then kept in a boiling water bath. Then the tubes were cooled in an ice bath for 5 min (tubes without drug concentration were used as a control, without 4% yeast as blank and without distilled water as a test). 20 μ l of the above tubes were removed in another set of tubes containing 1.5 ml glucose reagent.

These tubes were of glucose estimation. Tube for glucose blank was prepared by using 20 μ l distilled water and 1.5 ml glucose reagent. Tube for glucose standard was prepared by taking 20 μ l glucose standard along with 1.5 ml glucose reagent. All these tubes were shaken and incubated at 37 °C for

15 min. After incubation, the tubes were diluted with 2 ml of distilled water. The absorbance was measured at 530 nm using a colorimeter.

Release Efficiency of Encapsulated Spice:¹⁹ The release efficiency of encapsulated powder was determined by taking 0.5 gm of encapsulated powder and 10 ml of phosphate buffer in a conical flask and shaking the flask on a rotary shaker. 50 μ l of this solution was removed from the flask and taken in a test tube labeled T0. The α -glucosidase inhibitory activity of the tube was assessed. Every 30 min, the readings were taken for 6 h. Hence, the above procedure was repeated for other tubes (T1/2, T1, T1 1/2, T2....T6). 13 readings were noted each is indicating the release efficiency of encapsulated spice.

Release Efficiency of Drug and Spice:¹⁹ To determine the release efficiency of the drug along with spice, 0.5 g of encapsulated powder and 10 ml of phosphate buffer were taken in a conical flask. The flask was shaken on a rotary shaker and from this 50 μ l of the solution was removed in a test tube labeled T0 containing 50 μ l of drug concentration (Acarbose or Metformin) was added to it. The solution in the test tube was analyzed for α -glucosidase inhibitory activity and glucose estimation. The readings were taken every 30 min for 6 h. Hence, the above procedure was repeated for other tubes also (T1/2, T1, T1 1/2, ... T6) and 13 readings were noted each is indicating the release efficiency of drug and encapsulated spice. The entire procedure was performed for Acarbose and Metformin.

Release Efficiency:¹⁹ Release efficiency was assessed by taking 0.5 g of microencapsulated powder and 10 ml of phosphate buffer in a conical flask and shaking it on a rotary shaker. After every 30 min, readings were taken using a visible spectrophotometer for 6 h. 12 readings were noted each indicating release efficiency.

RESULTS AND DISCUSSION:

α -Glucosidase Inhibitory Activity: An increase in blood glucose has been linked to diabetes and its complications, which give a pointer to control of postprandial glucose. But, inhibition of carbohydrate-hydrolyzing enzymes (α -glucosidase) would slow down the absorption of glucose, reduce plasma glucose level, and consequently decrease

postprandial hyperglycemia^{22, 23}. The percentage α -glucosidase inhibitory activity was found to be 18.16 ± 0.809 , 15.76 ± 1.580 , 34.94 ± 2.490 , 40.86 ± 1.293 , and 47.30 ± 2.490 at concentrations 10 μ g/ml, 20 μ g/ml, 40 μ g/ml, 80 μ g/ml and 160 μ g/ml **Fig. 1**. With an increase in concentration, there was an increase in percentage inhibition. Lowest percentage inhibition was 15.76 at 20 μ g/ml. At 20 μ g/ml, the difference is 3 %, which could be because the difference of 0.01 OD leads to a 7% difference in α -glucosidase inhibition due to an instrumental error. The IC₅₀ value for α -glucosidase inhibitory activity of clove was found to be 154.36 μ g/ml, which showed that clove displays a strong inhibition on enzyme α -glucosidase at the studied concentrations, thereby showing antidiabetic activity. An earlier study has shown that the α -glucosidase inhibitory activity of clove in solvents like methanol, dichloromethane and *n*-hexane and the IC₅₀ value was found to be 0.3 ± 0.3 μ g/ml, 0.8 ± 0.4 μ g/ml and 1.1 μ g/ml²⁴.

