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Evaluation of Antidiabetic Activity and Antimicrobial Activity of Leaf Extract ofSyzygium Samarangense

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ABSTRACT: This research paper is about the Evaluation ofAntidiabetic Activity and Antimicrobial Activity of leaf extract of Syzygium Samarangense.The ethanolic extract of leaf of Syzygium Samarangense was assessed for the antidiabetic activitybyα-amylase invitro antidiabetic activity and glucose uptake in chicken ileum by everted gut sac method. Antimicrobial activity was assessed by Kirby Bauer test. The extract of leaf evaluated for α-amylase inhibition using acarbose as standard of various concentration of 10-100 µg/ml. Different concentration of HCE 200-800µg/mL was evaluated for inhibitory activity. The results showed promising antidiabetic activity. Glucose uptake in chicken ileum was performed with standard, control and the drug and the results were promising to accept the antidiabetic activity. The ethanolic extract of Syzygium samarangensehas demonstrated promising antimicrobial activity. The antibacterial activity was strong enough to inhibit E. coli (Gram negative bacteria) and S. aureus (Gram positive bacteria). The activity was strain and dose dependent.

KEYWORDS: SyzygiumSamarangense, α-amylase inhibitory activity, glucose uptake in chicken ileum by everted gut sac method, Kirby Bauer test.

I. INTRODUCTION

Diabetes mellitus is a group of metabolicdiseases characterized by hyperglycaemia, resulting from defects in insulin secretion, insulin utilization, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These includes autoimmune destruction of the β-cells of the pancreas with insulin deficiency to abnormalities that result in resistance to insulin action [1]

Diabetes is a metabolic condition characterised by hyperglycaemia (either fasting or postprandial state), glycosuria, hyperlipidaemia, negative nitrogen balance, and in certain cases, ketonemia [2,3]

Syzygium Samarangense, is commonly called as Wax apple, Wax Jambu and it belongs to the family Myrtaceae and generally called as eucalyptus family.In India, most commonly present species are namely Java Apple regionally it is called Jambul in Marathi, Jamun in Hindi, Jam in Bengali, Jambu in Gujarati, Nerale in Kannada, neereedu in Telugu, Jaman in Urdu, Navel or chammbakka in Malayalam, Neredam in tamil.The java apple leaves contain tannins, flavonoids, glycosides, terpenoids, chalcones. It may cause better therapeutic effect against diabetic infections, antioxidant and immunomodulation. The fruit of java apple consist of rich phenolic compounds like flavonoids, tannins etc [4]

S. **Samarangense**ishaving different morphological characters in different countries. In India, it is grown in sunny places. It requires normal water and it can tolerate more water content. It is long lived plant, having a special character like quick growing plant, ever green tree, it grows best in humid and warm region, and also it is an ornamental plant. It grows in elevation up to 1350 meters. The tree is around 6-8-meter height, with short and crooked trunk and it spread over 4 to 6 meters. The wood is reddish in colour, hard and coarse. The leaves are opposite, elliptical to elliptical-oblong, 10-25cm X 5-12 cm, leaves are dark bluish green in colour, coriaceous with a thin margin, pointed pellucid, very aromatic when bruised, 3-5 mm long petiole. Java apple normally lowers in the dry season in the month of January, February and March. The lowers tend to be self-compatible, pink in colour, the java apple may

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yield a crop of 700 fruits, it is cultivated two to three times per year. The fruit are pear shaped, matures in 40-50 days after anthesis. It is 10cm long, white to glossy red in colour.[5]

II. METHODOLOGY

COLLECTION OF PLANT MATERIAL

The plant **Syzygium samarangense**leaves were collected from our native place BG Nagar, Mandya, Karnataka. The fresh leaves of **S. Samarangense**were thoroughly washed with distilled water and shade dried. The dried leaves were subjected for size reduction into a coarse powder, and stored in airtight container at room temperature.

