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In-vitro Assessment of *Corchorus olitorius* in Inhibiting Antioxidant Activity and Enzyme Inhibition

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Abstract

Objective: The aim of the current study was to assess the effect of *Corchorus Olitorius* on Diabetes Mellitus induced by Streptozotocin (STZ) in experimental rats and how Corchorus *Olitorius* helps to mitigate the effects.

Methods: The leafs of *Corchorus Olitorius* was extracted using hot extraction method by using solvent like n-hexane, ethyl acetate, methanol and phytochemical screening was carried out. Wistar Albino rats of weight 120-130 grams were divided into 6 groups- normal, control, standard, treatment I, treatment II, treatment III with various dose and each group containing 5 animals. In-vitro screening methods were carried out to evaluate the various properties of Corchorus *Olitorius.*

Results: Methanolic extract of *C.olitorius* shows maximum inhibition rather than ethyl acetate and n-hexane extract of *C.olitorius.*

Conclusion: Collection, authentication & identification of *C.olitorius* was done and also successive extraction, phytochemical screening and in-vitro studies like DPPH, Alpha glucosidase activity and alpha amylase activity.

Key words: Antioxidant Activity, Enzyme Inhibition, in-vitro study, DPPH, Alpha glucosidase activity, alpha amylase activity, Streptozotocin etc.

Introduction:

The verdant vegetable *Corchorus olitorius*, which has a place to the Malvaceae family, is inborn to tropical and subtropical districts of Asia and Africa. Its consumable clears out, which are a wealthy source of vitamins, minerals, and dietary fiber, are the reason it is so commonly planted. In expansion to its wholesome substance, *Corchorus olitorius* has long been utilized in conventional medication for its charged wellbeing preferences.

Corchorus olitorius is credited for a number of therapeutic purposes within the writing survey. Its takes off have allegedly been utilized to remedy tumors, unremitting cystitis, gonorrhea, and inconvenience. The diuretic, demulcent, tonic, and gently febrifuge properties of the dried youthful clears out are utilized in an mixture to move forward craving and quality. Due to the nearness of noteworthy levels of dynamic cardiac standards, especially Olitoriside, which had an impact comparable to strophanthin in unremitting cardiac patients, its seeds are laxative and have been detailed to show estrogenic movement as well as being valuable in heart ailments. Jute fiber is generally delivered by the stem.

Additionally, it could be a component in creams for the hands, confront, and hair, as well as moisturizers. Also, the pharmacological properties of *Corchorus olitorius* incorporate anti-inflammatory, pain relieving, anti-tumor, hypoglycemic, antibacterial, anti-inflammatory, anti-obesity, gastroprotective, and wound mending activities. Encourage, more exhaustive phytochemical investigation uncovered the nearness of cardiac glycosides, alkaloids, flavonoids, tannins, etc. Phytochemicals are the non-nutritive parts of these substances. A vital organize in any shape of investigate on restorative plants is the subjective examination as well as the evaluation of the phytochemicals display.

Materials & Methods:

Plant Material: Whole plant of *Corchorus olitorius* **(Jute)** family is *Malavaceae* were collected from Goalpara distict of Assam.

Fig 1: Collection of *Corchorus olitorius*

Extraction of the compound:

About 3Kg of fresh plant were collected.After drying and grinding into coarsely powdered crude drug, it has been found that the total weight of the content is 800 g. Out of 800 g of crude drug, 40 g of the crude powdered drug per batch wise has been extracted by using the hot extraction method, where a thimble packing of the crude powdered drug was loaded into the Soxhlet chamber and extracted successively by using
hexane as first solvent (non-polar). After completion of the extraction cycle, the exhausted drug was taken out of the thimble and allowed to dry. In the second batch another 40g of crude drug was extracted by using hexane solvent and this is continued till the complete extraction of 800 g of the crude powdered drug. Further the next successive solvent were done separately for ethyl acetate (midpolar) & for methanol (polar) with another 800 gm of

each sample. Once the process was finished, hexane was evaporated from the crude extract using a water bath. After evaporation through water bath, the final concentration was lyophilized using lyophilizer & percentage yield were calculated by as follows.

$$\text{Percentage Yield: } \frac{\textit{Weight of the crude drug}}{\textit{Weight of the plant material}} \text{ X } 100$$

Fig 2: Extraction of *C.olitorius*

Phytochemical Screening:

To identify the chemical constituents of the aqueous plant extract of *Corchorus Olitorius* standard procedures are followed. Freshly prepared crude aqueous plant extracts of *Corchorus Olitorius.*

The qualitative tests for phytochemicals were performed according to several previously published standard protocols .10 gm of powdered material was mixed in 100 ml of distilled water and the mixture was placed in magnetic stirrer for 10 h. The mixture was filtered through Whitman filter paper No. 1 and the filtrate was used for the following phytochemical tests.

Alkaloids: A volume of 1cm^3 of 1% HCl was added to 3 cm^3 of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and then filtered. The filtrate was used as follows.

Test for tannins: About 10 ml of the aqueous extract was mixed with a few drops of 0.1% ferric chloride solution. Formation of a brownish-green or a blue-black precipitation indicated the presence of tannin. And tannin is present

Test for phlobatannins: 2 ml of concentrated HCl was added to 10 ml of aqueous extract and boiled for 2 min. Formation of red precipitate indicated the presence of phlobatannins.And pholbotanin is present.

Test for carbohydrates: 2 ml of aqueous extract was mixed with Molish's reagent (5% α-napthol in absolute alcohol) and shaken vigorously. Then 2 ml of concentrated H_2SO_4 was added carefully along the wall of the test tube. Formation of the reddish-violate ring at the junction of the liquid indicated the presence of carbohydrates.It is absent.

