EVALUATION OF ANTI-OBESITY AND ANTI-DIABETIC ACTIVITY OF ETHANOLIC SEED SPICES EXTRACT WITH LEMON JUICE BY *IN-VITRO* METHODS

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Abstract

Diabetes and obesity are major issues in today's world. Therefore, it is imperative that new therapies be developed. The present study is about anti-obesity and anti-diabetic drug which is formulated using the extracts of fennel, ajwain and cumin by using cell viability assay and α-Glucosidase inhibition activities of plant extracts. In the cell viability assay, 3T3-L1 cells were treated with different concentrations and experiment results suggested that extract showed dose-dependent cytotoxicity against the 3T3-L1, with an IC_{50} value of 7.55 µg/mL after 24 hrs of incubation. In the α-glucosidase inhibitory activity as the concentration increases, the percentage inhibition generally increases, indicating a dose-dependent response for both the extract and acarbose. The extract's inhibitory activity is demonstrated by its IC_{50} of 51.96 µg/mL. The work's outcomes unequivocally demonstrate these extracts' ability to exhibit anti-obesity and anti-diabetic properties.

Keywords: Anti-obesity, Anti diabetic, cell viability, α-glucosidase, 3T3-L1, IC_{50}

1. Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a health risk. A body mass index (BMI) over 25 is considered overweight, and over 30 is obese. Rates of overweight and obesity continue to grow in adults and children.[1] Obesity is the result of an imbalance between daily energy intake and energy expenditure, resulting in excessive weight gain. Obesity is characterized by increased size of adipocytes due to accumulation of lipids. Obesity is associated with multiple metabolic disorders such as type 2 diabetes, cardiovascular diseases, fatty liver disease and some cancers.[2] The usage of herbal medicine is growing worldwide in the treatment of obesity due to its low cost and less serious side effects. Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.

[3] Insulin is a hormone that regulates blood glucose. Hyperglycaemia, also called raised blood glucose or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.[4] Chronic hyperglycaemia also causes nonenzymatic glycation of proteins and lipids. Glycation leads to damage in small blood vessels in the retina, kidney, and peripheral nerves. Since diabetes mellitus is a complex illness, current medications only target one pathway to regulate blood sugar levels and have a number of negative side effects.[5]

Fennel (*Foeniculum vulgare)* is a hardy, perennial herb belongs to apiaceae family and widely distributed in Argentina, Bulgaria, Germany, Greece, India, and Lebanon. Fennel has been used to treat diseases such as cancer, abdominal pain, fever, gastralgia, gastritis, Diabetes, insomnia, liver pain, mouth ulcer, stomach ache, flatulence etc .[6]

Ajwain (*Trachyspermum ammi L*.) is an erect yearly herb with striate stem belongs to Apiaceae family and distributed in India and Eastern Persia. Ajwain has been used to treat diseases such as anti-fungal, anti-bacterial, anti-obesity, anti-diabetes etc .[7]

Cumin (*Cuminum cyminum)* is a small annual herbaceous plant belongs to Apiaceae family and widely distributed in India, Iran, Syria, Turkey, and the United Arab Emirates. Cumin has been used to treat diseases such as anti-microbial, anti-diabetic, antioxidant, anti-inflammation, anti-obesity etc .[8]

In the current study, three seed extracts fennel, ajwain, and cumin have been combined, and lemon juice has also been added to the extract. The current work used 3T3-L1 cell lines for obesity and alpha glucosidase inhibition for diabetes to investigate the inhibitory potentials of seed extracts.

Figure 1: Seeds of fennel, ajwain and cumin

2. Materials and Methods

Collection and authentication of the plant material

The Fennel, Ajwain, Cumin and lemon were procured from local store in the month June,2024, Tirupati. These seeds were cleaned by removing all the impurities and stored for further experiments. These seeds were crushed, sieved and stored in air tight container at normal room temperature. The plants were authenticated by Dr. M. Nagalakshmi Devamma, Head of Dept. of Botany, S.V. University, Tirupati with voucher specimen accession numbers Fennel 3433, Ajwain 3125, Cumin 3023.

