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Antibacterial activity and Antidiabetic activity of *Costus igneus, Gymnema sylvestre* and *Ocimum sanctum*

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Abstract : Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them.Indian medicinal plants have been found to be useful for successful management of diabetes. One of the great advantages of medicinal plants is that these are readily available and have very low side effects.The aim of the present study was to determine the antibacterial activity and antidiabetic activity of the leaf aqueous extracts of *Costus igneus*, *Gymnema sylvestre* and *Ocimum sanctum*. The bacterial cultures were used for screening antibacterial activity by disc diffusion method. *Costus igneus* showed the maximum zone inhibition against *P.aeruginosa*, they also showed minimum zone inhibition against *E.coli* and *E.aerogenes* bacterial strains. *Gymnema sylvestre* showed maximum inhibitory activity against *P.mirabilis* and minimum inhibitory activity against *E.coli* and *P.aeruginosa* and the *Ocimum sanctum* showed maximum zone of inhibition against the *E.coli* and the minimum zone inhibitory activity against *E.aerogenes*, *P.mirabilis* and *P.aeruginosa*. *Combined* Antidiabetic activity was carried out by α-amylase inhibitory assay. *Costus igneus* plant extract inhibited the α-amylase at 89.62 % and *Gymnema sylvestre* showed 63.52 % α-amylase enzyme inhibition whereas *Ocimum sanctum* showed 44.20 % of α-amylase inhibitory activity. The combined antidiabetic activity of the three plant extracts showed 46 % of the inhibitory activity. Hence, *Costus igneus* showed the maximum inhibition against most of the bacterial strains and also inhibited the α-amylase enzyme in highest concentration. Itis found to be more efficient natural source of medicine when compared to other two plants.
Keywords : *Costus igneus; Gymnema sylvestre; Ocimum sanctum;*antidiabetic activity;α-amylase; antibacterial.

Introduction

Microbial infections continue to scourge mankind for several million years. Infectious diseases are the leading cause of deaths world-wide. So, antibiotic resistance has become a global concern but the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens.

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The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specta of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs, which is safe, more dependable than costly drugs and have no adverse side effects.

Diabetes mellitus is a rampant lifestyle disease now-a-days. Due to hectic schedules, many people have taken to excessive consumption of fast foods resulting in this disorder. Furthermore, it is predicted that by 2030, India, China and US will have the largest number of people with diabetes. Diabetes mellitus is a spectrum of common metabolic disorders, arising from a variety of pathogenic mechanisms, all resulting in hyperglycemia. India is facing a diabetic explosion, according to the World Health Organization (WHO) estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030. The International Diabetes Federation (IDF) also reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025. Recently, the status of diabetes has changed from being considered as a mild disorder of elderly to one of the major causes of morbidity and mortality mainly due its chronic complications such as coronary artery disease, neuropathy, nephropathy, and retinopathy. These chronic complications of Diabetes mellitus translate into a significant economic burden on the individual and the community as the treatment is expensive. Although a number of allopathic medicines exist for diabetes, most of them have some harmful side effects. Synthetic drugs are likely to give serious effects in addition they are not suitable for intake during conditions like pregnancy. Apart from conventional diabetes therapy, several studies have shown that some plants used in traditional medicine have beneficial effects in diabetic patients.Management of diabetes without any side effect is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs.

Plant extracts has been used to treat for microbial disease from ancient time in traditional medical systems. Ability of using most of the medicinal plants for the treatments for various diseases may lie in the antioxidant and antimicrobial effect of phytochemicals. More than 40% of modern drugs are derived from natural sources using either the natural substance or a synthesized version[1]. Allopathy is the main stay of therapeutics for several years. Owing to development of drug resistance, various side effects, expensive medications and emergence of mutants, search for alternatives led to the use of medicinal plants for the betterment of the disease [2].A review on natural products by[3] states that the short term prospect for new NP (natural products) and Natural Products derived drug approvals is bright, with 31 compounds in phase III or in registration, which should ensure a steady stream of approvals for at least the next five years.

Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. One of the anti-diabetic therapeutic strategies is inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase. α-amylase hydrolyzes complex starches to oligosaccharides, while, α -glucosidase hydrolyzes oligosaccharides to glucose and other monosaccharides. Inhibition of these enzymes produces postprandial antihyperglycemic effect by reducing the rate and extent of glucose absorption. Currently, there are 5 classes of conventional anti diabetic drugs; however, these drugs are associated with various side effects. Hence there is urgent need to identify and explore natural sources with fewer side-effects for such inhibitors. The aim of the present study was to determine the qualitative phytochemical screening, antibacterial activity and antidiabetic activity of the leaf aqueous extracts of *Costus igneus, Gymnema sylvestre* and *Ocimum sanctum*.

Materials and Methods

Collection of plant materials

Fresh leaves of *Costus igneus*, *Gymnema sylvestre* were collected from Paramathi Velur, Namakkal Distict and *Ocimum sanctum* were collected from local area of Coimbatore, Tamil Nadu.

Preparation of plant extracts

The leaves of *Costusigneus, Gymnema sylvestre* and *Ocimum sanctum* were used for the experiment. Fresh plant leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles for further use.

Plant extracts preparation

10 grams of air-dried leaves powder was added to 100ml of distilled water and kept in heating mantle for 2 hours. It was then filtered through Whatmann No: 1 filter paper to collect the aqueous extract and it was stored at 4°C.

Antibacterial Assay

Collection of Bacterial strains

Six bacterial strains in which two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aures*) and four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Enterobacter aerogens*) were used in this study. *Pseudomonas aeruginosa* and *Bacillus subtilis* they were collected from the Department of Microbiology and other four cultures were obtained from the Department of Biotechnology, Dr. N.G.P. Arts and Science College, Coimbatore. Bacterial strains were cultured in nutrient agar slant and allowed to grow at 37℃then it was stored in 4°C for future studies.

Antibacterial Activity Test

The method suggested by [4]is widely used for the antibacterial susceptibility testing. The *invitro* antibacterial activity of the leaf extracts were evaluated by disc diffusion method using Nutrient agar. Filter paper (Whatmann No: 1) discs of 5mm diameter were prepared and sterilized. The discs were impregnated with 1mg of plant extract in 1 ml of Dimethyl Sulfoxide (DMSO) and then they were placed on previously inoculated Nutrient agar plates. Plates were kept for incubation at 37℃ for 24 hours. Tetracycline disc was used as the positive control and the DMSO as the negative control. The diameter of inhibition zones were used as a measure of antibacterial activity and each assay was repeated twice.

In vitro Antidiabetic Activity

α-Amylase Inhibition Assay

The α-amylase inhibition assay was performed using the chromogenic Dinitro Salicylic Acid(DNSA) method [5]. The total assay mixture was composed of 1400µl of 0.005 M sodium phosphate buffer (pH 6.9), 50µl of α-amylase and 1000µlsamples were incubated at 37℃ for 10 min. 1400µl of 0.005 M sodium phosphate buffer (pH 6.9) and 50µl of α-amylase was used as standard. After pre-incubation 500µl of 1% (w/v) starch solution in 0.005 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated again at 37℃ for 10 min. The reaction was terminated by adding 1.0ml of DNSA reagent and placed in boiling water bath for 5 min. It was then cooled to room temperature and the absorbance was measured at 540nm. The percentage of α-amylase inhibited by plant samples were calculated by using the following formula

$$\%\ \text{inhibition} = \frac{Abs_{control} - Abs_{extracts}}{Abs_{control}} \times 100$$

3. Results and Discussion

Antibacterial activity:

The potential for developing antimicrobials from higher plants appeared to be rewarding as it would lead to the development of phytomedicines to act against [6]. The antibacterial activity of *Costus igneus*, *Gymnema sylvestre* and *Ocimum sanctum* were evaluated against various gram positive and gram negative bacterial strains.Aqueous extract of *Costus igneus* showed maximum zone of inhibition of about 20mm against *Pseudomonas aeruginosa*. It also showed potential inhibitory zone(16mm,16 mm and 8mm) against *Escherichia coli, Enterobacter aerogenes* and *Staphylococcus aureus* respectively whereas they did not show inhibition against *Bacillus subtilis* and *Proteus mirabilis* (Table 1& Figure. 1).