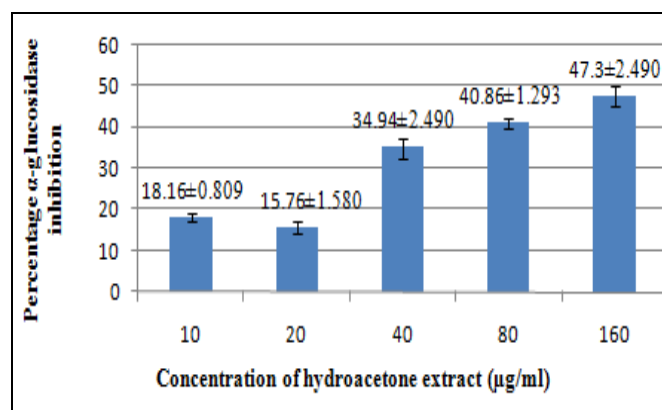


FIG. 1: PLOT OF CONCENTRATION (μ g/ml) vs. PERCENTAGE INHIBITION

Phytochemical Analysis: The clove extract was subjected to phytochemical analysis, which revealed the presence of tannins, sterols, terpenoids, and flavonoids. A similar study was carried out to analyze the phytochemical constituents of clove in ethanolic extract and concluded positive results for glycosides, tannins, and reducing sugars²⁵. Another study was conducted to detect the phytochemicals of clove and concluded positive results for terpenoids, tannins, alkaloids, and glycosides²⁶.

Microencapsulation: Microencapsulation refers to packaging an active ingredient inside a capsule ranging in size from one micron to several millimeters. The capsule protects the active

ingredient from its surrounding environment until an appropriate time. Then, the material escapes through the capsule wall by various means, including rupture, dissolution, melting, or diffusion¹¹. Clove extract was microencapsulated in β -cyclodextrin. The microencapsulated powder was light yellow in color, crystalline in texture, and it had an aromatic odor of clove. The particle size of the powder was also determined using a photographic microscope, **Fig. 2**.

The largest particle observed was of size 13.2 μm while the smallest particle observed was of size 5.6 μm . The percentage yield of the microencapsulated powder was found to be 76.8%. In the present study, the surface oil was found to be 12.08 mg/g while the total oil was found to be 68.02 mg/g. The microencapsulated oil is the difference between total oil and surface oil. The microencapsulated oil was found to be 25.94 mg/g. The percent surface oil was estimated to be 31.75%. The percentage of microencapsulated oil was estimated to be 68.22%. A decent amount of oil is contained in the β -cyclodextrin molecule, which is indicative of the fact that β -cyclodextrin is hydrophobic on the interior forming host-guest complexes, while on the exterior it is hydrophilic, thereby dissolving easily in water.

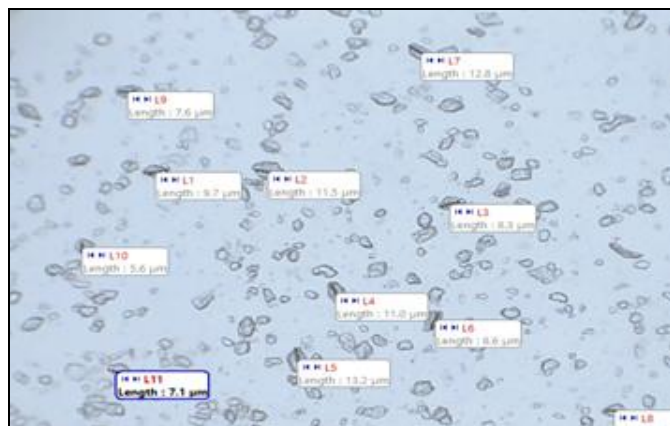


FIG. 2: PARTICLE SIZE OF MICROENCAPSULATED POWDER.

