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Pharmacognostical, Phytochemical and Pharmacological Evaluation of Hydroalcoholic Extract of Seeds of *Sesamum indicum* (Linn.) for its In-Vitro Anti-Diabetic Activity

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ABSTRACT

Black Sesame seeds (*Sesamum indicum* Linn.) belong to the family Pedaliaceae. *Sesamum indicum* Linn. seeds contain various active phytoconstituent components with good therapeutic values. The present study deal with the Pharmacognostic, physicochemical & pharmacological evaluation of the hydroalcoholic extract of seeds of *Sesamum indicum* Linn. The macroscopic, microscopic, physicochemical parameter, fluorescence analysis & phytochemical investigation of Seeds of Sesamum indicum Linn. was carried out. The phytoconstituent like alkaloids, flavonoids, and glycosides are present in seeds of *Sesamum indicum* Linn. The increased postprandial blood glucose is related to carbohydrate digestion. The α-amylase is the primary enzyme responsible for the digestion of carbohydrates. Therefore, it is important for the prevention and treatment of diabetic patients as well as the development of diabetes to strictly regulate postprandial blood glucose levels by inhibiting α-amylase. Amylase inhibitors (AIs) have the ability to slow the absorption and digestion of carbohydrates in the gastrointestinal tract. In-vitro anti-diabetic activity is evaluated by α – amylase inhibitory assay. Acarbose was used as a standard drug.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is a member of the Flax genus, a significant historic oil crop with a lengthy history of cultivation dating back more than 2200 years.[(1)]Sesame seeds are classified as white sesame, black sesame, and yellow sesame based on the hues of the seed coat, with white sesame and black sesame seeds being the most widely available.[(2)] Consuming black sesame seeds frequently may lower blood oxidation, raise blood pressure, and supply antioxidants and other plant substances that aid in the battle against cancer.[(3)] Sesame includes compounds that may aid in reducing swelling, speeding up the healing of wounds, and decreasing the rate at which sugar is absorbed from the diet.[(4)] Sesame seed and its products (oil, flour, and dietary supplement) are good sources of lignan compounds (sesamin, sesamolin, sesamol, and epi sesamin).[(5)]

Diabetes has grown in importance as a worldwide health issue in recent years, placing a heavy financial strain on many nations. Clinical studies examining the hypoglycemic effects of sesame intake have yielded mixed findings. Over 420 million people worldwide suffer from diabetes mellitus, one of the most prevalent metabolic diseases.[(6)] In 2016, this illness caused 28.8 million fatalities.[(7)]Type 2 diabetes (T2D) is characterized by insulin resistance and is diagnosed by biochemical markers such as high concentrations of insulin, glycosylated hemoglobin (HbA1C), and serum blood sugar levels.[(8)&(9)] Finding a workable solution to lower the prevalence of diabetes is crucial for the welfare of the world economy and population. Due to its cheaper costs and fewer side effects, herbal therapy, a part of complementary medicine (CAM), is frequently used to prevent and cure chronic illnesses.[(10)]

On review of literature, it is confirmed that there was no systematic evaluation performed on anti-diabetic adventure on *Sesamum indicum* L. So their substantial need is to carry out the evaluation of *Sesamum indicum* L. for its anti-diabetic potential.

MATERIAL AND METHODS

Drugs and Chemicals:-

- ✓ **Drug used as the standard for pharmacological screening:-**

Table No. 1: List of drugs used as standard for pharmacological screening.

Sr. no.	Name of drug	Company Name
1	Acarbose	Research-Lab Fine Chem Industries Mumbai 400 002

✓ **Chemicals used for extraction, phytochemical analysis, and pharmacological evaluation:-**

Table No.2: List of chemicals used for extraction, phytochemical analysis, and pharmacological evaluation.