Table.1 Antibacterial activity of crude extract of *Costus igneus*

Pathogens	Positive Control (mm)	Negative Control (mm)	*Costus igneus* Extract (mm)
B.subtilis	13	---	---
E.coli	16	---	16
E.aerogenes	16	---	16
P.mirabilis	17	---	---
P.aeruginosa	23	---	20
S.aureus	10	---	---

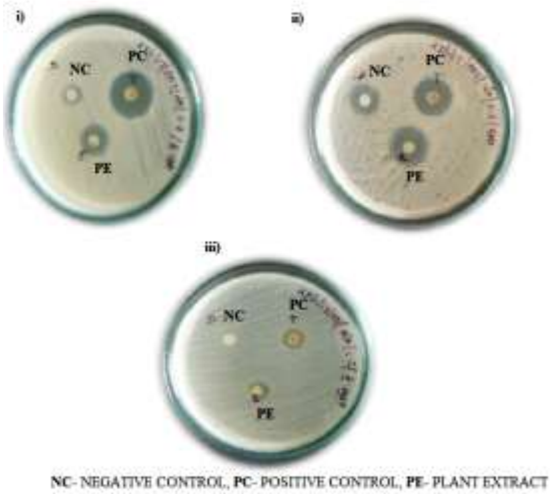


Figure.1 Antibacterial activity of crude aqueous extract of *Costus igneus*: (i) *E.coli*, (ii)*E.aerogenes* and (iii) *S.aureus*

Gymnema sylvestre aqueous extract showed very good antibacterial activity of around 20 mm against *Proteus mirabilis* whereas they produce inhibition zone of 14mm against *Escherichia coli* and *Pseudomonas aeruginosa*. But they did not inhibit *Bacillus subtilis, Enterobacter aerogenes* and *Staphylococcus aureus* (Table 2& Figure. 2). *Ocimum sanctum* aqueous extract showed maximum inhibitory zone of 13mm against *Escherichia coli* and showed 11 mm, 12 mm and 11 mm zone of inhibition against *Enterobacter aerogenes, Proteus mirabilis*, *Pseudomonas aeruginosa* whereas they did not inhibit *Bacillus subtilis* and *Staphylococcus aureus* (Table 3& Figure. 3).Antibacterial activity of the three plant extracts (*Costus igneus, Gymnema sylvestre, Ocimum sanctum*) combined together showed maximum zone of inhibition of about 16mm against *Proteus mirabilis* where as this combined extract showed inhibition zone of 15 mm, 15 mm, 14 mm and 15 mm against *Eschericia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacter aerogenes*.They did not show any inhibition against *Bacillus subtilis*(Table 4& Figure.4).

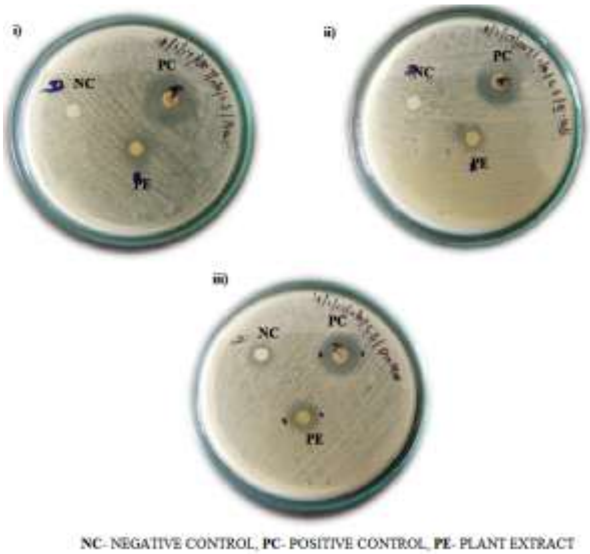


Figure.2 Antibacterial activity of crude extract of *Gymnema sylvestre*: (i) *E.coli*, (ii) *P.mirabilis* and (iii) *P.aeruginosa*