Test for proteins: 2 ml of aqueous extract was mixed with 1 ml of 40% NaOH. Then 1-2 drop of 2% $CuSO_4$ added to the solution. Change in the colouration of the solution into violet indicated the presence of peptide linkage in a solution which in turn is an indication of the presence of protein. 10 gm of powdered sample was taken in 250 ml conical flask and mixed with 100 ml of 70% methanol and stirred in a magnetic stirrer for 10 h at room temperature. The mixture was then filtered through Whatman filter paper No. 1 and the filtrate was used for the following phytochemical tests. Absent of protein.

Test for terpenoids: 5 ml of methanolic leaf extract was added with 2 ml of chloroform and then 3 ml of concentrated H_2SO_4 was added carefully along the wall of the test tube to form

a layer. Formation of reddish-brown colouration at the junction of two liquid indicated the presence of terpenoids. It is absent.

Test for cardiac glycosides: 5 ml of methanolic extracts was mixed with 2 ml of glacial acetic acid containing one drop of 2% ferric chloride solution. Then 1 ml of concentrated H2SO4 was added carefully along the wall of the test tube. Formation of a brown ring at the interface of the two liquids indicated the presence of glycosides.Presents of glycosides.

Test for steroids: 5 ml of sample extract was added with 0.5 ml anhydrous CH3COOH and cooled in ice bath for 15 min. After that 0.5 ml of chloroform and 1 ml of concentrated H2SO4 were added to the solution carefully along the wall of the test tube. Presence of a reddish-brown ring at the separation level of two liquids indicated the presence of steroids.

Test for cholesterol: 2 ml of sample extract was added with 2 ml of chloroform. Then 10-12 drops of anhydrous CH3COOH was added and the mixture was shaken well. After that, 2 drops of concentrated H2SO4 was added to the solution. The change of reddish-brown color into the blue-green confirmed the presence of cholesterol. Present of cholesterol.

Test for alkaloids: 2 ml of sample solution was taken into a test tube and 2 ml of 2N HCl was added to it. Then the test tube was shaken vigorously to mix that solution and kept in room temperature for 5 min. The aqueous phase was separated from the two liquid phases and then few drops of Mayer's reagent (HgCl2+KI in water) was added to it and shaken. Formation of creamy coloured precipitation indicated the presence of alkaloids.

Test for phenol: 10 ml of the sample was added with 4-5 drops of 2% FeCl3 solution. changes in colour indicate the presence of phenol.

Test for flavonoids: 2 gm of powdered material was added to10 ml of ethyl acetate and heated in a water bath for 5 min. After cooling, the solution was filtered and the filtrate was mixed with to 4 ml of liquid ammonia solution (10%) and shaken vigorously. The appearance of the yellow colouration indicated the presence of flavonoids. Flavanoids are present.For further confirmation Shinoda test & alkaline reagent test were been performed.

Shinoda Test:

The test solution of the extracts was dissolved in 95% ethanol. To this, a small piece of magnesium foil metal was added; this was followed by 3-5 drops of the concentrated HCl. The intense cherry red color indicated the presence of flavonoids.

Alkaline reagent test: Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Test for anthraquinone: 0.5 gm of powdered material was shaken with 20 ml of benzene in a magnetic stirrer for 4 h. at room temperature. The mixture was then filtered and 10 ml of the filtrate was mixed with 0.5 ml of 10% ammonia solution and shaken well. The presence of violet colour at the layer phase indicated the presence of anthraquinones.

Test for saponin: 0.5 gm of powdered material was added with 15 ml double distilled water and shaken well. Formation of intensive and persistent froth indicated the presence of saponin .

Table 1 : Phytochemical Presence of *Corchorus olitorius* solvent Extract

Tests	Hexane	Ethyl Acetate	Methanol	Methano
Alkaloids	-	-	+	
Glycosides	-	+	+	
Tannins	+	-	-	
Phenols	+	+	+	
Proteins and Amino acids	-	-	+	

Carbohydrates	-	-	+
Diterpenes	-	-	+
Phytosterols	-	-	+
Flavonoids	+	+	+
Cholesterol	-	-	+

+ indicates presence of the compound,-indicates absence of the compound

In-vitro Screening Methods:

DPPH methods:

Principle:

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical compound widely used in antioxidant assays to determine the antioxidant activity of various substances, including drugs, natural compounds, and food extracts. It is a purple-colored compound that undergoes a color change when it reacts with an antioxidant. The DPPH radical scavenging assay is a simple and commonly employed method to evaluate the ability of a substance to neutralize free radicals and thus determine its antioxidant capacity.

The principle behind the DPPH radical scavenging assay is based on the fact that DPPH is a stable free radical with an unpaired electron, making it highly reactive. However, when it reacts with an antioxidant, the antioxidant donates an electron to the DPPH radical, neutralizing it and causing the purple color to fade. The reaction can be monitored spectrophotometrically by measuring the decrease in absorbance at a specific wavelength, typically **517 nm**, which is associated with the purple color of the DPPH radical.