Sr. no.	Drug / Chemicals	Company Name
1	Ethanol	Research-Lab Fine Chem Industries, Mumbai 400 002
2	Chloral hydrate	Research-Lab Fine Chem Industries, Mumbai 400 002
3	Glycerin	Research-Lab Fine Chem Industries, Mumbai 400 002
4	Hydrochloric acid	Research-Lab Fine Chem Industries, Mumbai 400 002
5	NaOH	Research-Lab Fine Chem Industries, Mumbai 400 002
6	HCL	Research-Lab Fine Chem Industries, Mumbai 400 002
7	H_2SO_4	Research-Lab Fine Chem Industries, Mumbai 400 002
8	HNO_3	Research-Lab Fine Chem Industries, Mumbai 400 002
9	Ammonia	Research-Lab Fine Chem Industries, Mumbai 400 002
10	Iodine	Research-Lab Fine Chem Industries, Mumbai 400 002
11	$FeCL_3$	Research-Lab Fine Chem Industries, Mumbai 400 002
12	Acetic acid	Research-Lab Fine Chem Industries, Mumbai 400 002
13	α-naphthanol	Research-Lab Fine Chem Industries, Mumbai 400 002
14	Fehling's A & B	Research-Lab Fine Chem Industries, Mumbai 400 002
15	Benedict's reagent	Research-Lab Fine Chem Industries, Mumbai 400 002
16	Barfoed's reagent	Research-Lab Fine Chem Industries, Mumbai 400 002
17	$CuSO_4$	Research-Lab Fine Chem Industries, Mumbai 400 002
18	Million's reagent	Research-Lab Fine Chem Industries, Mumbai 400 002

19	Chloroform	Research-Lab Fine Chem Industries, Mumbai 400 002
20	Sodium picrate	Research-Lab Fine Chem Industries, Mumbai 400 002
21	Pyridine	Research -Lab Fine Chem Industries, Mumbai 400 002
22	Sodium nitroprusside	Research-Lab Fine Chem Industries, Mumbai 400 002
23	Glacial acetic acid	Research-Lab Fine Chem Industries, Mumbai 400 002
24	Benzene	Research-Lab Fine Chem Industries, Mumbai 400 002
25	Lead acetate	Research-Lab Fine Chem Industries, Mumbai 400 002
26	Dragendroff's reagent	Research-Lab Fine Chem Industries, Mumbai 400 002
27	Mayer's reagent	Research-Lab Fine Chem Industries, Mumbai 400 002
28	Hager's reagent	Research-Lab Fine Chem Industries, Mumbai 400 002
29	Bromine water	Research-Lab Fine Chem Industries, Mumbai 400 002
30	Potassium dichromate	Research-Lab Fine Chem Industries, Mumbai 400 002
31	NH_4OH	Research-Lab Fine Chem Industries, Mumbai 400 002
32	Potassium ferricyanide	Research-Lab Fine Chem Industries, Mumbai 400 002
33	Dil. potassium permanganate	Research-Lab Fine Chem Industries, Mumbai 400 002
34	Sodium phosphate monobasic monohydrate	Research-Lab Fine Chem Industries, Mumbai 400 002
35	Sodium phosphate dibasic hyptahydrate	Research-Lab Fine Chem Industries, Mumbai 400 002
36	Sodium phosphate dibasic anhydrous	Research-Lab Fine Chem Industries, Mumbai 400 002
37	α-amylase	Research-Lab Fine Chem Industries, Mumbai 400 002
38	Starch	Research-Lab Fine Chem Industries, Mumbai 400 002
39	3,5 –nitrosalicylic acid	Research-Lab Fine Chem Industries, Mumbai 400 002
40	Sodium potassium tartrate tetrahydrate	Research-Lab Fine Chem Industries, Mumbai 400 002

✓ **Instruments used for extraction, phytochemical analysis and pharmacological evaluation.**

Table No.3: Instruments used for extraction, phytochemical analysis and pharmacological evaluation.

Sr. no.	Instrument Name	Company name
1	Hot air oven	Lab line
2	Microscope	Lab line
3	Incubator	Biotech
4	Muffle furnace	Lab line
5	Weighing balance	Tapsons
6	UV Cabinet	Lab line
7	Desiccator	Polylab
8	Heating mental	Sunbim
9	Mixer	Rajesh
10	Electric water bath	Lab line

❖ **Collection and Authentication of plant material:-**

Most sandy loam soils that are shallow or medium in depth are used to cultivate *Sesamum indicum* Linn. It can grow in soils with salinities up to 10,000 ppm since it is salt-tolerant. The seeds of *Sesamum indicum* Linn. collected from Pandharpur, Dist-Solapur, Maharashtra. After collection, it can be identified from Mr. Gore Ram (HOD of the Department of Botany) at Walchand College of Art & Science, Solapur. After being verified, the *Sesamum indicum* Linn. seeds were maintained for drying and to eliminate any undesired material before being ground into a fine powder. This powder passed through sieve number 30. In order to prevent contamination and moisture, this powder is maintained in an airtight container.