AUTHENTICATION OF PLANT MATERIAL

The plant material brought by Ms. Spoorthi, T.C., Ms. Nandana Satheesh, B.S., And Mr. Anugrah, A. from Bharathi collegeof Pharmacy, Bharathinagara, Maddur Tq, Mandya Dist. Is identified and authenticated as **Syzygium samarangense** belongs to family **Myrtaceae**

EXTRACTION METHOD

Maceration by stirring using magnetic stirrer, was used for extraction using hydro-alcohol as a solvent (distilled water: ethanol: 20:70). The sample and solvent were taken in the ratio of 1:20. 100 grams of dried leaves powder was accurately weighed and transferred into 1L conical flask. 500ml of solvent was added and stirred continuously for 8 hours and the filtrate was collected and the mark again extracted using 500ml of fresh solvent for 8 hours. The same process was followed twice. The obtained filtrate was evaporated by heating. Then the condensed filtrate was dried using water bath at 55^0 C. The obtained extract was transferred into an airtight container and stored in refrigerator.

Fig.1: Fresh leaves of s.samarangense

Fig.2: Extraction by maceration

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Fig.3: Filtration of menstruum

Fig. 4: Dried extract OF S. SAMARANGENSE

ALPHA AMYLASE INHIBITORY ACTIVITY

Principle: Principle of alpha amylase inhibitory activity is based on colorimetric method. The enzyme α-amylase converts starch in to maltose. This maltose released from the starch is measured by the reduction of 3, 5 DNSA. Maltose reduces the pale-yellow colour of alkaline 3, 5 DNSA to orange red colour. The intensity of the colour is directly proportional to the maltose present in the sample.

Intensity of colour change is measured using spectrophotometer at 540 nm [6].

Preparation of Phosphate buffer: 2.8 g of Disodium hydrogen Phosphate and 3.1 g of Sodium dihydrogen Phosphate were accurately weighed and dissolved in 100 ml distilled water to produce 0.2 M Phosphate buffer. The PH was adjusted to 6.9 using 3N NaOH and 3N HCL.

Preparation of DNS reagent: Solution A-0.8 g of NaOH was dissolved in 3ml of water and 0.1g of DNS was dissolved in 2ml of water and mix both the solution to get 5ml of solution A. Solution B-3g of sodium potassium tartarate was dissolved in 5ml of water. Mix both solution A and Solution B to get DNS reagent.

Procedure: The enzyme, test, standard was prepared as 1 mg/ml solution using distilled water. Required amount of Sodium Phosphate buffer (0.2M PH 4.5) (for reaction volume 100 microliter), Alpha amylase enzyme 10 µl and Test samples 200 µl ,400 µl, 600 µl and 800 µl were added to the 1.5 ml micro centrifuge tubes and incubated at room temperature for 20 minute. 100 µl of starch solution (1% Starch in PBS) was added to all the tubes except reagent blank and incubated for 3 minutes at room temperature. Then 100 µl of freshly prepared DNS reagent was added to all the test tubes and heated on water bath at 95 °C for 5 min to stop the reaction. Similarly Control (with 100% enzyme activity) and standard was prepared and the absorbance is recorded at 540 nm. The experiment is repeated thrice. The alpha amylase

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inhibitory activity was calculated by using the formula [7].
Percentage of Inhibition= (Absorbance of test – Absorbance of control) X100/ Absorbance of test

GLUCOSE UPTAKE IN CHICKEN ILUEM BY EVERTED GUT SAC METHOD

Procedure: In this study, the method of viviyan Therasa et. al., has been followed with some modification.