Anti-diabetic Potential of Acarbose and Metformin: On studying the anti-diabetic potential of Acarbose, for concentrations 156.25 $\mu\text{g/ml}$, 312.5 $\mu\text{g/ml}$, 625 $\mu\text{g/ml}$, 1250 $\mu\text{g/ml}$ and 2500 $\mu\text{g/ml}$, the values of percentage α -glucosidase inhibition calculated were 11.24, 16.26, 26.18, 34.63, and 46.08 respectively. There was an increase in percentage α -glucosidase inhibition

with an increase in concentration. This proved that Acarbose is a good inhibitor of α -glucosidase. However, in case of Metformin, for concentrations 10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$, 80 $\mu\text{g/ml}$, 160 $\mu\text{g/ml}$ the percentage α -glucosidase inhibition was 18.26, 17.32, 18.01, 13.32 and 20.32 respectively. From the above results, it can be concluded that Metformin though anti-diabetic, is not a good α -glucosidase inhibitor when compared to Acarbose.

Release Efficiency of Microencapsulated Spice:

The α -glucosidase activity of the microencapsulated extract was assessed for a period of 6 h. It was seen that there was an increase in percentage α -glucosidase inhibition concerning time. At 4 h time, the percentage of α -glucosidase inhibition was 55.05%, but after that, there was no increase in the percentage, and it remained constant at 55.05%. This resulted in the formation of a plateau, which indicated that the release had reached a constant rate. The enhancement between the lowest and highest concentration studied and calculated from 0 h to 6 h was 405%. 55.05% of inhibition was observed by 4 h.

This indicates that the α -glucosidase inhibitory activity was extended over some time. Hence, this can show delayed digestion of carbohydrates in the system. The delay in digestion of carbohydrates can result in the reduced need for diabetics to satisfy their hunger. The IC_{50} value of the microencapsulated extract was found to be 89.414 $\mu\text{g/ml}$. The IC_{50} value of hydroacetone extract of the spice is higher than microencapsulated extract, proving that microencapsulating *Syzygium aromaticum* extract enhanced the α -glucosidase inhibition effect.

Release Efficiency of Herb and Drug:

Herb-drug interaction involving the α -glucosidase inhibitory activity of microencapsulated spice extract along with Acarbose and α -glucosidase inhibitory activity of microencapsulated spice extract along with Metformin was studied. In the case of α -glucosidase inhibitory activity of microencapsulated extract with Metformin, there was an increase in the values of percentage α -glucosidase inhibition concerning time, from 0 h to 2 ½ h. However, from 3 h there was no increase in the percentage α -glucosidase inhibition, and it remained constant at 57.89%.

There was a plateau formation. The enhancement between the highest and lowest concentration studied was 150.71%. The IC₅₀ value of microencapsulated spice extract with Metformin was 72.28 µg/ml. The α-glucosidase inhibitory activity of micro-encapsulated spice extract along with Acarbose showed an increase in percentage α-glucosidase inhibition values. From 0 h to 2 ½ h there was an increase in the percentage α-glucosidase inhibition, however, from 3 h, there was no increase seen in percentage α-glucosidase inhibition, and it remained constant at 59.71 %. The IC₅₀ value was 76.71%. This also resulted in the formation of a plateau. Formation of a plateau indicated that release had achieved a constant rate. Hence, there was some herb-drug interaction involved.

Release Efficiency: Released efficiency of the microencapsulated extract was determined using a UV visible spectrophotometer over a period of 6 h. From 0 h to 3 ½ h, there was an increase in absorbance. However, from 4 h onwards, there was no increase observed and the absorbance remained constant **Fig. 3**. A plot of concentration (µg/ml) vs time (in h) was plotted. It was seen that with an increase in time, there was an increase in concentration.

