IN VITRO ANTIDIABETIC ACTIVITY OF METHANOLIC AND ACETONE LEAF EXTRACTS OF CERATOTHECA SESAMOIDES

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DEPARTMENT OF ZOOLOGY
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DECLARATION

I, **ALFIA T S (210021037711),** hereby declare that the dissertation work entitled **"IN VITRO ANTIDIABETIC ACTIVITY OF METHANOLIC AND ACETONE LEAF EXTRACTS OF *CERATOTHECA SESAMOIDES* "** submitted for the award of a Bachelor's degree in Bharata Mata College, Thrikkakara, is a partial fulfillment of the requirements. This work was done by me during the period from December 2023 to February 2024 under the supervision and guidance of Dr. Simi Joseph P, Head of the Zoology Department, Bharata Mata College, Thrikkakara. I affirm that this thesis is original and has not been submitted for any degree, fellowship, or similar qualification by any other candidate to any university.

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ALFIA T S

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Initially and primarily, I wish to commence by invoking the name of the Almighty, whose blessings have guided and empowered me in the realization of this project. At this juncture, I wish to convey my deep appreciation to all those who have assisted me in the finalization of this scholarly work. It gives me immense pleasure to recognize and acknowledge the encouragement and backing offered by these individuals, whose invaluable input has been pivotal in the successful culmination of this endeavor. I am sincerely grateful to Prof. Johnson K. M, the Principal of Bharata Mata College, Thrikkakara, for his assistance in facilitating the necessary resources for the students to effectively carry out the project.

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In conclusion, I wish to convey my profound appreciation to my parents and friends who have assisted me in the completion of this project report.

ALFIA T S

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ABBREVIATIONS

gm	Grams
ml	Millilitre
α	Alpha
E. C. 3.2.1,20	Alpha glucosidase
pNPG	4-Nitrophenyl-alpha-D-glucopyranoside
Na2CO3	Sodium carbonate
°C	Degree celsius
mM	Millimolar
nm	Nanometer
%	Percentage
et al	Latin expression meaning “& others”
µl	Microlitre
UV	Ultraviolet

INDEX

1. ABSTRACT

Diabetes mellitus is a multifactorial chronic metabolic syndrome resulting from deficiencies in insulin production. One strategy for managing Diabetes involves the inhibition of alpha glucosidase and alpha amylase activities. A commonly held belief is that plants represent rich reservoirs of phytochemical compounds that enhance their therapeutic efficacy. The primary objective of the present investigation was to evaluate the Antidiabetic properties of methanolic and acetone leaf extracts derived from *Ceratotheca sesamoides* under in vitro conditions. *Ceratotheca sesamoides* is a flowering plant that blooms annually, belonging to the Pedaliaceae family indigenous to Africa. This botanical family encompasses several members renowned for their noteworthy medicinal attributes. To conduct the investigation, fresh leaves of the plant was collected and subjected to extraction with methanol and acetone. The experiment involved the use of specific chemicals such as 4-Nitrophenyl-alpha-D-glucopyranoside (pNPG) and alpha-glucosidase enzyme (E.C. 3.2.1, 20). Potassium dihydrogen phosphate and Dipotassium hydrogen phosphate were employed in the preparation of Potassium phosphate buffer. Sodium carbonate was utilized to stop the chemical reaction. Assessment of the plant's antidiabetic properties was performed through an alpha-glucosidase inhibition assay utilizing a Visible spectrophotometer. The findings demonstrated a dose-dependent escalation in inhibitory activity against alpha-glucosidase enzymes for both the methanolic and acetone extracts. Notably, the acetone extract exhibited superior efficacy, manifesting a substantial percentage inhibition (82.56% - 50µl) in contrast to the methanolic extracts (66.17% - 50µl).

2. INTRODUCTION

Human societies have maintained close interactions with their surrounding environments since their inception, utilizing natural resources for sustenance and healing purposes. The utilization of botanical elements for nourishment and healing has been a result of systematic experimentation, leading to a gradual evolution of knowledge and self-sufficiency within human communities. Knowledge regarding the therapeutic properties of plants has been disseminated across generations, contributing to the accumulation of expertise as societies advanced and developed. The utilization of medicinal plants as a healthcare resource is prevalent across diverse cultural landscapes. Recently, the assurance of safety, quality, and efficacy of medicinal plants and herbal remedies has emerged as a significant concern in both developed and developing nations (Jamshidi et al., 2017).