Preparation of plant extracts

Fennel, Ajwain and cumin were obtained and shade dried. The crude ethanolic extract were obtained by extracting 150 grams in 450 ml ethanol on a magnetic stirrer for 72 hrs. Extract was further concentrated using rotary vacuum evaporator.

% of yield = Extract obtained / Total powder x100

4/75x100 = 5.3 % w/w

S.NO	EXTRACT	AMOUNT
1.	Fennel	25 grams
2.	Ajwain	25 grams
3.	Cumin	25 grams
4.	Lemon juice	50 ml

Table 1: Quantities of extract taken

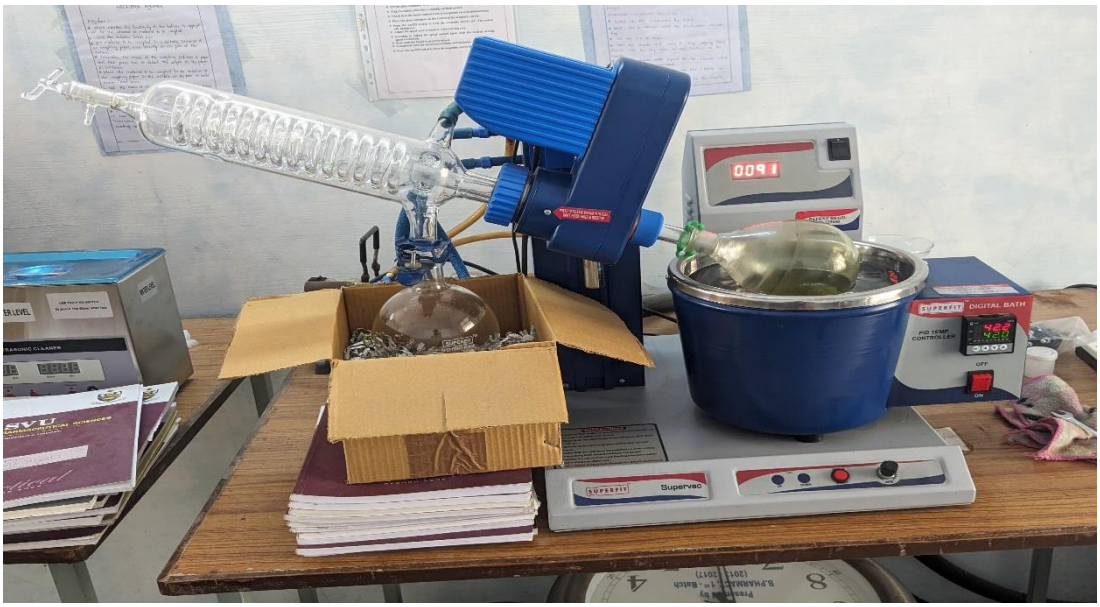


Figure 2: Extraction through rotary vacuum evaporator

Chemicals

Cell line: 3T3-L1 (Mouse Fibroblast Cell Line (NCCS, Pune), Cell culture medium: DMEM high glucose media (#AL066A, Himedia), Streptomycin sulphate (#MB287, Himedia) Pencillin G sodium salt (#TC020, Himedia), Trypsin-EDTA solution (#TCL155, Himedia) Standard drug: Orlistat (#O4139, Sigma), Adjustable multichannel pipettes and a pipettor (Benchtop, USA),Fetal Bovine Serum (#RM10432, Himedia), MTT Reagent (#TC191, Himedia), DMSO (#PCT1303, Himedia), D-PBS (#TL1006, Himedia), 96-well plate for culturing the cells (Corning, USA),

T25 flask (#12556009, Biolite- Thermo),50 ml centrifuge tubes (# 546043 TARSON), 1.5 ml centrifuge tubes (TARSON) 10 to 1000µl tips (TARSON), Acarbose (#A8980, Sigma), α-glucosidase (#G5003, Sigma), Phosphate buffer (#1218, Sigma), PNPG (#N1627, Sigma), DMSO (#PHR1309, Sigma), Pipettes: 2-10µl, 10-100µl, and 100-1000µl(TARSON) Microplate reader (#EC800, Biotek)