Procedure:

1. Preparation of DPPH solution: (4mg) DPPH is dissolved in a solvent like (100ml of 99%) methanol to create a purple-colored solution. The concentration of DPPH in the solution is usually known.
2. Preparation of sample solutions: The substance to be tested for antioxidant activity, such as a drug or a natural extract (1mg), is dissolved in a suitable solvent like (25 ml of 99% methanol) to make different concentrations of the sample solution.
3. Reaction between DPPH and the sample: Small volumes of the sample solutions are mixed with the DPPH solution, and the reaction is allowed to occur in the dark for a specific period, usually around 30 minutes.
4. Measurement of absorbance: After the reaction time, the absorbance of each reaction mixture is measured using a spectrophotometer at the wavelength of 517 nm.
5. Calculation of antioxidant activity: The antioxidant activity is determined by comparing the absorbance of the sample solutions with that of a control containing only DPPH without any antioxidant. The percentage of DPPH radical scavenging activity is calculated using the formula:

Antioxidant activity (%) = [(Abs_control - Abs_sample) / Abs_control] × 100

Abs_control is the absorbance of the control (DPPH without any sample), and Abs_sample is the absorbance of the sample with DPPH.

A higher percentage of DPPH radical scavenging activity indicates a stronger antioxidant capacity of the substance being tested. It means that the substance has the ability to neutralize free radicals and protect cells from oxidative damage. Oxidative stress caused by free radicals is associated with various health issues, including aging, inflammation, and chronic diseases, so substances with high antioxidant activity are considered beneficial for health.

In summary, the DPPH radical scavenging assay is a valuable tool to evaluate the antioxidant properties of drugs and other substances. By measuring the absorbance of the reaction mixture, we can quantify the ability of a substance to scavenge free radicals and protect cells from oxidative damage, providing valuable insights into its potential health benefits.

Results & Discussion for DPPH method:

Absorbance Measurement Data:

Table : 2 : DPPH free radical scavenging activity (Methanolic Extract)

Concentration ($\mu g/ml$)	**Absorbance at 517 nm**		**% RSA**
	Control **(DPPH in Methanol)**	Sample **(Methanolic Extract)**	
50	0.52	0.313	**39.80**
100	1.04	0.692	**42.30**
150	1.54	0.852	**44.80**
200	2.01	1.103	**45.12**
250	2.50	1.301	**47.96**

Table: 3:DPPH free radical scavenging activity (Ethyl Acetate Extract)

Concentration ($\mu g/ml$)	**Absorbance at 517 nm**		**% RSA**
	Control (DPPH in Ethyl Acetate)	**Sample (Ethyl Acetate Extract)**	
50	0.42	0.29	**30.95**
100	0.84	0.55	**34.52**
150	1.20	0.76	**36.66**

200	1.59	0.99	**37.73**
250	2.13	1.25	**41.31**

Table: 4 :DPPH free radical scavenging activity (n- Hexane Extract)

Concentration ($\mu g/ml$)	**Absorbance at 517 nm**		**% RSA**
	Control (DPPH in n-Hexane)	**Sample (n-Hexane Extract**	
50	0.35	0.25	**28.57**
100	0.71	0.50	**29.57**
150	1.01	0.70	**30.69**
200	1.35	0.91	**32.59**
250	1.56	1.19	**33.93**