❖ **Pharmacognostic Evaluation:-**

I. Macroscopic Characters:-

The seed of *Sesamum indicum* Linn. was subjected to macroscopic identification based on colour, odour, taste, shape, length, width, thickness, hilum and ridges.

II. Microscopic Characters:-

a. Transverse section:-

Procedure:-Take dried black sesame seed into a clean beaker. Allow it to soak into 70% alcohol for 24h.After 24h take free hand section of the seed with the help of the blade. Then clear it with chloral hydrate and water solution. Take staining agent solution i.e. safranin in a clean watch glass. With the help of a brush transfer the section form chloral hydrate and water solution to staining agent solution and wait for 10 min. Pick up the section after 10 min and place it in a glass of plain water to remove any remaining discoloration. At this point, the section is prepared for mounting on a slide with the help of brush.To make the portion easier to see, add a 1-2 of drops of glycerin. Place the cover slip on the section gently using forceps and a needle. Wipe the excess glycerin present outside the coverslip and observe the section under 10 x microscope.[11,12&13]

b. Power characters:-

The seeds were separated & powdered. Using chloral hydrate, mounts, microscopic characteristics were investigated in powders of various components.[14]

❖ **Physicochemical parameters:-**

A) Moisture Content :-

Procedure:-1.5 g of the powdered drug sample were put onto a Petri plate that had previously been weighed. Place the porcelain dish into a hot air oven for drying at 100° C for 1h.The petriplate then cool into desiccators for 30 min. After cooling note down the weight of the petriplate. The difference in weight was taken. By using the following formula calculate moisture content.

$$LOD = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where,W1 = weight of empty Petri dish

W_2 = weight of Petri dish +drug

W3 = weight of Petri dish +drug (after drying)

B) Ash values:-

a) Total ash value:-

Procedure:- Take a 2g of powdered drug sample into a previously weighted tarred silica crucible.In a tarred silica crucible, the sample was added in a thin layer. Then place the crucible into muffle furnace at 450-500° C until all the carbon is burnt off. Then remove the crucible from the muffle furnace and allow it to cool into desiccators for 20 min. After cooling take a weight of the crucible. Calculate the percentage of total ash using the following formula.

$$\text{Total Ash value in \%} = \frac{(b - c)}{a} \times 100$$

Where, a= weight of sample

b= weight of crucible with ash

c= weight of crucible after drying

b) Acid- insoluble Ash value:-

Procedure:-Proceed in accordance with the steps outlined in the technique for calculating a crude drug's total ash value.

Add 20 ml of hydrochloric acid and 25 ml water to a silica crucible after this covered with a watch glass. After that, it was gently boiled for 10 min on a burner and cool it. Then filter it with 'ashless' filter paper. Hot water was used to rinse this filter paper several times until the filtrate was acid-free & become neutral. In the previous crucible, place the filter paper that contains insoluble material. Dry it on a hot plate, then let to cool it. A crucible should be ignited in the muffle furnace at 450°C for 30 min. The crucible was removed from the

muffle furnace and placed it 30min into the desiccator. Weight the crucible and calculate the acid – insoluble ash value of the sample in percentage.

$$\text{Acid} - \text{insoluble Ash value in \%} = \frac{(b - c)}{a} \times 100$$

Where, a= weight of sample

b= weight of crucible with ash

c= weight of crucible after drying

c) Water- soluble ash:-

For water –soluble ash value similar procedure follows as mentioned in acid-insoluble ash value, use 25 ml of water, instead of diluting hydrochloric acid.