The utilization of medicinal flora was initiated by the Japanese for the purpose of conducting ceremonies, enhancing food taste, and managing various ailments. Global research endeavors have been undertaken to validate their efficacy, leading to the emergence of botanical remedies. These plants, serving as a crucial medicinal reservoir, have consistently held significant value across diverse historical cultures. Regarded as abundant reservoirs of conventional therapeutics, medicinal plants not only fulfill this role but also function as vital contributors to numerous contemporary pharmaceuticals. The distinctive characteristics of these plants are attributed to the presence of bioactive compounds (Rasool et al., 2020).

Herbal plants are useful in both treating and preventing illnesses in people. Plants are thought to be abundant suppliers of the phytochemical components that give them their therapeutic efficacy. The development of novel herbal medications may benefit from the use of medicinal plants. The pharmacological properties of medicinal plants have been viewed as a promising new drug or medicine for the management of healthcare in the twenty-first century. Rediscovered medicinal plants have piqued curiosity as a possible source of novel drugs in recent years (Shakya A K., 2016).

Diabetes mellitus is a significant chronic metabolic disorder impacting the metabolism of carbohydrate, fat, and protein, encompassing a cluster of metabolic conditions marked by hyperglycemia. This arises when blood sugar levels are elevated due to insufficient insulin production by the pancreas or ineffective response of the body's cells to the insulin generated. The consequences of diabetes mellitus encompass enduring complications such as heart diseases, stroke, and dysfunction or failure of diverse organs (Banerjee et al., 2017).

Traditionally, diabetes has been categorized into three forms: Type 1 DM or insulin-dependent diabetes mellitus (IDDM), characterized by the body's inability to produce insulin, necessitating insulin injections or the use of an insulin pump. Commonly known as "juvenile diabetes," Type 2 DM or non-insulin-dependent diabetes mellitus (NIDDM) emerges from insulin resistance, where cells do not efficiently utilize insulin, with or without a complete insulin deficiency. This type was previously labeled as "adult-onset diabetes." The third principal type is gestational diabetes, occurring in women without prior diabetes history who develop elevated blood glucose levels during pregnancy. It may precede the onset of type 2 DM (Deshmukh et.al., 2015).

The incidence of T1DM globally has been steadily increasing by 3 to 5% each year, notably among children under the age of 5. T1DM constitutes over 90% of cases in children and teenagers, with 50 to 60% diagnosed before 15 years, predominantly in the Western world. In 2010, the global T1DM patient count was documented at 285 million individuals, encompassing 90% of those with T2DM. The projected diabetes rate for 2030 is approximately 439 million worldwide, representing 7.7% of the adult population aged 20-79 (Mahnashi et al., 2022).

Recent epidemiological investigations indicate that the occurrence of diabetes mellitus is nearly equivalent in malnourished populations compared to well-nourished populations, despite the absence of obesity as a causative factor in the former. The underlying risk factor in such populations predisposing them to diabetes may be attributed to undernutrition. It is recognized that protein-energy malnutrition leads to glucose intolerance, which is linked to structural alterations in the beta cell. In a considerable number of undernourished individuals, this condition is deemed irreversible even after prolonged and intensive nutritional therapy. It is plausible that persistent undernourishment throughout one's lifetime could significantly contribute to the development of diabetes by gradually compromising beta cell functionality or heightening susceptibility to various genetic and environmental factors that promote diabetes (Rao, R. H., 1984).

The global prevalence of type 2 diabetes presents a significant challenge to public health worldwide, particularly impacting developing nations. This crisis is fueled by rapid urbanization, shifts in dietary patterns, and increasingly sedentary behaviors, closely paralleling the escalating rates of obesity on a global scale. Asia, with its vast population and rapid economic growth, has emerged as a focal point of this epidemic. Notably, individuals of Asian descent tend to manifest diabetes at younger ages and lower body mass index levels compared to Caucasians. Multiple factors contribute to the accelerated spread of diabetes in

Asian populations, including unique metabolic profiles such as the "normal-weight metabolically obese" phenotype, high rates of tobacco and alcohol consumption, elevated consumption of refined carbohydrates like white rice, and a substantial decline in physical activity levels. Furthermore, inadequate nutrition during fetal development and early childhood, coupled with excessive food intake in later stages of life, may also contribute to the escalating diabetes rates in Asia. Findings from epidemiological studies and clinical trials suggest that type 2 diabetes is largely preventable through modifications in diet and lifestyle practices (Hu, F. B., 2011).