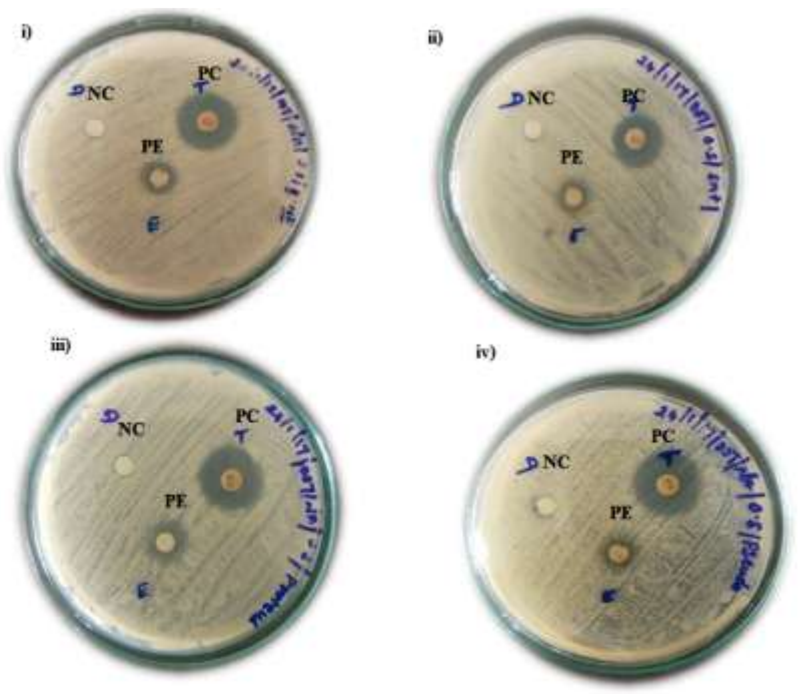
Table 2: Antibacterial activity of crude extract of *Gymnema sylvestre*

Pathogens	Positive Control (mm)	Negative Control (mm)	*Gymnema sylvestre* Extract (mm)
B.subtilis	16	---	---
E.coli	20	---	14
E.aerogenes	17	---	10
P.mirabilis	18	---	20
P.aeruginosa	24	---	14
S.aureus	15	---	---

The crude methanolic extract of *Ocimum sanctum* showed strong antimicrobial activity against *S.aureus* and *C.albicans* and moderate activity against *E. coli* and *B. subtilis*. The crude methanolic extract of *Ocimum kilim and sacharicum* showed strong antimicrobial activity against *S. aureus, E. coli* and *C. albicans* at higher concentration. It showed moderate activity against *B. subtilis*. The crude aqueous extracts of *Ocimum sanctum* showed strong antimicrobial activity against *S.aureus* and moderate activity against others[7]. Bilal *et al.*, (2016)reported that the phytochemical compounds of methanolic extract of *Ocimum sanctum* played a major role in their antibacterial activities[8]. Though the methanolic extract of *Ocimum sanctum* exhibited antibacterial activity against *Enterococcus faecalis, Enterobacter cloacae, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus* and *Staphylococcus saprophyticus,* they showed strong antibacterial activity against *Staphylococcus aureus* and *Staphylococcuss aprophyticus.*

Table 3: Antibacterial activity of crude extract of *Ocimum sanctum*

Pathogens	Positive Control (mm)	Negative Control (mm)	*Ocimum sanctum* Extract (mm)
B.subtilis	13	---	---
E.coli	19	---	13
E.aerogenes	12	---	11
P.mirabilis	12	---	12
P.aeruginosa	21	---	11
S.aureus	11	---	---



NC- NEGATIVE CONTROL, PC- POSITIVE CONTROL, PE- PLANT EXTRACT

Figure.3 Antibacterial activity of crude extract of *Ocimum sanctum:* (i) *E.coli*, (ii) *E.aerogenes*, (iii) *P.mirabilis* and (iv)*P.aeruginosa*

Murugan *et al.,* (2012)screened two medicinal plants namely *Gymnema sylvestre* and *Morinda pubescens* var. *pubescens* for potential antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*[9]. The chloroform and methanol extract of leaf of *Gymnema sylvestre* showed highest inhibition against *Escherichia coli* and *Klebsiella pneumoniae* respectively; whereas, acetone extract of *Morinda pubescens* var. *pubescens* leaf exhibited maximum inhibition against *Pseudomonas aeruginosa*. Bishnu *et al.,* (2011) used the aqueous ethanolic extract of four medicinal plants for*in vitro* antibacterial assay against human pathogenic bacterial strains such as *Escherichia coli, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* by employing cup diffusion method. Among four plantstested, *Eugenia caryophyllata* (Clove) was found to be the most effective against *S. typhi*. All the plants were found to be ineffective against *E. coli* and *K. pneumonia*[10]. *Achyranthes bidentata* was found to be ineffective against all the tested organisms. The largest zone of inhibition (22 mm) was obtained with *E.caryophyllata* against *S. typhi*.