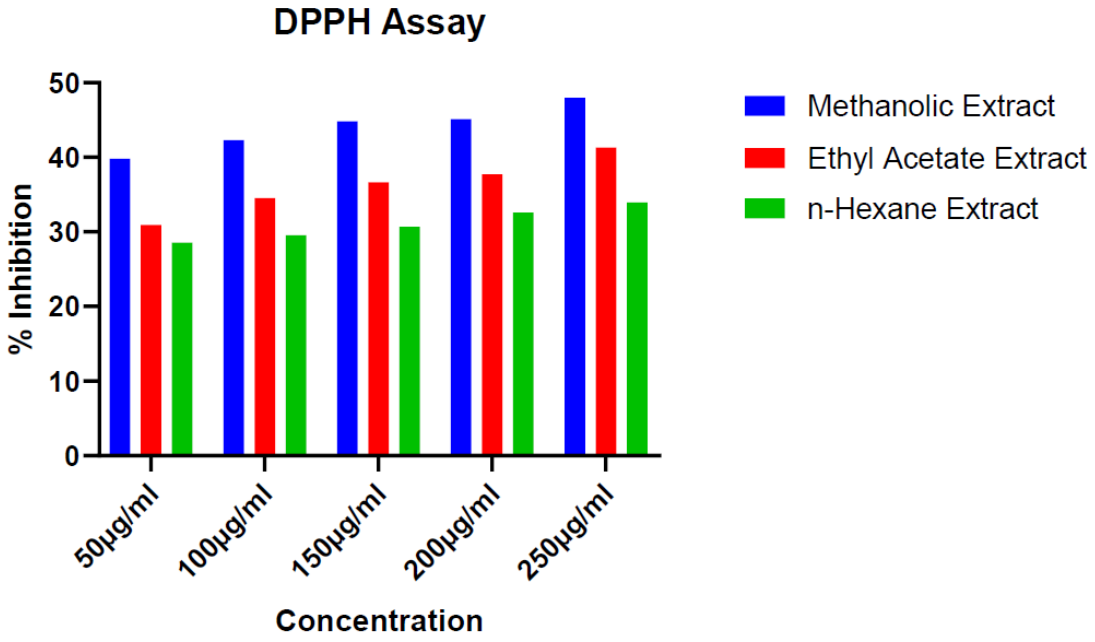

Fig. 3: Represents bar chart showing comparisons of three extracts

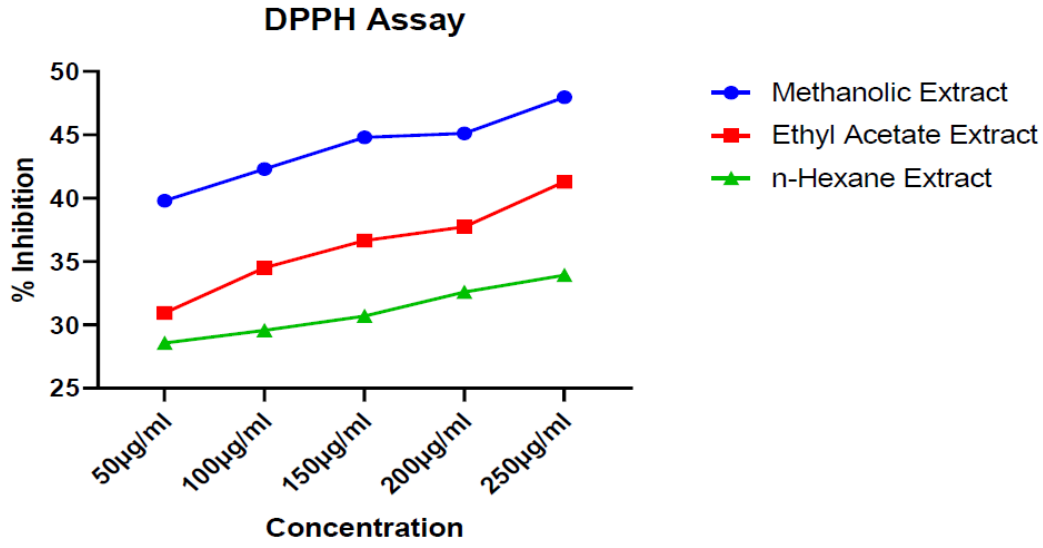

Fig. 4: Represents linear charts of %inhibition Vs Concentration

Determination of alpha amylase inhibitor activity :

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the whole plant extracts of C. Olitorius in concentration range 20-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts. The percentage inhibition calculated by as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{540}(\text{Control}) - \text{Abs}_{540}(\text{Extract}) * 100}{\text{Abs}_{540}(\text{Control})}$$

Table 5: The percentage inhibition of alpha amylase by methanol ,ethyl acetate & n-hexane extracts of *C. Olitorius*

Concentration (µg/ml)	% Inhibition by *C. Olitorius* (methanol)	% Inhibition by *C. Olitorius* (ethyl acetate)	% Inhibition by *C. Olitorius* (n-hexane)
50	45.45	12.5	10
100	45.94	25.00	15
150	46.87	33.33	20
200	52.27	37.50	25
250	57.69	40.00	28

Table 6: The percentage inhibition of alpha amylase by Ethyl Acetate extract of *C. Olitorius*

Concentration ($\mu g/ml$)	Control (Without extract)	Sample (Ethyl Acetate Extract)	% Inhibition by *C. Olitorius* (Ethyl Acetate Extract)
50	0.40	0.35	**12.5**
100	0.80	0.60	**25.00**
150	1.20	0.80	**33.33**
200	1.60	1.00	**37.50**
250	2.00	1.20	**40.00**

Table 7: The percentage inhibition of alpha amylase by methanol extracts of *C. Olitorius*

Concentration ($\mu g/ml$)	**Control (Without extract)**	**Sample (Methanolic Extract)**	**% Inhibition by *C. Olitorius* (methanol)**
50	0.55	0.30	**45.45**
100	1.11	0.60	**45.94**
150	1.60	0.85	**46.87**
200	2.20	1.05	**52.27**
250	2.60	1.10	**57.69**

Table 8: The percentage inhibition of alpha amylase by n-hexane extracts of *C. Olitorius*

Concentration ($\mu g/ml$)	**Control (Without extract)**	Sample **(n-hexane)**	**% Inhibition by *C. Olitorius* (n-hexane)**
50	0.50	0.45	**10**
100	1.00	0.85	**15**
150	1.50	1.20	**20**
200	2.00	1.50	**25**
250	2.50	1.80	**28**