Insulin replacement therapy constitutes the primary approach for individuals diagnosed with type 1 diabetes mellitus, whereas dietary adjustments and modifications in lifestyle are fundamental elements in the management of type 2 diabetes mellitus. In cases of type 2 diabetes mellitus where conventional methods such as dietary regulation, physical activity, and oral medications fail to adequately control blood glucose levels, the inclusion of insulin becomes imperative. The utilization of oral hypoglycemic agents also plays a significant role in the therapeutic regimen for type 2 diabetes mellitus. These agents encompass a variety of medications including sulphonylureas, biguanides, alpha glucosidase inhibitors, meglitinide analogues, and thiazolidinediones.
The primary aim of these pharmacological interventions is to address the underlying metabolic irregularities associated with insulin resistance and insufficient insulin secretion. It is recommended that these medications be prescribed in conjunction with appropriate dietary adjustments and modifications in lifestyle. The dietary and lifestyle strategies aim to facilitate weight reduction, enhance glycemic control, and mitigate the likelihood of cardiovascular complications, which contribute to a substantial percentage of fatalities in individuals with diabetes. Optimal management of diabetes involves either non-pharmacological approaches such as diet and exercise, or a combination of dietary modifications with herbal remedies, oral hypoglycemic agents, or insulin. Common adverse effects associated with these medications include weight gain and hypoglycemia with sulfonylureas, gastrointestinal disturbances with metformin, weight gain, gastrointestinal disturbances, and hepatotoxicity with thiazolidinediones, gastrointestinal disturbances, weight gain, and hypersensitivity reactions with meglitinides, as well as flatulence, diarrhea, and abdominal bloating with alpha-glucosidase inhibitors (Bastaki, S., 2005)

Ceratotheca sesamoides, classified within the genus Ceratotheca, is an annual flowering plant native to Africa, thriving both in the wild and under local cultivation, and colloquially known as false sesame due to its striking resemblance to common sesame (Sesamum indicum). Predominantly grown in the African savannah and other semi-arid regions on the continent, this plant is distributed across Africa within tropical and sub-tropical latitudes, typically favoring sandy soils located south of the Sahara. Distinguished by the presence of numerous hairs on its stem, pinkish flowers often displaying brown and purple patterns, and an inclination towards a partially upright growth pattern, Ceratotheca sesamoides is recognized for its unique characteristics. The leaves and flowers of this plant are frequently utilized as a culinary ingredient or incorporated into sauces. While the leaves are believed to possess medicinal properties, the seeds are utilized for the extraction of cooking oil. Despite its diverse applications and the increasing trend of local cultivation, Ceratotheca sesamoides remains largely underutilized and underappreciated (Falusi et al., 2002).

Ceratotheca sesamoides typically attains a stature of approximately 60 cm, although it has been documented to grow up to 100 cm in height. It is characterized as a slender, erect herb featuring blossoms in shades of pink, mauve, or lilac. The fruit generated by this plant bears resemblance to that of Sesamum, manifesting as a laterally-compressed capsule adorned with delicate protrusions at the terminal end. Generally, Ceratotheca sesamoides yields ten or more shoots stemming from its foundational cluster of leaves, often reclining horizontally; the recurrent elimination of nascent shoots fosters prolonged vegetative expansion and blooming, thereby extending the period of productivity. C. sesamoides predominantly engages in self-pollination, with its blooms unfurling at daybreak. Upon the culmination of the pollination process, it requires approximately six weeks from flowering to achieve full fruit maturation. The germination of seeds occurs at the advent of the wet season. Analogous to other members of the Pedaliaceae family, C. sesamoides is coated with mucilage glands, which enhance the species' capacity to endure desiccation without cellular demise, thus imparting a degree of resistance to drought. False sesame exhibits considerable adaptability concerning habitat and growing circumstances, thriving as a weed or in cultivated fields, thriving optimally in well-drained sandy soils exposed to substantial sunlight, and exhibiting lesser prosperity in rocky terrains.