Table 4: Combined antibacterial activity of the crude extracts of *Costus igneus, Gymnema sylvestre* and *Ocimum sanctum*

Pathogens	Positive Control (mm)	Negative Control (mm)	Combined Extract (mm)
B.subtilis	21	---	---
E.coli	21	---	15
E.aerogenes	21	---	---
P.mirabilis	21	---	16
P.aeruginosa	19	---	15
S.aureus	21	---	15

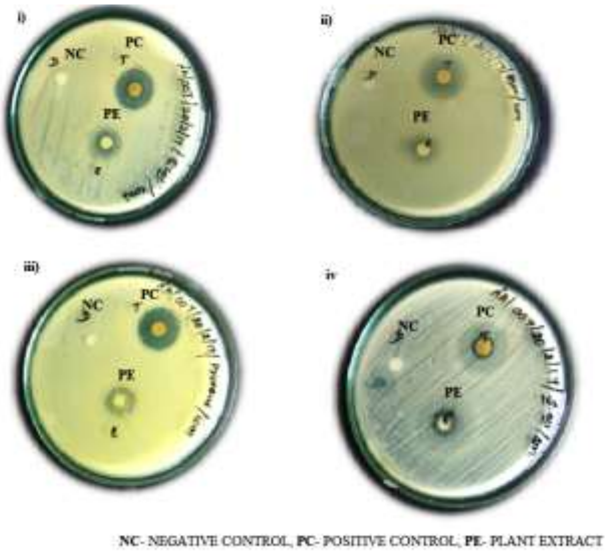


Figure.4 Antibacterial activity of the crude extracts of *Costus igneus, Gymnema sylvestre* and *Ocimum sanctum* (i) *E.coli*, (ii) *P. aeruginosa*, (iii) *P. mirabilis* and (iv) *S.aureus*

Antidiabetic activity:

Many herbal extracts had been reported to have antidiabetic activities and were used in Ayurveda for treatment of diabetes. The α-amylases were calcium metalloenzymes, completely unable to function in the absence of calcium. The excess conversion of starch to sugars (remaining sugar moieties which left by brain) will increase the sugar level in blood then the role of insulin come in action here which will order cells to metabolize the excess sugar moieties and store it as energy sources i.e. glycogen. This cycle will continuously going on in healthy person. The role of α-amylase is to cleave the large starch molecules into smaller fragments of sugars which in terms utilized by the brain as its energy source because the large molecules like starch cannot pass through blood brain barrier. To overcome this problem larger starch molecules are converted into smaller fragments in order to pass through the blood brain barrier. The excessive conversion of starch to sugar molecules leads to diabetes mellitus. By inhibiting the enzyme α-amylase this problem can be solved.

The assay performed by the DNSA method to inhibit the enzyme α-amylase by the aqueous leaf extract indicated that the inhibition was indeed dose dependent. The aqueous extracts of *Costus igneus, Gymnema sylvestre* and *Ocimum sanctum*, at 1000 µl inhibited 89.62%,63.25%,44.20% ofα-amylase. Three plants (*Costus igneus, Gymnema sylvestre, Ocimum sanctum*) combined inhibited46.37% of α-amylase enzyme at 540nm (Figure.5).