C) Extractive Values:

i. Alcohol- soluble extractive value:-

Procedure:-5g of the powdered drug sample should be weighed in a weighing bottle while being transferred to a dry 250ml conical flask. The solvent (90% alcohol) should be poured into a 100 ml graduated flask to the delivery mark. In the conical flask, add the remaining solvent, the washed-out weighing bottle, and the washings. For 24 h, shake frequently and allow the flask corked (Maceration). Filter into a 50 ml bottle. Transfer 25 ml of the filtrate to the weighted, thin porcelain dish that was used to determine the ash value once enough filtrate has accumulated. Evaporate to dryness on a water bath, then finish drying in an oven set to 100 $^{\circ}$C. For cooling place it into a desiccator for 30 min and weigh it. Calculate the extractive w/w percentage using the air-dried drugs as a reference.

$$\text{Alcohol} - \text{soluble extractive value in \%} = (b - a) \times 4 \times \frac{100}{w}$$

Where, a = weight of the empty dish

b = weight of dish + residue

w = weight of plant residual taken

ii. Water- soluble extractive value:-

Procedure:-For water-soluble extractive value similar procedure follow as mentioned in alcohol-soluble extractive value.[13]

❖ **Fluorescence Analysis:-**

Procedure:-Take 2g powdered sample of crude drug in a crucible. First observe the colour under ordinary light and note down colour of sample then place it into under UV cabinet (366nm) and observe the colour change of sample and note down it. Take another crucible with 2 g powder and 2ml 1N NaOH (aqueous) solution, observe the colour under ordinary light and note down result after that place it into under UV cabinet (366nm) and observe the colour change of sample and note down it.A similar procedure follow for 1N NaOH (alcoholic), 1N HCL, H_2SO_4(1:1), HNO_3, Ammonia, Iodine, 5% FeCL, Acetic acid.[15]

❖ **Extraction:-**

Procedure: - The seeds*Sesamum indicum*Linn.were ground using mixture grinder .The maceration process follows for extract preparation. The hydroalcoholic extract is created by 50 g of seed powder dissolved in 200 ml of hydroalcoholic solvent (distilled water-ethanol 96% mixture; 60-40). Kept on magnetic stirrer at room temperature for 72h.The containers contents were then filtered using a Buchner funnel and Whatman's filter paper no. 1.After extracting the solvent and drying at room temperature, a semisolid mass was obtained and maintained at 4°C before use. % yield of extract was calculated by following formula.[16&17]

$$\% \text{ Yield} = \frac{\text{weight of extract}}{\text{weight of drug taken}} \times 100$$

❖ **Phytochemical Investigation:-**

A. Test for carbohydrates:-

1) Molish's test:-4-5 drops of alpha-naphthol solution dissolved in alcohol should be added to 2-3 ml of hydroalcoholic extract. The mixture should be shaken before adding from the test tube's sides. The intersection of two liquids generates a violet ring.

2) Fehling's test:-Add 1 ml of the Fehling's A and B solution, and then boil for 1 minute. Add 2 ml of the test solution. Heat with boiling water for 5 to 10 min. A yellow and then a brick red colour is first noticed so indict presence of reducing sugars.

3) Benedict's test:-In a test tube, put 2 ml of the test solution and 2 ml of Benedict's reagent. 5 min heat on a water bath. The presence of reducing sugar is indicated by the colour red.

4) Barfoed's test:-Add 2ml of Barfoed's reagent and 2 ml test solution on test tube. Heat on water bath for 3-5 min then cool it. The presence of monosaccharides is indicated by red ppt.

B.Test for Proteins:-

1) Biuret test:-Add 3 ml of test solution with 4% NaOH into the test tube then add 3- 4 drops of 1% $CuSO_4$ solution. violet or pink colour observed.

2) Million's test:-5 ml of Million's reagent should be added to 3 ml of test solution. it form a white ppt. The warm ppt turns brick red, or the ppt dissolves, producing a reddish-colored solution.

3) Xanthoprotein test:-When 3 ml of test solution is added along with 1 ml of concentrated H_2SO_4, white ppt is produced. This ppt then turns yellow by heating on water bath. When conc. H_2SO_4 is added and converted it into orange**.**

C. Tests for Steroid:-

1) Salkowski reaction:-In a test tube, combine 2 ml extract, 2 ml chloroform, and 2 ml concentrated H_2SO_4 and shake it. The acid layer gives greenish-yellow fluorescence, whereas the chloroform layer appears red.

D. Tests for Glycoside:-

Hydrolyze the extract with HCL and then used for the glycosides test.