The leaves and blooms of false sesame are not solely ingested as edibles but also utilized for medicinal purposes. An infusion of the plant is employed in combatting diarrhoea. Leaves are soaked in water, and the gelatinous fluid is administered to the eye for treating conjunctivitis. The mucilage is sporadically utilized as a soothing agent and lubricant. The maceration of leaves aids in administration to humans and animals. Leaves are heated, pulverized, combined with ash, and applied to inflamed cervical lymph nodes. False sesamum is reputedly utilized as an aphrodisiac, for addressing jaundice, snakebites, and dermatological ailments. The vegetation is grazed upon by camels, cattle, and goats. The oil derived from the seeds bears a resemblance in composition to sesame oil, containing the phenylpropanoid, lignan, sesamin. This particular compound has exhibited various bioactivities such as antioxidant, anti-inflammatory, antihypertensive, cytotoxic (including antitumor), and insecticidal properties (Bedigian, D. & Adetula, O. A., 2022)

Ceratotheca sesamoides harbors significant nutritional potential, being rich in energy content, fat concentration, as well as protein and carbohydrate levels (Masondo et al., 2016).

The phytochemical analysis of Ceratotheca sesamoides reveals the presence of glycosides, carbohydrates, and alkaloids in all extracts, with only flavonoids detected in aqueous and ethanol extractions. This indicates that C. sesamoides is deemed safe for consumption and is abundant in polyphenols, flavonoids, and tannins (Silas et al., 2020).

3. REVIEW OF LITERATURE

The significance of herbal remedies, which have long been regarded as a highly valuable source of medicine, was assessed by Rao et al. (2010). In addition to their natural therapeutic benefits against a range of illnesses—many studies have been conducted on medicinal plants to treat diabetes mellitus—it is reported that the phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents of these plants that exhibit a decrease in blood glucose levels are the cause of their antidiabetic activity. Since there is no known treatment for diabetes mellitus, medicinal herbs are crucial in managing the condition.

A study by Mujtaba et al. (2023) examined the antidiabetic properties of *Pedalium murex* seed oil, which is a member of the Pedaliaceae family. It was done using an Accu-Chek glucose meter. To test the antidiabetic effects of seed oil at 100 mg/kg and 200 mg/kg in combination with the conventional medication glibenclamide at 5 mg/kg, albino mice in good health were used. On days 1, 7, 14, 21, 27, and 30, there was antidiabetic action. Using One-way and Two-way analysis of variance, statistical computations and significant results were achieved, which were then confirmed using Tucker's test. The study indicates that the antidiabetic action of the seed oil may be attributed to the phytochemical n-hexadecanoic acid (19.53%).

An investigation was conducted by Basha and Kumari (2012) to assess the glucose absorption of crude n-hexane, ethanol, and aqueous leaf extracts of *P. guajava* (antidiabetic efficacy).Using a certain standard in vitro technique, *P. guajava* leaf extracts were treated to an inhibitory effect of glucose utilization. Out of the four distinct extracts, the results showed that the methanol extract, at a concentration of 50g plant extract/l, was more powerful than the other extracts with the lowest mean glucose concentration after 27 hours, at 201+1.69 mg/dl.

In their study, Narkhede et al. (2011) sought to determine the in vitro antidiabetic efficacy of a methanol extract of *Caesalpinia digyna* roots. Methanol was used to extract *Caesalpinia digyn*a Rottler's dried root. 10.15 percent was discovered to be the extract yield percentage. Upon doing a preliminary phytochemical screening, the CD extract was found to include triterpenoids, glycosides, tannins, polysaccharides, and flavonoids. The findings indicate that a dose-dependent increase in the % inhibitory activity on αglucosidase enzymes (IC50 402.23±10.14 μg/ml) and α-amylase (IC5 of

686.94 ± 3.98 µg/ml) was observed in the methanol extract of *Caesalpinia digyna*. Acarbose was a common medication.

The phytochemicals and antibacterial properties of *Cerathoteca sesamoides* leaf extracts were studied by Abubakar et al. (2020) in order to determine their potential use in herbal medicine. The plants' fresh leaves, which were collected from Lafia in Nasarawa State, Nigeria, were dried, ground into a powder, and then put through normal procedures for methanolic extraction, partitioning, phytochemical, and antimicrobial analyses. *C. sesamoides* contains flavonoids, tannins, alkaloids, terpenoids, saponins, steroids, and cardiac glycosides in its methanolic and ethyl acetate extracts. Both saponins and flavonoids were missing from the *C. sesamoides* ethyl acetate extract. No anthraquinone or reducing sugar was found in any of the *C. sesamoides* solvent extracts.