***MTT* assay**

Seeded 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent and allowed the cells to grow for about overnight. Appropriate concentrations of the test agents were added to respective wells. Incubated the plate for 24hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, spent media was removed from the wells and MTT reagent to a final concentration of 0.5mg/mL of total volume was added to each well and incubated for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.) For the semi-adherent cell line, centrifuged the plate at 1800rpm for 5 min and removed the MTT reagent, added 100µl of solubilisation solution (DMSO). Read the absorbance on a spectrophotometer or an ELISA reader at 570nm. The IC 50 value was determined by using a logarithmic equation i.e., Y= Mln(x)+C. [9,10,11,12,13]

% inhibition = 100 (control – treatment) / control

α-Glucosidase inhibition assay:

In 96 well plate reaction mixture containing 20µL phosphate buffer (50mM, pH 6.8), 20µL α-glucosidase solution (1U/mL in 0.1 M phosphate buffer, pH 6.9), 20µL of varying concentrations of the sample (10, 25, 50, 75, 100µg/ml) and 20µL p- nitrophenyl-α-D-glucopyranoside (5mM) added as a substrate were incubated for 20 min at 37 ^{0} C. The absorbance was measured at 405nm by using a microplate reader (#EC800, Biotek). Acarbose at various concentrations (10, 25, 50, 75, 100µg/ml) was used as positive control. The activity of the test sample was calculated by the following formula.

%inhibition = Mean OD of control – mean OD of samples/ Mean OD of control x 100

The IC_{50} value was determined by using a linear regression equation i.e. Y =Mx+C (14,15).

3. Results:

Phytochemicals	Fennel	Ajwain	Cumin
Steroids	+	+	+
Flavonoids	+	+	-
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Coumarin	+	+	+
Anthocyanins	-	-	-

Table 2: Phytochemical analysis of ethanolic seed spices extract

Effect of seed extracts on cell viability in 3T3-L1 Cells

To examine intracellular toxicity, 3T3-L1 cells were treated with wide range of concentrations (5,10,25,50,100 µg/mL) and the cell viability was determined using the MTT assay. Cell population treated with seed and lemon extracts decreased the viability of obesity cells (3T3-L1) significantly when compared to control cells at 24 hrs. The results are presented in Table 1and figure 1, 2. The percentage of cell viability decreased steadily as concentration increased. At 10 µg/mL concentration, we observed 50% growth inhibition. As a result, the IC_{50} dose (10 µg/mL) was chosen for future investigations.

In this study, the samples were evaluated to analyse the cytotoxicity effect on 3T3-L1 cell line. The concentrations of the test compounds used to treat the cells are as follows

Concentration (µg/ml)	% Cell Viability	IC_{50} (µg/ml)
Untreated	0	
Standard	49.1369	
5	53.7305	
10	47.5501	
25	38.0846	
50	29.6492	
100	22.4944	7.55

Table 3: The percent inhibition of cell viability by ethanolic extracts of seeds at varying concentrations