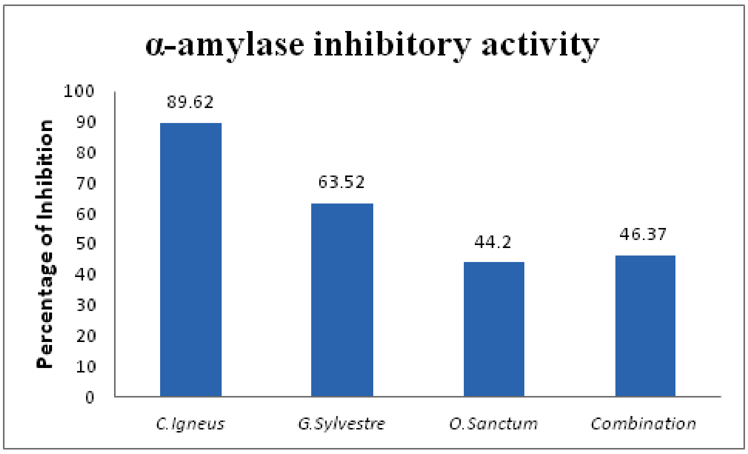


Figure.5 α-amylase inhibitory activity of *Costus igneus, Gymnema sylvestre* and *Ocimum sanctum*

Manikandan *et al*., (2013) described the phytochemical bioactive compounds of the methanolic extract of *Psidium guajava* leavesand *in vitro* antidiabetic activity (α-amylase inhibition) [11]. The assay results suggested that the presence of bioactive compounds could be responsible for the versatile medicinal properties of *Psidium guajava* including antidiabetic property. The extract exhibited the dose-dependent increase in inhibitory effect on α-glucosidase enzyme (upto 89.4%) and α-amylase enzyme (upto 96.3%).Mutiu *et al.*, (2013) evaluated the anti-diabetic potential of *Picralima nitida* leaf via *in vitro* inhibition of α-amylase and α-glucosidase using the acetone, water and ethanol extracts[12]. The α-amylase inhibition was also investigated by reacting different concentrations of the extracts with α-amylase and starch solution while α-glucosidase inhibition was determined by pre-incubating α-glucosidase with different concentrations of the extracts followed by the addition of p-nitrophenyl glucopyranoside (pNPG). The acetone extract of *Picralima nitida* displayed the most effective inhibition of both α-amylase and α-glucosidase activities. Anuya *et al*., (2014) evaluated the effect of aqueous and ethanol extract of *Ocimum sanctum* on α-amylase and α-glucosidase enzymes using *in vitro* assays. Aqueous extract showed the highest α-glucosidase inhibitory activity than ethanol extract. However, these extracts showed no α-amylase inhibitory activity[13]. Satyanarayan *et al.*, (2015) reported the inhibition of α-amylase by the methanol leaf extract of *Costus igneus* by Chromogenic DNSA method. The leaf methanol extract has displayed inhibition at 500, 250 and 100 μg concentrations. Whereas, the standard Acarbose inhibited the enzyme at all concentrations screened[14]. They indicated that the α-amylase inhibition by the methanol extract of *C.igneus* is dose dependent.

Arunachalam *et al.*,(2013) investigated the *in vitro* anti-diabetic effect ofaqueous extracts of the medicinal plants *Nigella sativa*, *Eugenia jambolana, Andrographis paniculata, Gymnema sylvestre*[15]. Aqueous extracts of the plants were tested for inhibition of α-amylase activity by DNSA color reagent. They were tested for their ability to hinder diffusion of glucose across a dialysis membrane. The aqueous extract of *Nigella sativa* showed maximum inhibition of α-amylase activity and a strong hindrance to diffusion of glucose across a dialysis membrane. *Andrographis paniculata* showed both strong inhibition of α-amylase and a significant hindrance to the diffusion of glucose across the dialysis membrane. *Gymnema sylvestre* showed low inhibition of α-amylase activity, but it showed maximum hindrance to the diffusion of glucose across the dialysis membrane. *Nigella sativa* was found to possess maximum anti-diabetic properties. The findings indicated that all the above plants possess anti diabetic properties too varying degrees. They commonly used strong allopathic drugs which possess a number of harmful side effects. Bhutkar and Bhise (2012) used the aqueous extracts from leaves, stems, seeds and roots of selected plants such as *Tamarindus indica*, *Catharanthus roseus* and *Caesalpinia bonducella* which are used in the ayurvedic traditional system of medicine to treat diabetes, for their inhibitory effect on α-amylase[16]. The results revealed that the aqueous extracts of leaves of *T.indica*, extracts from the stems and roots of *C. roseus* and *C. bonducella* exhibited significant (more than 70%) reduction in α-amylase activity. The highest inhibition of about 87.26% was observed with the aqueous extract of the seeds of *C.bonducella*.

Conflict of Interest: None declared.

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