Studies on the antibacterial susceptibility of *C. sesamoides* residue extracts and n-hexane revealed no antimicrobial action against the examined microorganisms. The clinical bacteria *K. spp., E. coli, P. aeruginosa, S. aureus*, and *C. albicans* were all susceptible to the chloroform and ethyl acetate extracts of *C. sesamoides*. The plant extracts in methanol shown activity against the bacterial isolates, but not against *Candida albicans*.

The minimal inhibitory doses varied from 12.5 mg to > 50 mg for plant extracts. The results indicated that urinary tract infections might be treated with chloroform or ethyl acetate extracts from the leaves of these plant medications.

In an effort to preserve and domesticate this species, Masondo et al. (2016) carried out a study to highlight the untapped nutritional and pharmacological potential of the native leafy vegetable *Ceratotheca sesamoides* in Africa. Good nutritional potential can be found in *Ceratotheca sesamoides*. The species has high quantities of protein, fat, energy, and carbohydrates. Additionally, the species is said to possess strong antiviral, antidiabetic, antiplasmodial, antibacterial, and antidiarrheal qualities. When extracted at high concentrations, these species exhibited modest toxicity and cytotoxic action, but no mutagenesis activity was found.

The toxicity and phytochemical analysis of Ceratotheca sesamoides leaf extracts in aqueous, ethanol, and pet ether extracts were assessed in albino wistar rats by Silas et al. (2019). Ceratotheca sesamoides was screened for phytochemicals using both qualitative and quantitative methods. Aqueous extracts of *Ceratotheca sesamoides* leaf did not exhibit any fatalities within the dosage range of 300 mg-5000 mg, according to the results of the toxicity test. The phytochemical analysis reveals that all of the extracts contain glycoside, carbohydrate, and alkaloid; only the ethanol and aqueous extracts contain flavonoids. This demonstrates that C. sesamoides is rich in tannins, flavonoids, and polyphenols and is safe to eat.

Abiodun (2017) studied *Ceratotheca sesamoides's* chemical and antioxidant characteristics. The contents of minerals, carotene, amino acid profiles, proximate, and antioxidants were examined. The vegetable's approximate composition was as follows: ash (9.15%), protein (28.92%), crude fiber (6.21%), fat (1.79%), and carbohydrates (44.11%). The vegetable extract's capacity to scavenge free radicals using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) demonstrated increased antioxidant activity (72000 μ moles TE/100g).

The primary non-essential amino acids in the amino acid profile were glutamic acid (2.010%) and aspartic acid (1.927%), while the major essential amino acid was leucine (1.436%). The vegetable's main mineral concentrations were potassium and calcium, with a notable proportion of iron. Trans beta carotene had a higher value than total carotene, which was observed in C. sesamoides at 32000 IU/100g. Vegetable *C. sesamoides* is a source of antioxidants and nutrients that support healthy metabolism and illness prevention.

In a study published in 2012, Toyin et al. examined the antidiarrheal effects of *C. sesamoides* aqueous leaf extract at 25, 50, and 100 mg/kg body weight in female rats utilizing models of gastrointestinal transit, diarrhea, and enteropooling caused by castor oil. The extract tested positive for flavonoids, phenolics, alkaloids, and saponins.
In rats administered with 50 and 100 mg/kg body weight, the extract at a dose of 25 mg/kg body weight significantly ($p<0.05$) extended the time it took for diarrhea to start and decreased the fecal parameters (number, water content, fresh weight, total number of wet feces) without causing an episode. While nitric oxide, intestinal fluid volume and mass, and the distance the charcoal meal traveled all declined, the activity of the small intestine Na^+-K^+ ATPase increased ($p<0.05$).

The antioxidant and antiproliferative properties of hydromethanolic extracts of *Ceratotheca sesamoides* Endl from Burkina Faso were the subject of a study conducted by Bayala et al. in 2020. The ABTS+ radical cation decolorization test and the DPPH radical scavenging assay were used to evaluate the antioxidative activity of hydromethanolic plant extracts. Using the MTT assay, antiproliferative activity was assessed. It demonstrated that the *Ceratotheca sesamoides* leaf stem hydromethanolic extract exhibited the most antiproliferative efficacy against cervical cancer HeLa cell lines.