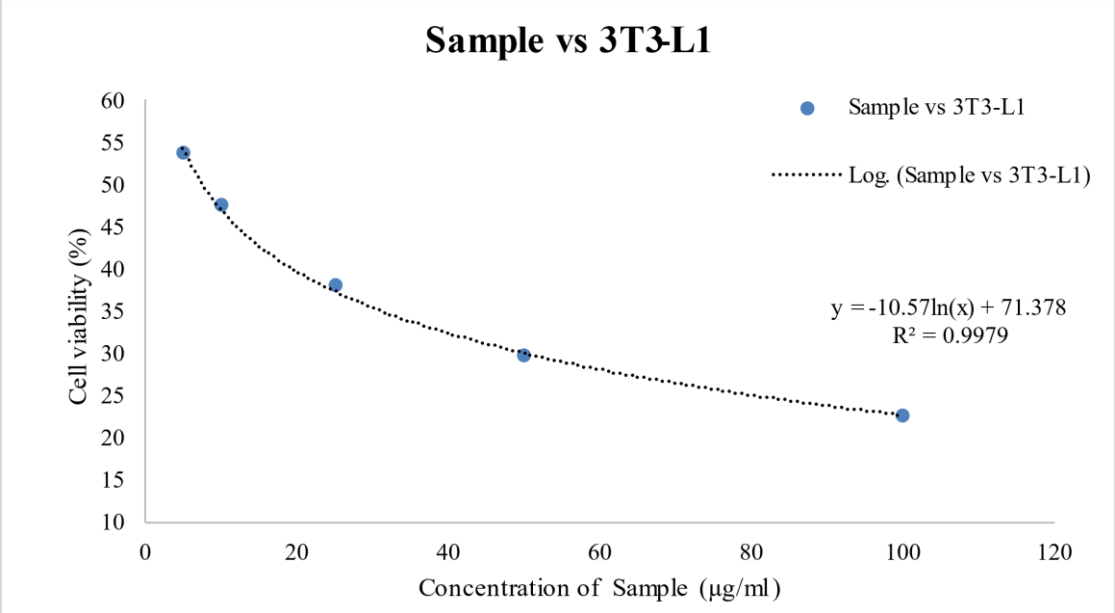


Figure 3: Graph representing the % of cell viability treated with different concentrations

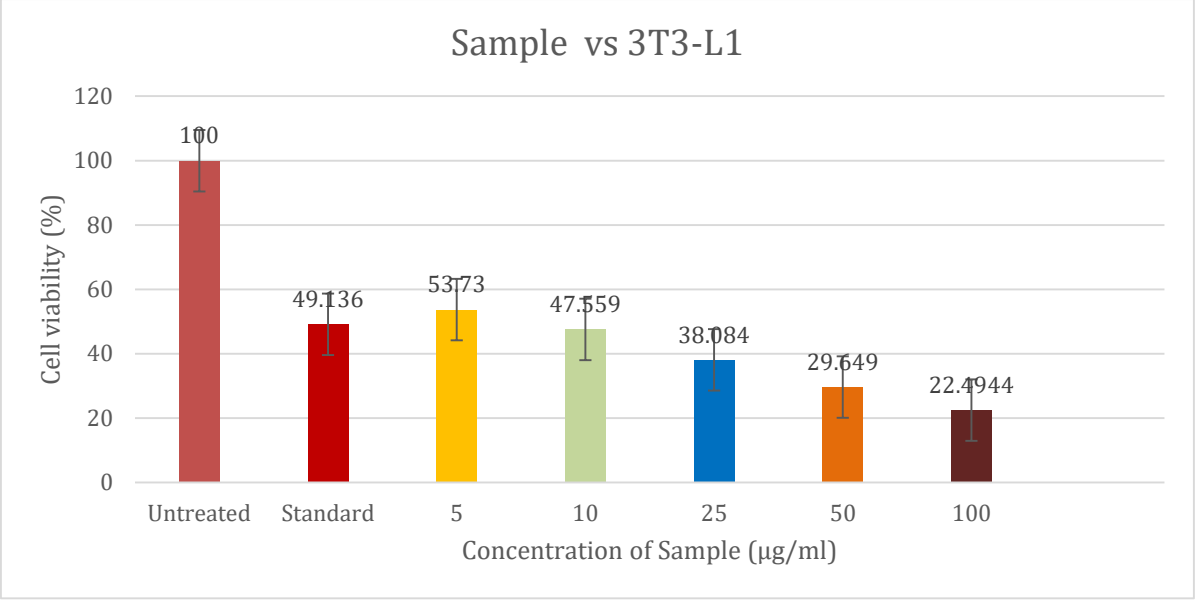


Figure 4: Plot depicting the cytotoxic effect of the sample (Extract) compared to the Standard

Morphology study

The morphological changes observed in the 3T3-L1 cells with increasing concentrations of Seeds extract suggest a dose-dependent cytotoxic effect. At lower concentrations (5 µg and 10 µg), the cells show minimal stress, while higher concentrations (25 µg, 50 µg, and 100 µg) lead to significant cell damage and death. These visual results align with the quantitative data provided earlier, where increasing concentrations of the extract led to higher percentages of cell inhibition and lower percentages of cell viability. The high degree of morphological changes and cell death at 100 µg suggests that this concentration is highly effective in reducing cell viability in 3T3-L1 cells as shown figure 3.