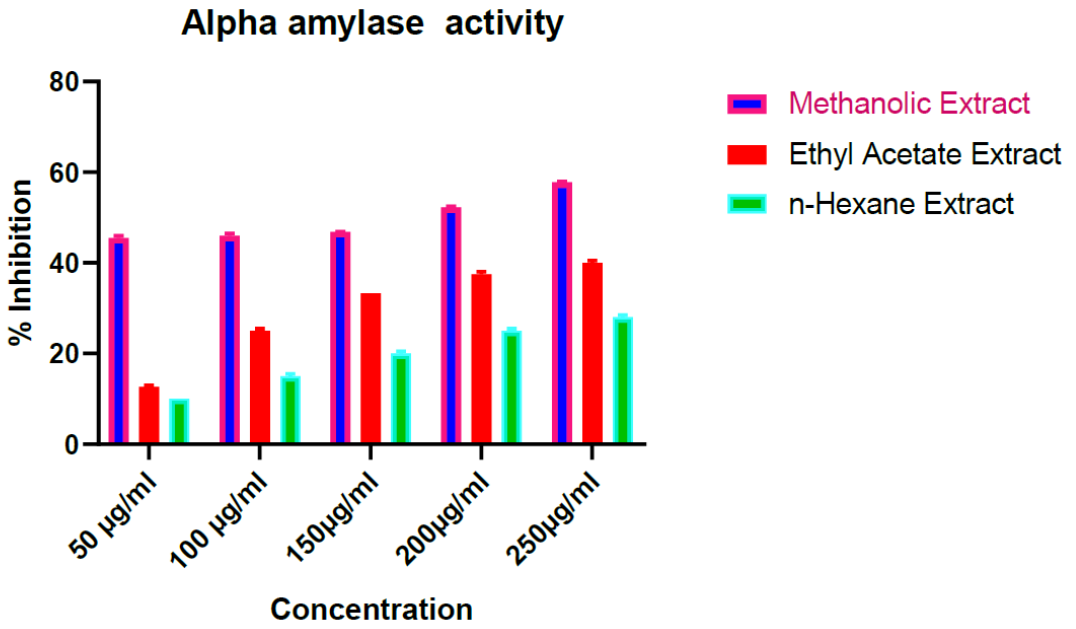

Fig. 5: Represents bar chart showing comparisons of three extracts

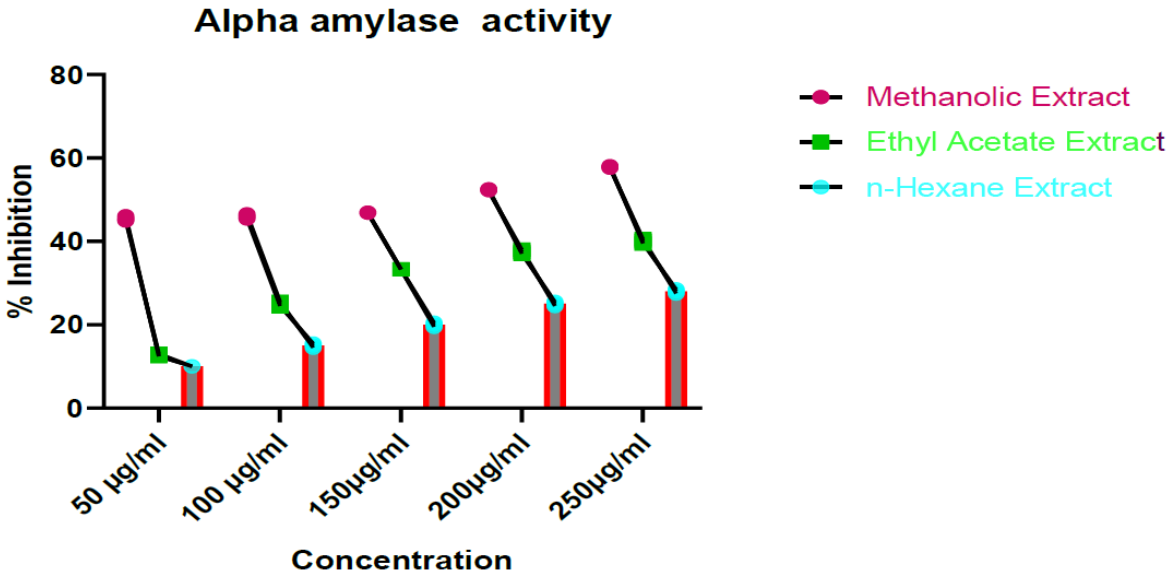

Fig. 6: Represents linear charts of %inhibition Vs Concentration

Determination of alpha glucosidase inhibitor activity:

The alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts were used in the concentration ranging from 20-100 µg/ml. Different concentrations of plant extracts were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 minutes.40µl of p-NPG was added to the mixture & incubate for another 15 minutes and the absorbance was read at 410 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula.

$$\textbf{Inhibition (\%)} = \frac{\text{Abs}_{410}(\text{Control})-\text{Abs}_{410}(\text{Extract})*100}{\text{Abs}_{410}(\text{Control})}$$

Table 9: The percent inhibition of alpha glucosidase by methanolic, ethyl acetate & n-hexane extracts of *C. Olitorius.*

Abs 410 (Control) **Concentration (µg/ml)**	**% Inhibition by *C. Olitorius* (methanol)**	**% Inhibition by *C. Olitorius* (ethyl acetate)**	**% Inhibition by *C. Olitorius* (n-hexane)**
50	30.00	10	**4**
100	35.00	15	**10**
150	40.00	33.33	**13.33**
200	47.50	35	**15**
250	55.76	40	**20**

Table 10: The percent inhibition of alpha glucosidase by methanolic extracts of *C. Olitorius*

Concentration ($\mu g/ml$)	**Control (Without extract)**	**Sample (Methanolic Extract)**	**% Inhibition by *C. Olitorius* (methanol)**
50	0.50	0.35	**30.00**
100	1.00	0.65	**35.00**
150	1.50	0.90	**40.00**
200	2.00	1.05	**47.50**
250	2.60	1.15	**55.76**

Table 11: The percent inhibition of alpha glucosidase by ethyl acetate extracts of

C. Olitorius

Concentration ($\mu g/ml$)	**Control (Without extract)**	Sample **(Ethyl acetate Extract)**	**% Inhibition by *C. Olitorius* (Ethyl acetate Extract)**
50	0.50	0.45	**10**
100	1.00	0.85	**15**
150	1.50	1.00	**33.33**
200	2.00	1.30	**35**
250	2.50	1.50	**40**

Table 12: The percent inhibition of alpha glucosidase by n-hexane extracts of *C. Olitorius*

Concentration ($\mu g/ml$)	**Control (Without extract)**	Sample **(n-hexane Extract)**	**% Inhibition by *C. Olitorius* (n-hexane Extract)**
50	0.50	0.48	**4**
100	1.00	0.90	**10**
150	1.50	1.30	**13.33**
200	2.00	1.70	**15**
250	2.50	2.00	**20**