1) Test for Cardiac Glycosides: -

a) Balijet's test:-Add 2 ml hydrolysed extract with 2 ml sodium picrate solution. A thick section appears yellow to orange in colour.

b) legal's test :- Hydrolised extract was treated with 2 ml pyridine and 1 ml sodium nitroprusside. Red colour shows presence of glycosides.

c) Test for Deoxysugars :- 2 ml of hydrolyzed extract and 2 ml of glacial acetic acid should be added with 1 drop of H_2SO_4 and 5% $FeCL_3$ .Reddish brown colour develops at the intersection of two liquid layers, and the upper layer displays bluish green colour .

2) Test for Anthraquinone Glycosides:-

a) Borntrager's test:-Add 3 ml of the hydrolyzed extract with dilute H_2SO_4 to the test tube, boil it over water bath and then filter it. Add an equal volume of benzene or chloroform to the cold filtrate and thoroughly shake. Add ammonia after separating the organic solvent. Anthraquinone glycosides are present when the ammonia layer turns pink or red.

b) Modified Borntrager's test:-Add 5 ml of 5% FeCL3 and 5 ml of diluted HCL to 5 ml of hydrolized extract. Heat on boiling water for five minutes. Add any organic solvent, such as benzene, after cooling. Stir thoroughly. Add the same volume of dil. ammonia to the organic layer which is separated. The ammonia layer is pinkish-red in colour.

3) Test for Saponin Glycosides:-

a) Foam test:-4 ml extract was added into 4 ml water and shake it. The persistent foam observed indicate presence of saponin glycosides in extract.

E) Test for Flavonoids:-

1) Shinoda test:-5ml of 95% ethanol, 3-4 drops of concentrated HCL and 0.5 g of magnesium turnings should be added to 2 ml of extract. Flavonoids are present as shown by the colour pink.

2) Lead acetate test:-2 ml extract was added into 2 ml lead acetate solution. Yellow colored ppt indicate presence of flavonoids.

F) Test for Alkaloids:-

Separately evaporate the aqueous, alcoholic, and chloroform extracts. Add dilute HCL to the residue. Shake vigorously and filter . Perform the following test on the filtrate:

1) Dragendorff's test few drops of Dragendroff's reagent were added to the 2 ml filtrate. Formation of orange brown ppt indicate presence of alkaloids.

2) Mayer's test:-A few drops of Mayer's reagent were added to the 2 ml filtrate. Formation of cream ppt indicate presence of alkaloids .

3) Hager's test:-A few drops of Hager's reagent were added to the 2 ml filtrate. Formation of yellow ppt indicate presence of alkaloids.

4) Wagner's test:-A few drops of Wagner's reagent were added to the 2 ml filtrate. Formation of reddish brown ppt indicates presence of alkaloids.

5) Murexide test for purine alkaloid:-Add 3–4 drops of concentrated HNO_3 to 3 ml test solution dry up through evaporation. After cooling, add 2 drops of NH_4OH. Alkaloids are present as evidenced by the purple colour seen.

G) Tests for Tannins and Phenolic Compounds:-

1) 5% $FeCL_3$ solution few drops of 5% $FeCL_3$ solution were added to the 2 ml extract. Formation of deep blue–black color indicate presence of tannins and phenolic compounds.

2) Lead acetate solution:-A few drops of lead acetate solution were added to the 2 ml extract. The formation of white ppt indicate presence of tannins and phenolic compounds.

3) Gelatin solution:-A few drops of gelatin solution were added to the 2 ml extract. The formation of white ppt indicates the presence of tannins and phenolic compounds.

4) Bromine water:-A few drops of bromine water solution were added to the 2 ml extract. Decoloration of bromine water indicate presence of tannins and phenolic compounds.

5) Acetic acid solution:-A few drops of acetic acid solution were added to the 2 ml extract. The formation of red color indicates the presence of tannins and phenolic compounds.

6) Potassium dichromate:-A few drops of potassium dichromate solution were added to the 2 ml extract. Formation of red ppt indicates the presence of tannins and phenolic compounds.[13]

❖ **Pharmacological screening:-**

In-vitro Anti-diabetic Activity by α – amylase inhibitory assay:-

Reagents preparation:-

1) 0.20 mM phosphate buffer:-Take a 1000ml container into it add 800 ml water, 3.569 g sodium phosphate dibasic heptahydrate and 0.922 g sodium phosphate monobasic monohydrate. Adjust the solution to pH 6.9 using HCL or NaOH. To make 1 L, gradually add distilled water.