To help find alternatives to the chemical warfare against NGIs, Dicko et al. (2021) tested the anthelmintic activity of aqueous extracts of *S. hermonthica* and *C. sesamoides* on adult *H. contortus* worms in vitro. Each extract has been tested at three different concentrations: 100 mg/ml, 50 mg/ml, and 25 mg/ml. There was a positive control (levamisole at 2.5 mg/ml) and a negative control (PBS 1X).The findings indicate that, in comparison to the negative control P (<0.05), *C. sesamoides* aqueous extracts produced a significant mortality rate of adult *H. contrortus* worms. At a high dose of 100 mg/ml, it demonstrated 79.86% of adult worm mortality, whereas lesser doses showed a low mortality rate. The LC50 for *C. sesamoides* aqueous extracts was found to be 78.74 mg/ml.

4. AIMS & OBJECTIVES

- To collection and extraction of the leaves of *Ceratotheca sesamoides.*
- To evaluate the in vitro antidiabetic efficacy of methanolic and acetone leaf extracts of *Ceratotheca sesamoides* using alpha-glucosidase inhibition assay.
- To compare the percentage inhibition activity exhibited by methanolic and acetone leaf extracts on alpha-glucosidase enzyme.

5. MATERIALS AND METHOD

ANTIDIABETIC PROPERTY

Preparation of plant extract

For the preparation of the extract, the fresh leaves of *Ceratotheca sesamoides* were collected and cleaned with water. The leaves were then kept in shaded area away from direct sunlight for about a week. The dried leaves were then made into powder with the help of a motor and pestle. About 10gm of the powder was weighed and extracted with 60ml of methanol and acetone solution. After which, it was kept undisturbed in a dark place for 24 hours. The solution was then centrifuged, and the supernatant was taken and stored till further use.

Figure 1 :
CERATOTHECA SESAMOIDES

Figure 2 :
DRIED LEAF POWDER

Figure 3 :
WEIGHING MACHINE

Figure 4 :
EXTRACTION OF POWDERED LEAVES

Figure 5 :
CENTRIFUGE

Figure 6 :
METHANOLIC AND ACETONE LEAF EXTRACTS

Chemicals used

4-Nitrophenyl-α-D-glucopyranoside (pNPG) and α-glucosidase (E.C.3.2.1, 20) were used. Potassium dihydrogen phosphate, dipotassium hydrogen, Sodium chloride were utilized in the preparation of the Potassium phosphate buffer. Na_2CO_3 served as the solution for stopping the reaction.

Alpha Glucosidase Inhibition Assay

A phosphate buffer of pH 6.8 was formulated by mixing 1 gram of potassium dihydrogen phosphate with 2 grams of dipotassium hydrogen phosphate and 8.5 grams of sodium chloride in 900ml of water. The pH of the solution was then adjusted to 6.8, followed by the addition of more solvent to reach a total volume of 1000 ml.

In a controlled laboratory environment, 50 microliters of α–glucosidase solution of concentration of 0.5 units per ml was combined with 50 microliters of 0.2 Molar Potassium phosphate buffer of pH 6.8. The resulting mixture was introduced to the plant extract and placed in a shaded area for 15 minutes, maintaining a temperature of 37℃. Then 100 microliters of 3mM pNPG solution was added and the reaction was allowed to proceed for another 10 minutes under the same condition. Then to stop the reaction 750 microliters of 0.1 molar Na_2CO_3 solution was added.

The absorption rate of 4-nitrophenol was measured using a spectrophotometer of 405 nm. The controls were then set up using the reaction mixtures which excluded the test samples and the substrate, and then the experiment was repeated three more times for precision (Kim et al.,2011)

The percentage of α-glucosidase inhibition was calculated using the following equation :

Alpha glucosidase inhibition (%) = (A control – A sample)/A control × 100
Where, A control is the rate of absorption of the control

A sample is the rate of absorption of the experimental sample (Narkhede et al., 2011).