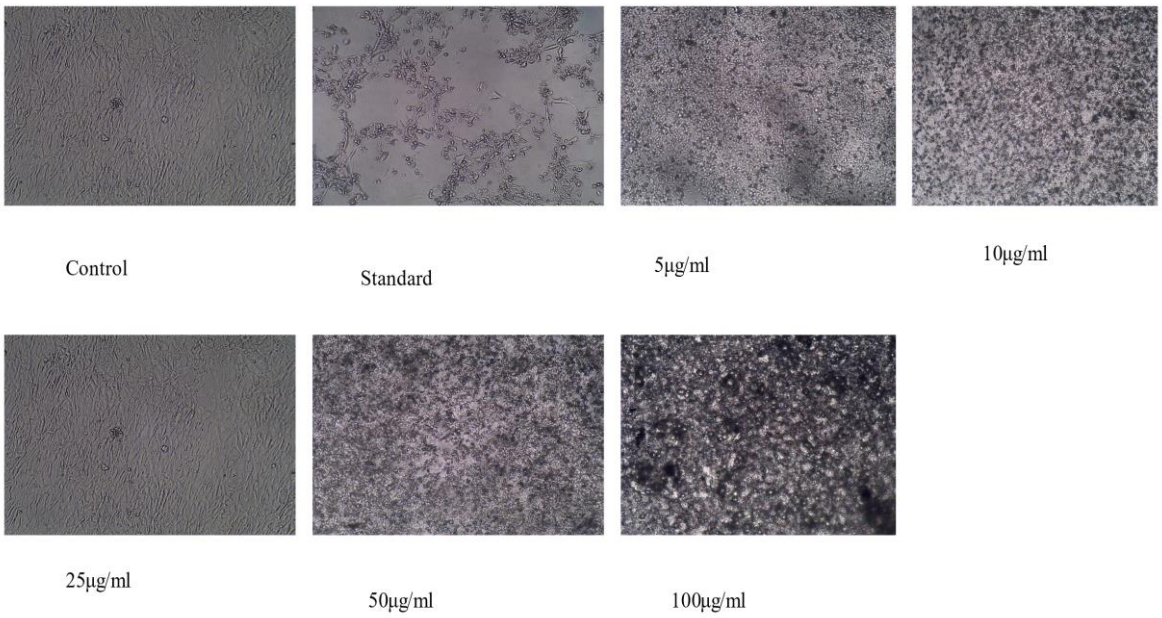


Figure 5: 3T3-L1 cell viability at various concentrations of seed extract

In vitro α-glucosidase inhibitory activity

The seed extracts were prepared with ethanol solution. Each extract was evaporated using rotary evaporator, under reduced pressure. Different concentrations (10,25,50,75,100 µg/ml) were taken and subjected to α – glucosidase inhibitory activity using 20µL p- nitrophenyl-α-D-glucopyranoside as a substrate. As concentration increases, the percentage inhibition generally increases, indicating a dose-dependent response for both the extract and acarbose. The extract's IC_{50} of 51.96 µg/mL shows its inhibitory effect, comparable to acarbose which has an IC_{50} of 45.61 µg/mL These results suggest that the extract exhibits inhibitory activity against the target, although, slightly less potent than acarbose under the conditions tested. [13,14]

Concentration (µg/ml)	**% Inhibition**	**IC_{50} (µg/ml)**
Control	0	
10	28.3232	
25	35.6565	
50	49.1515	
75	60.5454	
100	76.4444	51.96