2) 0.02M Sodium phosphate buffer:-Prepare two stock solution. For the first stock solution add 28.4 g sodium phosphate dibasic anhydrous and 85 g NaCL to 1 L distilled water and for the second stock solution add 27.6 gm sodium phosphate monobasic monohydrate and 85 g NaCL to 1L distilled water. Make a 1:10 dilution of each stock solution to get 0.02 M phosphate-buffering saline (0.85%). Titer diluted solution 1 to pH 6.9 using a pH meter by adding around 65ml of diluted solution 2.[18]

3) α – amylase:-0.5 g alpha –amylase add into 100 ml 0.20 mM phosphate buffer solution.

4) 1% starch solution:-Add 1 g starch into 100 ml 0.02 M sodium phosphate buffer solution.

5) Dinitrosalicylic acid color reagent:-50 ml of distilled water and 1gm of 3, 5-dinitrosalicylic acid are combined to create the preparation. 30 g of sodium potassium tartrate tetrahydrate should be added gradually. 20 ml of 2N NaOH should be added. Using distilled water, dilute to a final volume of 100 ml.[19]

6) Preparation of test solution:-Stock solution of various seed extracts of 1000µg/ml was prepared by using hydroalcoholic (6:4) as a solvent. From this stock solution 5 different concentrations of 200, 400, 600, 800, 1000 µg/ml were prepared.

7) Preparation of standard solution:-Acarbose is used as a standard. Stock solution of acarbose in hydroalcoholic was prepared. From this stock solution 5 different concentrations of 200, and 400,600,800,1000 µg/ml were prepared.

α – amylase inhibitory assay procedure:-500µl of a 0.20 mM phosphate buffer solution (pH 6.9) containing 0.5 mg/ml of alpha-amylase was combined with 500µl of test samples and the standard drug (200, 400, 600, 800, 1000 µg/ml). Then it was incubated for 10 min at

25°C.Following these, each tube add 500 µl of a 1% starch solution in a 0.02M sodium phosphate buffer (pH 6.9).Following that, the reaction mixture was incubated for 10 min at 25°C.Then add 1 ml of 3, 5 dinitro salicylic acid as a color reagent in each test tube. The test tube was then cooled at room temperature after 5 min of incubation in a boiling water bath.10 ml of distilled water were then added to the reaction mixture, and the reactions absorbance was calculated at 540 nm. By substituting vehicle for extract, control experiments were carried out with 100% enzyme activity. [20]

$$I\% = \frac{(Ac - As)}{Ac} \times 100$$

Where,

I = Percentage inhibition

Ac = Absorbance of the control

As = Absorbance of the sample. [34]

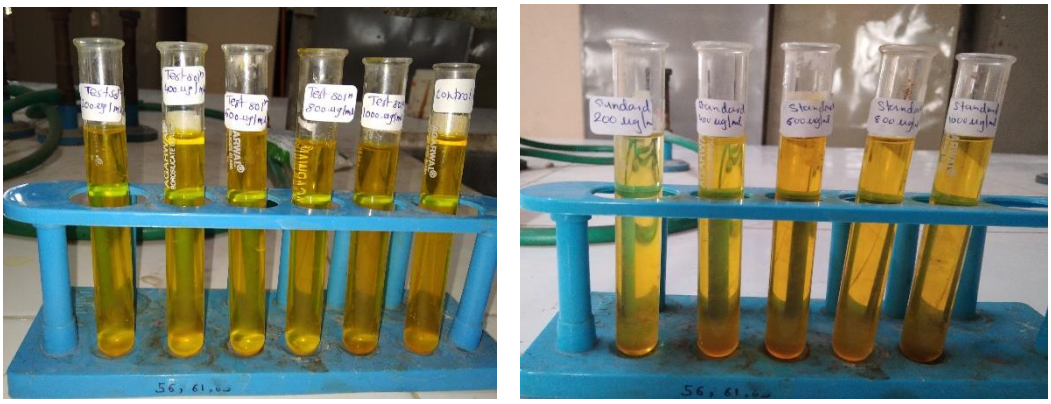

Figure No.1:- α – amylase inhibitory assay of Seed of *Sesamum indicum* Linn.