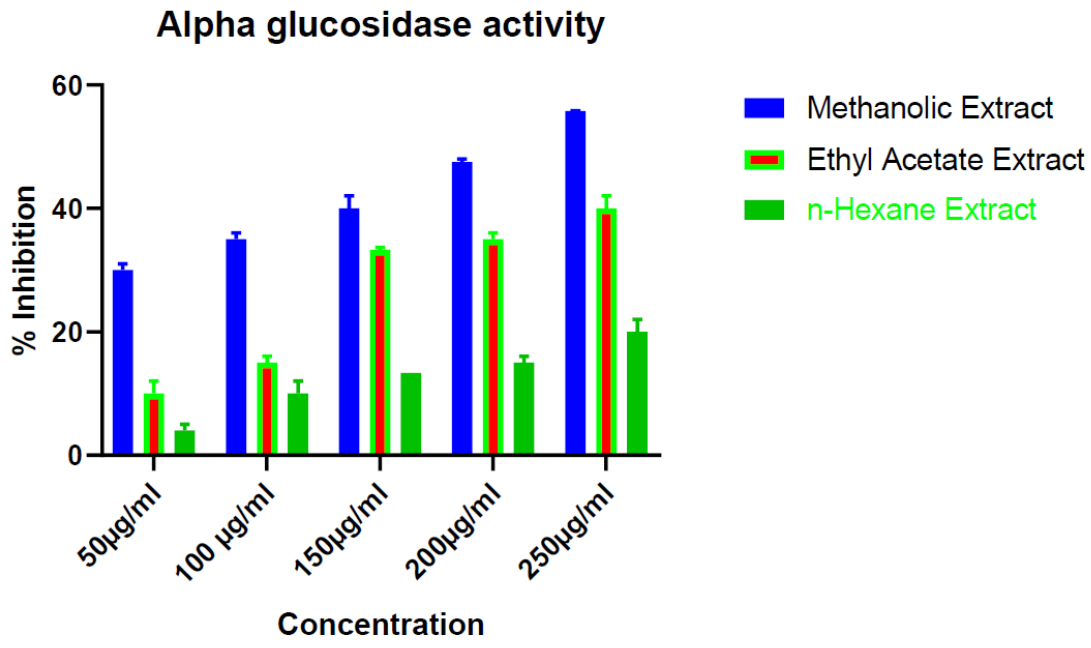

Fig. 7: Represents bar chart showing comparisons of three extracts

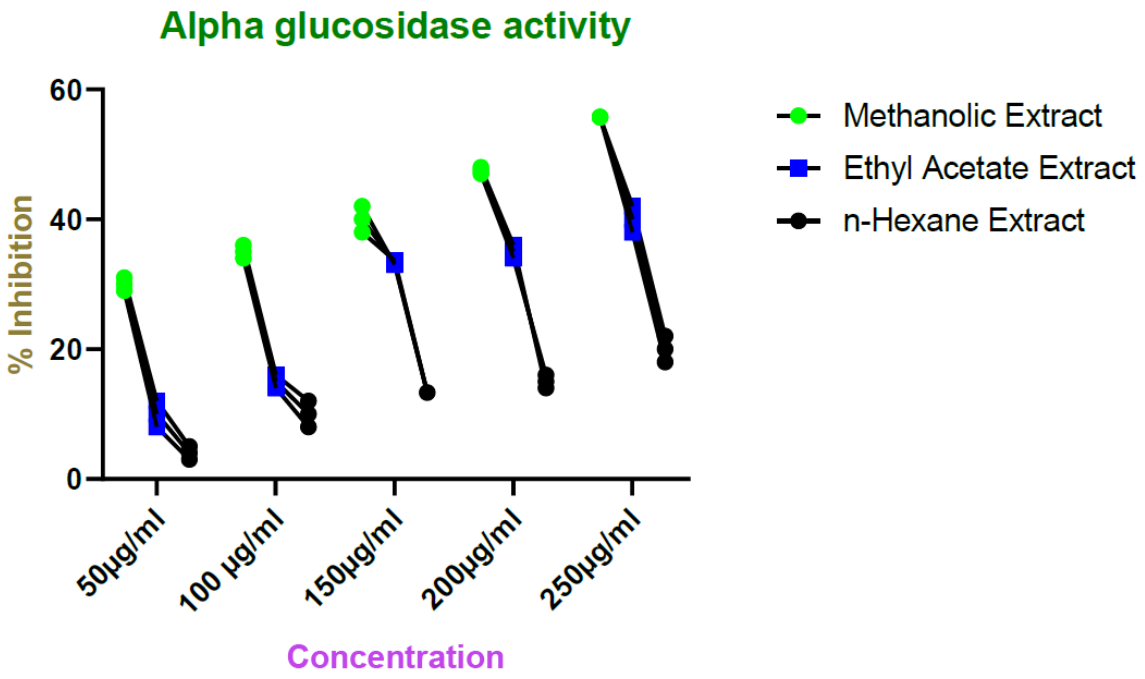

Fig. 8: Represents linear charts of %inhibition Vs Concentration

Results and Conclusion:

The methanolic extract of *C. olitorius* exhibited the greatest inhibition activity, outperforming the ethyl acetate and n-hexane extracts. This suggests that the methanolic extract contains compounds with higher inhibitory potential, making it more effective in the tested assays. Methanolic, ethyl acetate, and n-hexane extracts of *C. olitorius* were prepared through successive extraction. Identified the presence of various bioactive compounds. Evaluated antioxidant activity confirming by using DPPH method. Examined potential for carbohydrate digestion inhibition using a method of alpha amylase and alpha glucosidase activity. Indicated that the percentage inhibition increases with the concentration of the extracts. Displayed the lowest inhibition among the three Extract compounds using different solvents and identify the phytochemicals present. Evaluate the inhibitory effects of the extracts on various enzymes. This summary captures your findings and organizes the information clearly, highlighting the effectiveness of different extracts and the methods used in the study.