Figure 7 :
pNPG SOLUTION

Figure 8 :
ALPHA GLUCOSIDASE SOLUTION

Figure 9 :
POTASSIUM PHOSPHATE BUFFER

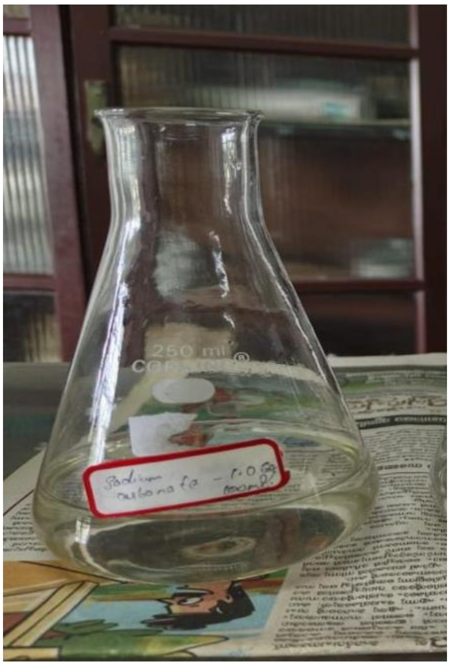

Figure 10 :
SODIUM CARBONATE
SOLUTION

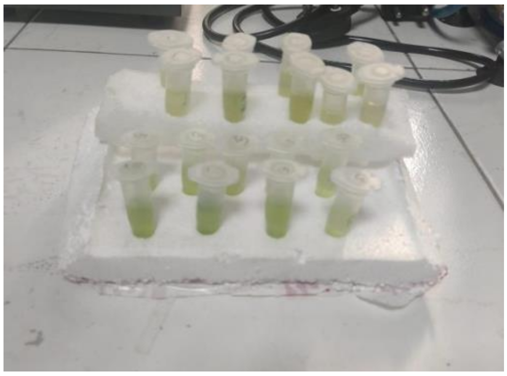

Figure 11 :
TEST SAMPLES

Figure 12 :
VISIBLE SPECTROPHOTOMETER

6. RESULT

Alpha glucosidase inhibition activity

Natural products represent the most abundant reservoirs of alpha glucosidase inhibitors (Kim et al., 2011). Therefore, the present study as undertaken for investigating the antidiabetic effects of methanolic and acetone leaf extracts of *Ceratotheca sesamoides*. Antidiabetic assay was conducted using a visible spectrophotometer, set at 405 nm to measure the absorption rates of the extracts. This allowed for the calculation of the inhibition percentage, indicating the extent of alpha glucosidase inhibition by the samples taken at different concentrations.

The obtained absorption rates were as follows :

Concentration of the sample	Absorption rate of the methanol extract
15µl	1.578
15µl	1.719
15µl	1.726

25µl	0.899
25µl	0.944
25µl	0.976
50µl	0.595
50µl	0.634
50µl	0.61

TABLE 1 : Table showing the absorbance rate of Methanolic leaf extracts of *C. Sesamoides*.

Concentration of the sample	Absorption rate of the acetone extract
15µl	1.267
15µl	0.953
15µl	1.156
25µl	0.574
25µl	0.55
25µl	0.744
50µl	0.356
50µl	0.32
50µl	0.33

TABLE 2 : Table showing absorbance rate of Acetone leaf extracts of *C. Sesamoides*.

The absorbance value of Control obtained was;

Methanol control = 1.812

Acetone control = 1.921

The percentage of inhibition of alpha glucosidase was calculated using the following equation ;

Alpha glucosidase inhibition (%) = (A control – A sample)/A control × 100

The percentage of inhibition was:

- **Methanolic Extract**

Concentration of sample	Average rate of absorption	Inhibition percentage
15µl	1.674	7.62
25µl	0.939	48.14
50µl	0.613	66.17

- **Acetone Extract**

Concentration of sample	Average rate of absorption	Inhibition percentage
15µl	0.069	41.44
25µl	0.393	67.57
50µl	0.965	82.56