- ✓ Isolate the chick ileum and place it in a kreb's solution and provide aeration till further use.
- ✓ Locate the glass rod that you are going to use to evert the sac and note that it has a rounded end with a constriction a few millimetres from one end.
- ✓ Moisten the glass rod with kreb's solution and slip the end of the intestine over the glass rod until the intestine extends just past the constricted region of the rod.
- ✓ Next, tie a loop of moistened thread around the intestine and glass rod at the point of constriction. A double loop knot is desirable to prevent slippage. Make certain that the intestine is tied tightly onto the glass rod and then trim the thread on either side of the knot to leave 1-2 cm.
- ✓ Now, gently push the intestine over the knot and rod so that the intestine doubles over on itself. It is important that this is handled carefully as the intestine is very fragile and, as it is everted, the mucosalsurface of the intestine is expose. Therefore, it is very important that you do not touch the mucosal surface, as that will damage the epithelial tissue.

Continue this until the entire intestinal segment is turned inside out. When you have completed everting the intestine, if it hangs over the end of glass rod, trim it off with a pair of scissors so that everted iluem is slightly longer than the glass rod.

- ✓ Now , cut the everted intestine so that it is a 3-4cm long segment. You will use this piece of intestine to make a sac.
- ✓ Moulded the segmented pieces on to the everted gut sac apparatus .This apparatus is dipped into the beaker containing kreb's solution and give aeration , for the survival of tissue.
- ✓ The areainside the sac is considered as serosal fluid compartment and area outside the sac (in the beaker) is mucosal fluid compartment.
- ✓ Add a drop of phenol red inside the tiusse to check the leakage or damage happen to the tissue. Three set of beaker are arranged like this.
- ✓ In first beaker, along with kreb's solution glucose were added and it is the control.
- ✓ In second beaker , acarbose were added to glucose and kreb's solution and it is the standard.
- ✓ After two hours and five hours , determine the concentration of glucose with in the sac by using glucometer [8].

Fig.5: Glucose uptake by chicken iluem everted gut sac method

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ANTIMICROBIAL ACTIVITY BY KIRBY-BAUER TEST

Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance. Zone of Inhibition Testing is a fast, qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of microorganisms.

In Kirby- Bauer test, bacteria are placed on the plate of solid growth medium and wafers of antibiotic are added to plate. After allowing the bacteria to grow overnight, area of clear media surrounding the disks indicate that the antibiotic inhibits bacterial growth.

Procedure:

- Swab a muller-hinton plate with only 2 bacteria, dip a sterile swab into the broth and express any excess moisture by pressing the swab against the side of the tube.
- Swab the surface of the agar completley
- After completely swabbing the plate, turn it 90 degrees and repeat the swabbing process.
- Run the swab around the circumference of the plate before discarding.
- Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar.
- Placethe antibiotic disks on the agar, make sure that it is in good contact with the agar surface.

Fig 6: Inhibitory effect of Syzygium samarangenseleaf extract on E.coli

Fig 7: Inhibitory effect of Syzygium Samarangense leaf extract on S. Aureus

Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to other edge of the zone holding the plate up to the light. Use a millimetre measurement. The disk diameter will actually be part of that number. If there is no zone of inhibition the respective antibiotic is not having the anti-bacterial activity. Zone diameter is reported in millimetre. If the organism killed or inhibited by the concentration of antibiotic, there will be no growth in the immediate area around the disc, this is called as Zone of Inhibition [9].

III. RESULT AND DISCUSSIONS

ALPHA AMYLASE IN-VITRO ANTIDIABETIC ACTIVITY

The extract of leaves of Syzygium Samarangense was evaluated for invitro alpha amylase inhibition activity by using acarbose as a standard of various concentration of 10-100 µg/ml (Table-1 and Figure-8). Different concentration of the HCE were evaluated for antidiabetic potency. Various concentration of HCE at 200 µg/ml, 400 µg/ml, 600 µg.ml, 800 µg/ml were screened for the activity and the results indicated18.75±1.6, 26.05±2.3, 41.7±1.5 and 51.3±2.3 percentage of

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inhibition respectively. Among the estimated results, HCE at 800µg/ml had maximum amylase inhibition followed by 600 µg/ml, 400 µg/ml, and 200 µg/ml, the details are mentioned in the (Table-2).