RESULT AND DISCUSSION

❖ Pharmacognostic Evaluation:-

I. **Macroscopic characters:-**The *Sesamum indicum* Linn. seeds colour is black ,taste is bitter, odour is oily without any characteristic smell,shape is oval, length is 2-3mm, width is 1-5mm, thickness 1mm, hilum at pointed end ridges are longitudinal.

II. **Microscopic characters:-**

a)Transverse section:- The transverse section of the seed has an oval shape and has an outer epidermis characterized by thin-walled being more or less wavy, cells contain spherical mass of crystals of calcium oxalate crystals. The testa is made up of collapsed cells with a yellowish membrane within. Cellulosic, polygonal parenchyma with fixed oil and tiny aleuronic grains make up the endosperm and cotyledons.

b) Powder character:-There are observed in different fragment of tissues with abundant oil-gloubles, epidermal cells with crystal, cotyledon cells with oil-gloubles and aleurone grains.

❖ **Physicochemical parameters :-**

The physicochemical parameters evaluated like moisture content was found to be 4.76%, ash values in that total ash value was found to be 26%, acid-insoluble ash value 13.5%, water-soluble ash value 33%, Extractive value in that water-soluble extractive value 26.4 %, alcohol soluble extractive value 27.2%.

❖ **Fluorescence Analysis:-**

Table No.4:- Fluorescence Analysis of seeds powder of *Sesamum indicum* Linn.

Sr. No.	**Reagent**	**Particulars of the treatment**	
		Under ordinary light (366nm)	**Under UV light**
1	Powder as such	Whitish grey	Grey
2	Powder + 1N NaOH (aqueous)	Black	Grey
3	Powder + 1N NaOH (alcoholic)	Whitish grey	Blueish
4	Powder + 1N HCL	Whitish grey	Grey
5	Powder + H_2SO_4(1:1)	Blackish brown	Violet
6	Powder + HNO_3	Brown	Dark grey
7	Powder + Ammonia	Blueish black	Blueish grey
8	Powder + Iodine	Yellow	Grey
9	Powder + 5% $FeCL_3$	Brown	Blue
10	Powder + Acetic acid	Whitish brown	Grey

❖ **Extraction:-**

The percentage yield of hydroalcoholic extract of seeds of *Sesamum indicum* Linn.

$$\% \text{ yield of Seeds} = \frac{2_{gm}}{50_{gm}} \times 100$$

= 4%

❖ **Phytochemical Investigation:-**

Table No.5:- Qualitative test result of hydroalcoholic extract of seed of *Sesamum indicum* L.plant.

Chemical constituents	**Test**	**Extract**
Carbohydrate	Molish's	+
	Fehling's	+
	Benedict's	-
	Barfoed's	-
Proteins	Biuret	-
	Million's	-
	Xanthoproteic	-
Steroid	Salkowski	+
Glycosides	• Test For cardiac Glycosides	
	Balijet's	++
	Legal's	++
	Deoxysugars	++
	• Test For Anthraquinone Glycoside	
	Borntrager's	++
	Modified Borntrager's	++
	• Test for Saponin Glycosides	
	Foam	++
Flavonoids	Shinoda	++
	Lead acetate	++
Alkaloids	Dradendroff's	+++
	Mayer's	+++
	Hager's	+++
	Wagner's	+++
	Murexide for purine alkaloid	+++
Tannin and Phenolic compounds	5%$FeCl_3$	-
	Lead acetate solution	+
	Gelatin	-
	Borwine water	-
	Acetic acid	-
	Potassium dichromate	+

(-Absent, + Present)

❖ **Pharmacological Screening: -**

***In-vitro* Anti-diabetic Activity by α – amylase inhibitory assay**:-

Table No.6:- Percentage inhibition for α-amylase inhibitory assay of hydroalcoholic extract of seeds of *Sesamum indicum* Linn.