REFERENCE:

1. Tripathi, KD (2003).Essential of medical pharmacology.Jaypee brothers medical publishers(p) ltd. New delhi: India
2. Wadker, K A., Magdum, C S., Patit, S S., Naikwade, N S., (2008). Anti-diabetic potential and Indian medicinal plants. Journal of herbal medicine & toxicology, 2, 45-50.
3. Wang, T., Li, X., Zhou, B., Li, H., Zeng, J., & Gao, W. (2015). Anti-diabetic activity in type 2 diabetic mice and α-glucosidase inhibitory, antioxidant and anti-inflammatory

potential of chemically profiled pear peel and pulp extracts (Pyrus spp.). Journal of Functional Foods, 13, 276–288.
4. Petite S. E. (2018). Noninsulin medication therapy for hospitalized patients with diabetes mellitus. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists, 75(18), 1361–1368.
5. Li, X., Liu, J., Chang, Q., Zhou, Z., Han, R., & Liang, Z. (2021). Antioxidant and Antidiabetic Activity of Proanthocyanidins from Fagopyrum dibotrys. Molecules (Basel, Switzerland), 26(9), 2417.
6. Lauritano, C., & Ianora, A. (2016). Marine Organisms with Anti-Diabetes Properties. Marine drugs, 14(12), 220.
7. Saliu, JA., Ademiluyi, AO., Boligon, AA., Oboh, G., Schetinger, MR., Rocha, JB.(2019). Dietary supplementation of jute leaf (Corchorus olitorius) modulates hepatic delta-aminolevulinic acid dehydratase (δ-ALAD) activity and oxidative status in high-fat fed/low streptozotocin-induced diabetic rats. Journal of food biochemistry. 43(8), 12949

8. Kimani, C N., Mbariaj,M., Suleiman, M., Gakuya, D., Kiamas,G. (2015).Anti hyperglycemic activity of zanthoxylumchalybeum stem bark extract in diabetic rats. The journal of phytopharmacology, 4(3). 183-189.
9. Marreel, A., Hercinger, M. (2015) Implementation of a Quality Improvement Project to Improve Provider Adherence to the American Diabetic Association Standards of Care.
10. Joshi, SR., Parikhrm, (2007). India - diabetes capital of the world: now heading towards hypertension. J Assoc Physicians India, 55.323–334
11. Whiting, D. R., Guariguata, L., Weil, C., & Shaw, J. (2011). IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Research and Clinical Practice, 94(3), 311–321
12.Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA, 285(19), 2486–2497.
13. Satyanarayana, T., Dwarakanadhareddy, P., Swarnalatha, D., Anjana, A. Mathews. (2010).Hypoglycemic effect of a poly herbal extract on normal and streptozotocin induced diabetic rats. International journal of pharmacy and pharmaceutical sciences. 2(3), 56-57.
14. Valerón, P. F., & de Pablos-Velasco, P. L. (2013). Limitaciones de los fármacos dependientes de insulina para el tratamiento de la diabetes mellitus tipo 2 [Limitations of insulin-dependent drugs in the treatment of type 2 diabetes mellitus]. Medicina clinica, 141 Suppl 2, 20–25.
15.Olaokun, O. O., McGaw, L. J., Eloff, J. N., & Naidoo, V. (2013). Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African Ficus species (Moraceae) used traditionally to treat diabetes. BMC Complementary and Alternative Medicine, 13(1)
16. Tofighi, Z., Alipour, F., Hadavinia, H., Abdollahi, M., Hadjiakhoondi, A., & Yassa, N. (2014). Effective antidiabetic and antioxidant fractions of Otostegia persica extract and their constituents. Pharmaceutical biology, 52(8), 961–966.
17. Oztay, F., Sacan, O., Kayalar, O., Bolkent, S., Ipci, Y., Kabasakal, L., Sener, G., & Yanardag, R. (2015). Chard (Beta vulgaris var. cicla) extract improved hyperglycemia-induced oxidative stress and surfactant-associated protein alterations in rat lungs. Pharmaceutical biology, 53(11), 1639–1646.
18. Hu, X., Cheng, D., & Zhang, Z. (2016). Antidiabetic activity of Helicteres angustifolia root. Pharmaceutical biology, 54(6), 938–944
19. Ezzat, S. M., Abdel Motaal, A., & El Awdan, S. A. W. (2017). In vitro and in vivo antidiabetic potential of extracts and a furostanol saponin from Balanites aegyptiaca. Pharmaceutical biology, 55(1), 1931–1936.
20. Blat, A., Halabi, M. F., Mohamad, J., Hasnan, M. H., Hazni, H., Teh, S. H., Shilpi, J. A., Mohamed, Z., & Awang, K. (2017). Antidiabetic effects of Brucea javanica seeds in type 2 diabetic rats. BMC complementary and alternative medicine, 17(1), 94.
21. Markham A. (2018). Ertugliflozin: First Global Approval. Drugs, 78(4), 513–519.
22.Venkateswaran, M. R., Jayabal, S., Murugesan, S., & Periyasamy, S. (2019). Identification of polyphenolic contents, in vitro evaluation of antioxidant and antidiabetic potentials of a polyherbal formulation-Mehani. Natural Product Research, 1–5.
23. ARAS, A., Bursal, E., Türkan, F., Tohma, H., Kılıç, Ö., Gülçin, İ., & Köksal, E. (2019). Phytochemical content, Antidiabetic, Anticholinergic, Antioxidant Activities of Endemic Lecokia cretica Extracts. Chemistry & Biodiversity,16(10),e1900341
24. Shivanagoudra, S. R., Perera, W. H., Perez, J. L., Athrey, G., Sun, Y., Wu, C. S., Jayaprakasha, G. K., & Patil, B. S. (2019). In vitro and in silico elucidation of antidiabetic