Sl. No.	Concentration	Percentage of inhibition in %
1	10µg	11.6±2.5
2	20µg	28.2±3.5
3	40µg	35.8±2.8
4	60µg	44.2±1.5
5	80µg	68.2±2.6
6	100µg	81.5±2.8

Table no 1: α-amylase invitro antidiabetic activity of standard acarbose of various concentration

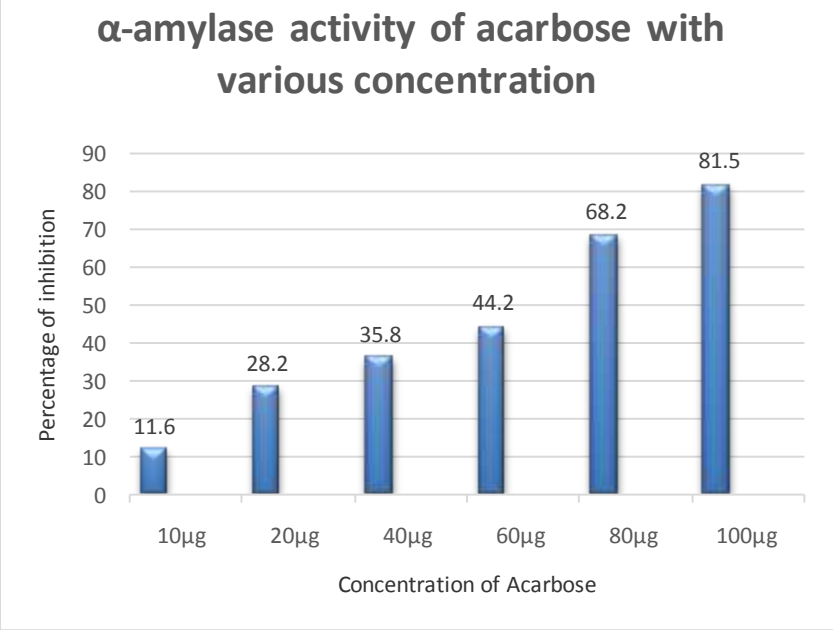

Fig8: α-amylase activity of acarbose with various concentration

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Sl. No.	Concentration	Percentage of inhibition (In %)
1	200µg/ml	18.75±1.6
2	400µg/ml	26.05±2.3
3	600µg/ml	41.7±1.5
4	800µg/ml	51.3±2.3

Table no 2: α-amylase activity of HCE with various concentration

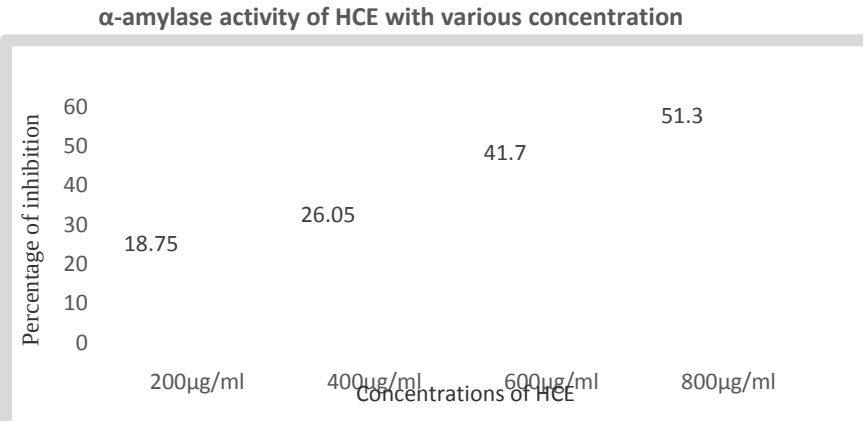

Fig 9: α-amylase activity of HCE with various concentration

GLUCOSE UPTAKE IN CHICKEN ILEUM BY EVERTED GUT SAC METHOD

SL NO:	DRUG	TIME INTERVAL (MINS).	GLUCOSE CONC IN SEROSAL COMPARTMENT (mg/dl)
1	Control	After 150 min	286.33mg/dl
		After 300 min	293.33mg/dl
2	Standard	After 150 min	235.33mg/dl
		After 300 min	258.66mg/dl
3	Syzygium samarangense silver nanoparticles	After 150 mins	250.5mg/dl
		After 300 mins	286.5mg/dl