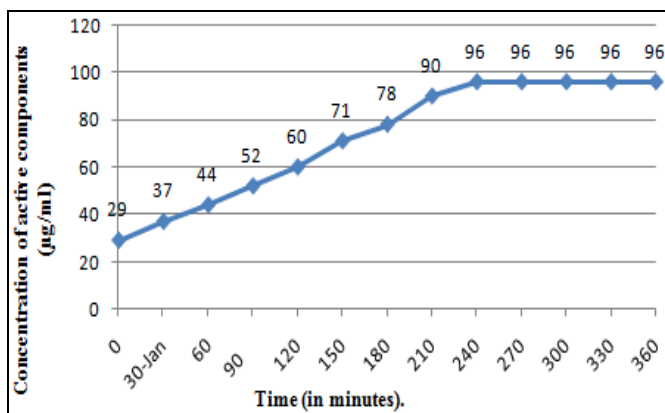


FIG. 3: PLOT: OF TIME (IN MIN) vs. CONCENTRATION (µg/ml)

The percentage α-glucosidase inhibitions of hydroacetone extract before microencapsulation and after microencapsulation were compared using comparative graphs **Fig. 4**. The percentage α-glucosidase inhibition of extract before microencapsulation were 18.16, 15.76, 34.94, 40.86 and 47.80 for concentrations 10 µg/ml, 20 µg/ml, 40µg/ml, 80 µg/ml and 160 µg/ml. The percentage α-glucosidase inhibition of extract after micro-

encapsulation were found to be 16.43, 32.08, 63.18, 125.38 and 249.78 respectively. It was found that microencapsulated extract showed more percentage of α-glucosidase inhibition in comparison to hydroacetone extract.

The percentage α-glucosidase inhibition of extract post microencapsulation (16.43) was less than that of before micro-encapsulation (18.16) at concentration 10 µg/ml. This can be due to the presence of fibers in the hydroacetone extract of *Syzygium aromaticum*. At concentrations 80 µg/ml and 160 µg/ml post microencapsulation, the percentage α-glucosidase inhibition was 125.38 and 249.78, respectively. As these values are more than 100, they cannot be considered. At 20 µg/ml and 40 µg/ml, the percentage α-glucosidase inhibition observed was 32.08 and 63.18, respectively. Hence, microencapsulated plant extract when incorporated into antidiabetic medications will show better inhibition.

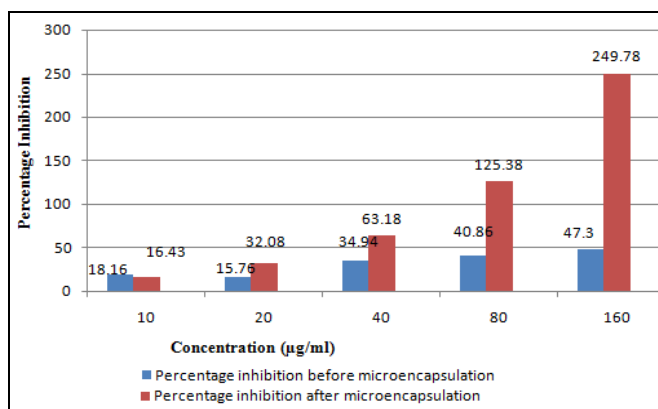


FIG. 4: PLOT OF CONCENTRATION (µg/ml) vs. PERCENTAGE α-GLUCOSIDASE INHIBITION

The α-glucosidase inhibitory activity of the microencapsulated extract was compared along with the α-glucosidase inhibitory activity of Acarbose and Metformin using comparative graphs **Fig. 5**. It was observed that microencapsulated extract, along with Acarbose and Metformin showed more inhibition than only microencapsulated extract. The IC₅₀ values of microencapsulated extract and Acarbose were 72.284 µg/ml, and that of microencapsulated extract and Metformin was 76.713 µg/ml. The reduced values of IC₅₀ shows that microencapsulation does enhance the efficiency of α-glucosidase inhibition. In combination with either Acarbose or Metformin, it increases the inhibition even more.