Sample	Concentration	Absorbance (Mean ± SEM)	% Inhibition
Standard (Acarbose 30 mg/ml)	200 µg/ml	0.0249 ± 0.00203*	55.45%
	400 µg/ml	0.0402 ± 0.001827*	57.44%
	600 µg/ml	0.0564 ± 0.002195***	60 .78%
	800 µg/ml	0.0722 ± 0.001652***	64.35%
	1000µg/ml	0.0838 ± 0.00193***	67 .56%
Test (Aq-Alc extacts of seeds of *Sesamum indicum* Linn.)	200 µg/ml	0.0195 ± 0.001136*	51.33%
	400 µg/ml	0.0330 ± 0.001943*	53.83%
	600 µg/ml	0.0475 ± 0.001927**	55.45%
	800 µg/ml	0.0666 ± 0.000664**	56.67%
	1000µg/ml	0.0777 ± 0.00155	59.69%

Date was analyzed using Graph Pad Prism 8.0.1 for windows as Mean ±SEM (n=3) Disease control Vs Treatment group (*p<0.05, *p<0.01,**p<0.001)

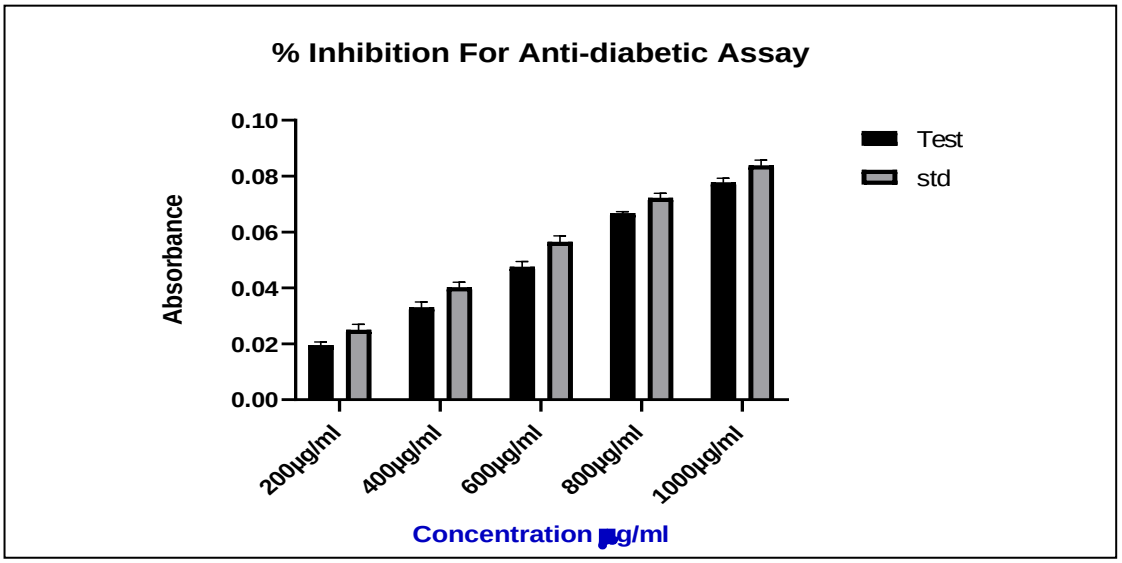

Figure No.2:-Percentage inhibition by α – amylase inhibitory assay of hydroalcoholic extract of Sesamum indicumL

CONCLUSION

One of the most significant medicinal plants with therapeutic characteristics is *Sesamum indicum* L., which may be found across India. Because recent research on medicinal plants has garnered a lot of attention, it is crucial to take into account the therapeutic benefit of this phytochemical study of *Sesamum indicum* L. extract by employing a hydroalcoholic solution plant. The research was based on the examination of phytochemicals and in-vitro activity of -amylase. Alkaloids, flavonoids, and glycosides were identified as bioactive compounds by the qualitative analysis. The presence of many bioactive components has justified the use of *Sesamum indicum* L. seeds extract by traditional physicians for a variety of ailments. The research also supports the efficacy of *Sesamum indicum* L. seeds to treat numerous chronic illnesses, including diabetes. Utilizing the hydroalcoholic extract of *Sesamum indicum* L. seed, the experiment was also conducted to test the in-vitro activity of -amylase. The outcome indicated that this plant's hydroalcoholic extract strongly inhibits -amylase. Further research is required to determine the precise chemical mechanism through which *Sesamum indicum* L. seeds exert their anti-diabetic effects.

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