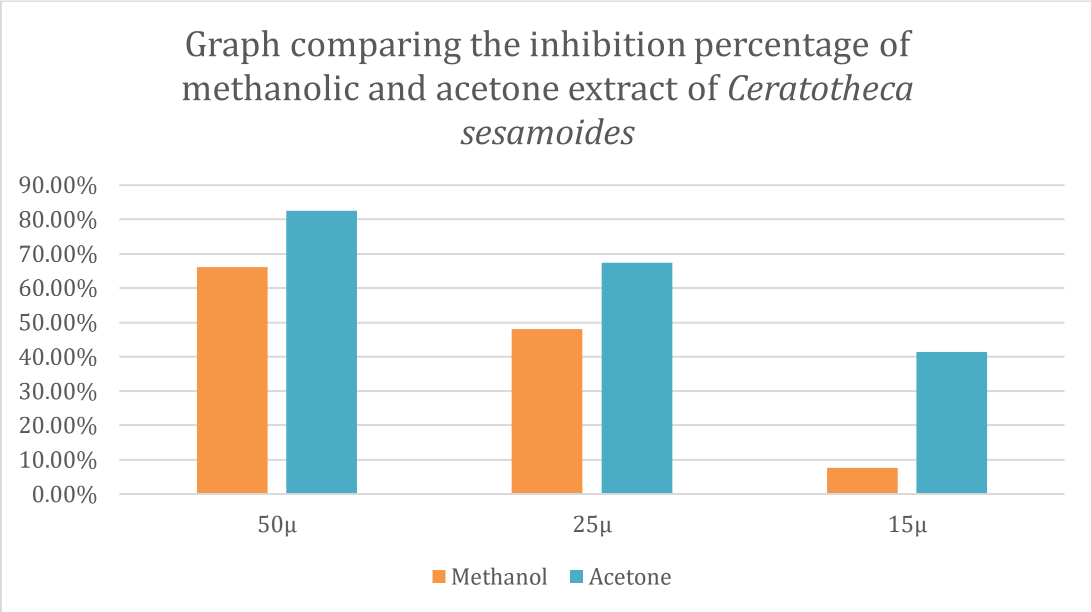

Figure 13 : Graph showing the inhibition percentage of Methanolic and acetone leaf extracts of *C. Sesamoides.*

7. DISCUSSION

In Vitro Alpha Glucosidase Assay

Natural compounds obtained from botanical sources are increasingly being recognized for their significance in the management of diabetes owing to their potent therapeutic properties and absence of adverse reactions. The primary aim of the current investigation is to elucidate the anti-diabetic efficacy exhibited by *Ceratotheca sesamoides*.

The research specifically delves into the assessment of alpha glucosidase inhibitory potential inherent in both methanol and acetone extracts of the plant's leaves. Upon analysis, it was evident that the acetone extracts displayed a notable dose-dependent escalation in inhibitory efficacy against the alpha glucosidase enzyme in comparison to the methanolic extracts. The methanolic extracts exhibited varying levels of percentage inhibition, ranging from 7.62% to 66.17%, across different concentrations (15µl to 50µl). Similarly, the acetone extract displayed percentage inhibition in the range of 41.44% to 82.56% for corresponding concentrations. The findings indicate the superior potency of the acetone extract over the methanolic extract. Moreover, it was observed that higher concentrations of the sample led to a decrease in absorbance rates, thereby enhancing the inhibitory effects of the extract.

Previous research focusing on the nutritional and pharmacological attributes of *C. sesamoides* revealed its rich content of energy, carbohydrates, fats, and proteins. This plant is believed to possess medicinal properties for various ailments and is recognized for its high nutritional significance (Masondo et al., 2016)

The examination of phytochemical components in *C. sesamoides* has unveiled the presence of glycosides, alkaloids, and various other chemical compounds (Silas et al., 2020). The inhibitory activity against alpha glucosidase exhibited by the plant may be attributed to the effects of flavonoids, polyphenols, tannins, alkaloids, and other bioactive compounds contained within them.

Consequently, the research on the antidiabetic properties of *C. sesamoides* has demonstrated that the acetone leaf extract displayed notable inhibition of the alpha glucosidase enzyme, proving to be more potent in comparison to the methanolic extract. The investigation was carried out utilizing a Visible spectrophotometer, although enhanced precision in values and outcomes could be achieved through the utilization of a UV spectrophotometer.

8. CONCLUSION

The evaluation of the antidiabetic properties of leaf extracts derived from *Ceratotheca sesamoides* was conducted within the confines of this particular investigation. Through the utilization of an alpha glucosidase inhibitory assay, both the methanolic and acetone extracts were scrutinized under laboratory settings. It was observed that both extracts exhibited the capacity to impede the functionality of the alpha glucosidase enzyme, thereby implying a heightened antidiabetic potential within them. Specifically, the methanolic extract demonstrated a considerable inhibitory efficacy of 66.17% at elevated concentrations, while the acetone extract displayed a higher inhibition rate of 82.56% at the same concentration level. Consequently, the acetone extract proved to possess greater potency in comparison to the methanolic extract.

The outcomes of the analysis highlighted the antidiabetic capabilities inherent in *Ceratotheca sesamoides*, with a significant emphasis on the increased effectiveness and pronounced inhibitory effects associated with the acetone extract when compared with the methanolic extract.

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