Table No3: Glucose uptake in chicken ileum

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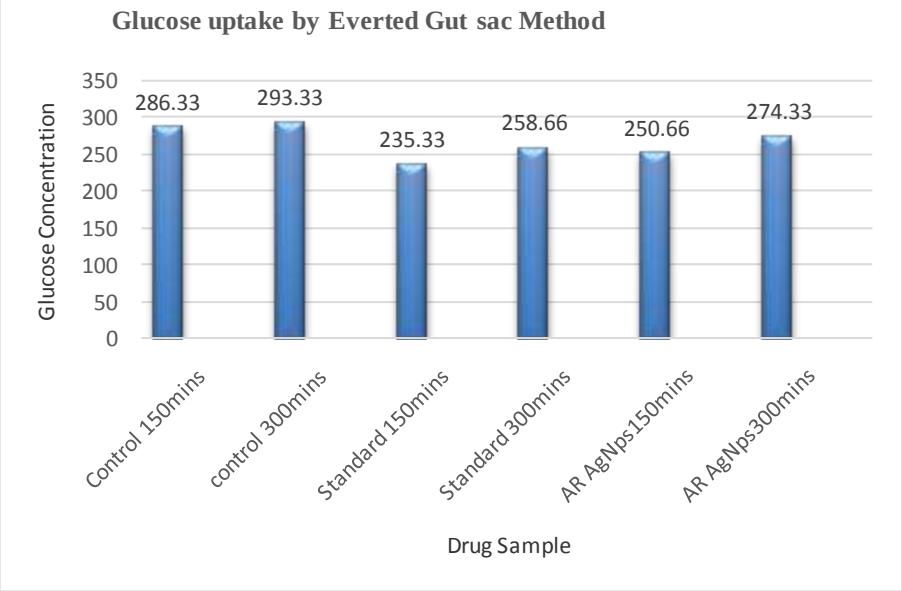

Fig 10:Glucose uptake by Everted Gut sac Method

ANTI-MICROBIAL ACTIVITY BY KIRBY-BAUER TEST

The ethanolic extract of Syzygium samarangense leaf has demonstrated promising antimicrobial activity. The antibacterial activity was strong enough to inhibit E. coli (Gram negative bacteria) and S. aureus (Gram positive bacteria). The inhibitory effect was strain and concentration dependent; Gram positive bacteria was more susceptible to the effect of leaf extract of Syzygium samarangense when compared to Gram negative.

The zone inhibition by the antibiotic was found to be 12.0mm against the gram-positive bacteria and 8.0mm against the gram-negative bacteria. These results show that respective antibiotic have more activity towards Gram positive bacteria than gram negative bacteria. And this study shows that our drug leaf extract of Syzygium samarangense have promising anti-microbial activity

IV. CONCLUSION

In the last few years, the usage of herbal drugs is more in India and worldwide than allopathic medicine because of less side effects and their potency. The pharmacological activity of herbal plantmaterial is mainly due to its phytoconstituents. The current study concludes that the dried leaves of **Syzygium samarangense** extract showed a significant potency in In-vitro Antidiabetic activity in a dose dependent manner. The extract of **S. Samarangense** wasevaluated for α-amylase inhibition assay and glucose uptake in chicken ileum by everted gut sac method, Anti-microbial activity and the results showed a promising antidiabetic activity and Anti-microbial activity. The activity reported, may be due to the presence of flavonoids and phenolic content in the leaves of the plant.

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