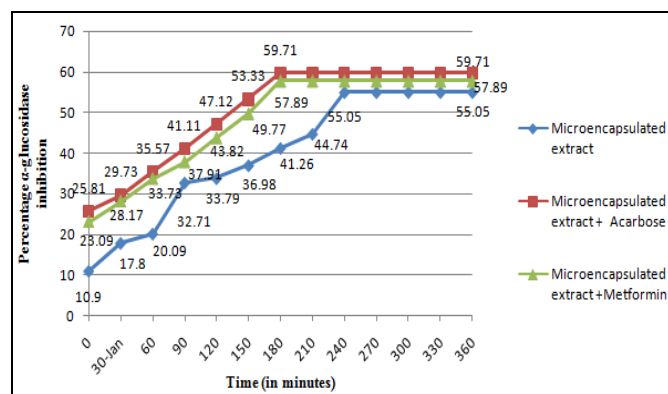


FIG. 5: PLOT OF TIME (IN MIN) vs. PERCENTAGE α -GLUCOSIDASE INHIBITION

CONCLUSION: From the above results, it can be concluded that clove is an inhibitor of α -glucosidase and hence, it is showing anti-diabetic properties. Microencapsulation is a remarkable technique, which helps in enhancing the α -glucosidase inhibitory activity. Microencapsulated clove extract in combination with antidiabetic medications Acarbose and Metformin shows an even better inhibition, and hence, a herb-drug interaction is seen between the microencapsulated powder and Acarbose and Metformin.

A major drawback associated with the drug Metformin is hypoglycemia. Herb-drug medication is taken along with the anti-diabetic medication. Antidiabetic medications aim at lowering blood glucose levels. If the herb-drug nutraceutical is taken along with regular antidiabetic medication without the advice of a physician, it can result in lowering the blood glucose to a huge extent, thereby causing hypoglycemia. Hypoglycemia is as detrimental to human health as is hyperglycemia.

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CONFLICT OF INTEREST: Nil

REFERENCES:

- Shobana S, Sreerama YN, and Malleshi NG: Composition and enzyme inhibitory properties of finger millet (*Eleusine corocana* L.) seed coat phenolics: Mode of inhibition of α -