and anti-inflammatory activities of bioactive compounds from Momordica charantia L. Bioorganic & medicinal chemistry, 27(14), 3097–3109.
25. Tafesse, T. B., Hymete, A., Mekonnen, Y., & Tadesse, M. (2017). Antidiabetic activity and phytochemical screening of extracts of the leaves of Ajuga remota Benth on alloxan-induced diabetic mice. BMC complementary and alternative medicine, 17(1), 243.
26. Sadat, A., Hore, M., Chakraborty, K., & Roy, S. (2017). Phytochemical analysis and antioxidant activity of methanolic extract of leaves of corchorus olitorius. International Journal of Current Pharmaceutical Research, 59–63.
27. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental health perspectives, 109 Suppl 1(Suppl 1), 69–75.
28. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary, and alternative medicines : AJTCAM, 8(1), 1–10.
29. Airaodion, AI., Ogbuagu, EO., Ogbuagu, U., Awosanya, OO., Airaodion, EO.(2019). Hypoglycemic and hypolipidemic effect of methanolic extract of Corchorus olitorius leaves in Wistar rats. Asian Plant Research Journal,7(6),1-2.
30. Dewanjee, S., Sahu, R., Karmakar, S., & Gangopadhyay, M. (2013). Toxic effects of lead exposure in Wistar rats: involvement of oxidative stress and the beneficial role of edible jute (Corchorus olitorius) leaves. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 55, 78–91.
31. Jonsson, M., Jestoi, M., Nathanail, A. V., Kokkonen, U. M., Anttila, M., Koivisto, P., Karhunen, P., & Peltonen, K. (2013). Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 53, 27–32.
32. Vivek, KS.,(2010). Streptozotocin: an experimental tool in diabetes and alzheimer's disease (A-Review). Int J Pharma Res Dev,2(1),1-7.
33.Njan, A. A., Adenuga, F. O., Ajayi, A. M., Sotunde, O., Ologe, M. O., Olaoye, S. O., Erdogan, O. N., & Iwalewa, O. E. (2020). Neuroprotective and memory-enhancing effects of methanolic leaf extract of Peristrophe bicalyculata in rat model of type 2 diabetes mellitus. Heliyon, 6(5), e04011.
34. Ambade, V. N., Sharma, Y. V., & Somani, B. L. (1998). Methods for estimation of blood glucose : a comparative evaluation. Medical journal, Armed Forces India, 54(2), 131–133.
35. Singh, A. K., Rana, H. K., Singh, V., Chand Yadav, T., Varadwaj, P., & Pandey, A. K. (2021). Evaluation of antidiabetic activity of dietary phenolic compound chlorogenic acid in streptozotocin induced diabetic rats: Molecular docking, molecular dynamics, in silico toxicity, in vitro and in vivo studies. Computers in biology and medicine, 134, 104462.
36. Singh, A. K., Rana, H. K., Singh, V., Chand Yadav, T., Varadwaj, P., & Pandey, A. K. (2021). Evaluation of antidiabetic activity of dietary phenolic compound chlorogenic acid in streptozotocin induced diabetic rats: Molecular docking, molecular dynamics, in silico toxicity, in vitro and in vivo studies. Computers in biology and medicine, 134, 104462.
37. El-Sayed M. I. (2011). Effects of Portulaca oleracea L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. Journal of ethnopharmacology, 137(1), 643–651.
38. Toora, B. D., & Rajagopal, G. (2002). Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. Indian journal of experimental biology, 40(3), 352–354.

39. Shaukat, A., Hussain, K., Bukhari, NI., Shehzadi, N. (2020). Acid Uric 3× Tablet: Standardization and pharmacological evidence of uric acid use as anti-gout medicine. Journal of Pharmacy & Pharmacognosy Research, 8(6), 501-14.
40. Quillfeldt, J. A. (2016). Behavioral Methods to Study Learning and Memory in Rats. Rodent Model as Tools in Ethical Biomedical Research, 271–311. doi:10.1007/978-3-319-11578-8_17
41. Gould, TD., Dao, DT., Kovacsics, CE.(2009). The open field test. Mood and anxiety related phenotypes in mice,1-20
42. Grandin, T., & Deesing, M. J. (2014). Genetics and Behavior During Handling, Restraint, and Herding. Genetics and the Behavior of Domestic Animals, 115–158.
43. Sakat, S., Juvekar, A. R., & Gambhire, M. N. (2010). In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. *Int J Pharm Pharm Sci*, *2*(1), 146-155.
44. Khan, M., Sohail, Raja, N. I., Asad, M. J., & Mashwani, Z. U. R. (2023). Antioxidant and hypoglycemic potential of phytogenic cerium oxide nanoparticles. *Scientific Reports*, *13*(1), 4514.
45. Sarian, M. N., Ahmed, Q. U., Mat So'ad, S. Z., Alhassan, A. M., Murugesu, S., Perumal, V., ... & Latip, J. (2017). Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study. *BioMed research international*, *2017*.

46. Tiong, S. H., Looi, C. Y., Hazni, H., Arya, A., Paydar, M., Wong, W. F., ... & Awang, K. (2013). Antidiabetic and antioxidant properties of alkaloids from Catharanthus roseus (L.) G. Don. *Molecules*, *18*(8), 9770-9784.
47. Ali, M. A., Wahed, M. I. I., Khatune, N. A., Rahman, B. M., Barman, R. K., & Islam, M. R. (2015). Antidiabetic and antioxidant activities of ethanolic extract of Semecarpus anacardium (Linn.) bark. *BMC Complementary and Alternative Medicine*, *15*(1), 1-10.