- glucosidase and pancreatic amylase. Food Chem 2009; 115: 1268-1273.
- American Diabetes Association: Economic Costs of Diabetes in the US in 2002. Diabetes Care 2003; 26: 917-932.
- Joshi SR and Parikh RM: India-diabetes capital of the world: Now heading towards hypertension. J Assoc Physicians India 2007; 55: 323-324.
- Wild S, Roglic G, Green A, Sicree R and King H: Global prevalence for diabetes-estimates for the year 2000 and projections for the year 2030. Diabetes Care 2004; 27(3): 1047-53.
- Whiting DR, Guariguata L, Weil C and Shaw J: IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Research and Clinical Practice 2011; 94: 311-321.
- Mathur ML, Gaur J, Sharma R and Haldiya KR: Antidiabetic properties of spice plant *Nigella sativa*. Journal of Endocrinology and Metabolism 2011; 1(1): 1-8.
- Nissen SE and Wolski K: Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. New Eng J of Med 2007; 356: 2457-71.
- Tzoulaki I, Molokhia M, Curcin V, Little MP, Millett CJ, Ng A, Hughes RI, Khunti K, Wilkins MR, Majeed A and Elliott P: Risk of cardiovascular disease and all-cause mortality among patients with type 2 diabetes prescribed oral anti-diabetes drugs: retrospective cohort study using UK general practice research database. BMJ 2009; 339: b4731.
- Obboh G, Akinbola I, Ademosun A, Sanni D, Odubanjo O, Olasehinde T and Oyeleye S: Essential oil from clove bud (*Eugenia aromatica* Kuntze) inhibit key enzymes relevant to the management of type 2 diabetes and some pro oxidant-induced lipid peroxidation in rat pancreas *in-vitro*. J Oleo Sci 2015; 64(7): 775-782.
- Lebovitz HE: Alpha-glucosidase inhibitors as agents in the treatment of diabetes. Diabetes Rev 1998; 6(22): 132-145.
- Bansode SS, Banarjee SK, Gaikwad DD, Jadhav SL and Thorat RM: Microencapsulation: A review. International Journal of Pharmaceutical Sciences Review and Research 2010; 1(2): 38-43.
- Helena M and Cabral M: A review on cyclodextrin encapsulation of essential oils and volatiles. Flavour and Fragrance Journal 2010; 25: 313-326.
- Villaverde J, Morillo E, Perez Martinez JI, Gines JM and Maqueda: Preparation and characterization of inclusion complexes of norflurazon and β -cyclodextrin to herbicide formulations. J of Agri-Food Chem 2004; 52: 864-869.
- Subbulakshmi G and Naik M: Indigenous foods in the treatment of diabetes mellitus. Bom Hosp Res J 2001; 43: 548-561.
- Parle M and Khanna D: Clove: A champion spice. International Journal of Research in Ayurveda and Pharmacy 2011; 2(1): 47-54.
- Musabayne CT, Tufts MA and Mapanga RF: Synergistic antihyperglycemic effects between plant-derived oleanolic acid and insulin in streptozotocin-induced diabetic rats. Renal Failure 2010; 32(7): 832-839.
- Adefegha S, Obboh G, Odubanjo T and Ogunsuyia OB: A comparative study on anti-oxidative activities, anti cholinesterase properties, and essential oil composition of clove (*Syzygium aromaticum*) bud. La Rivista Italiana Delle Sostanze Grazze 2015; 259-268.
- Prasad RC, Herzog B, Boone B, Sims L and Waltner-Law M: An extract of *Syzygium aromaticum* represses genes encoding hepatic gluconeogenic enzymes. Journal of Ethnopharmacology 2005; 96(1-2): 295-301.

19. Geng P, Yang Y, Gao Z, Yu Y, Shi Q and Bai G: Combined effect of total alkaloids from *Feculae bombycis* and natural flavonoids on diabetes. *Journal of Pharmacy and Pharmacology* 2007; 59: 1145-1150.
20. Arulpriya P, Lalitha P and Hemlatha S: Competence of different solvent extraction methods in the isolation of bioactive principles from *Samanea saman* (Jacq.) Merr. *Pharma Science Monitor. An International Journal of Pharmaceutical Sciences* 2010; 2(2): 184-199.
21. Petrovic GM, Stojanovic GS and Radulovic NS: Encapsulation of cinnamon oil in β -cyclodextrin. *Journal of Medicinal Plants Research* 2010; 4(14): 1382-1390.
22. Aryangat AV and Gerich JE: Type 2 diabetes: Post prandial hyperglycemia and increased cardiovascular risk. *Vasc. Health Risk Manag* 2010; 6: 145-155.
23. Narkhede MB: Investigation of *in-vitro* α -amylase and α -glucosidase inhibitory activity of the polyherbal extract. *Int J Pharm Res Dev* 2011; 3: 97-103.
24. Salehi P, Asghari B, Esameili MA, Dehghan H and Ghazi I: α -glucosidase and α -amylase inhibitory effect and anti oxidant activity of ten plant extracts traditionally used in Iran for diabetes. *Journal of Medicinal Plants Research* 2013; 7(6): 257-266.
25. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, Pandey J and Malla R: Phytochemical extraction and antimicrobial properties of different plant species: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achiranthos bidentata* (Datiwan), *Azadirachta indica* (Neem). *Journal of Microbiology and Antimicrobials* 2011; 3(1): 1-7.
26. Kumar B, Kumar U, Bhandari A and Kumar Y: Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom” *International Journal of Pharmaceutical Sciences and Research* 2010; 12(1): 138-147.

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