Table 4: The percent inhibition of α – glucosidase inhibition by ethanolic extracts of seeds at varying concentrations

Concentration (µg/ml)	**% Inhibition**	**IC_{50} (µg/ml)**
Control	0	
10	35.1159	
25	39.6908	
50	52.9141	
75	60.8523	
100	75.66323	45.61

Table 5: The percent inhibition of α – glucosidase inhibition by Standard (Acarbose) at varying concentrations

Sl. No	**Sample Code**	**IC_{50} (µg/mL)**
1	Extract	51.96µg/mL
2	Acarbose	45.61µg/mL

Table 6: IC_{50} values of the test compound and standard

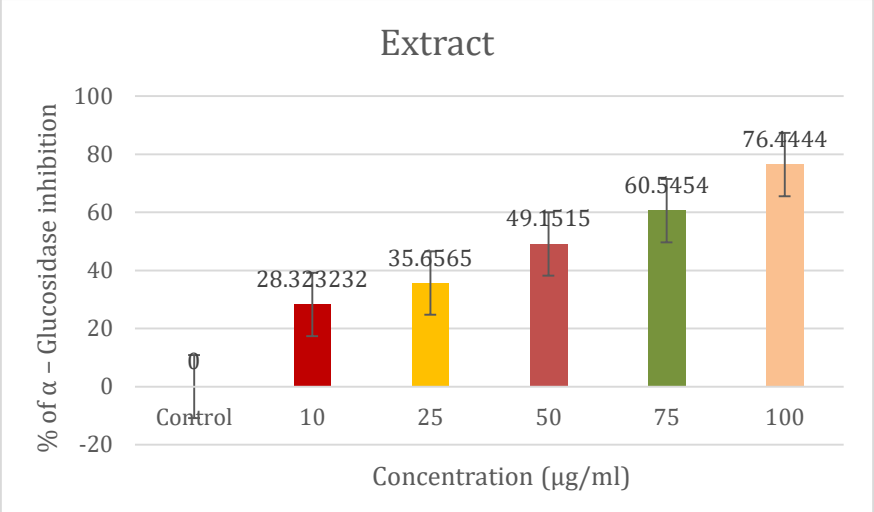


Figure 6: Plot depicting the percent inhibition of α – glucosidase inhibition by ethanolic extracts of seeds at varying concentrations

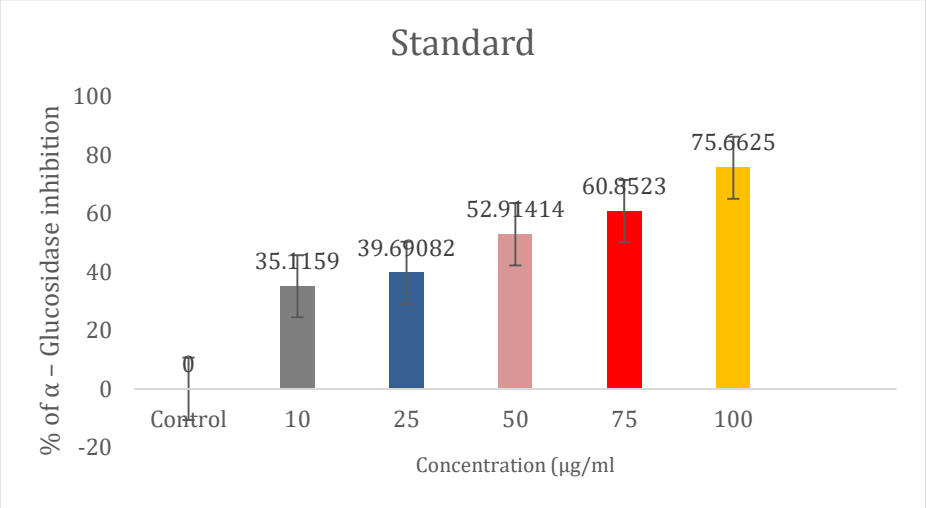
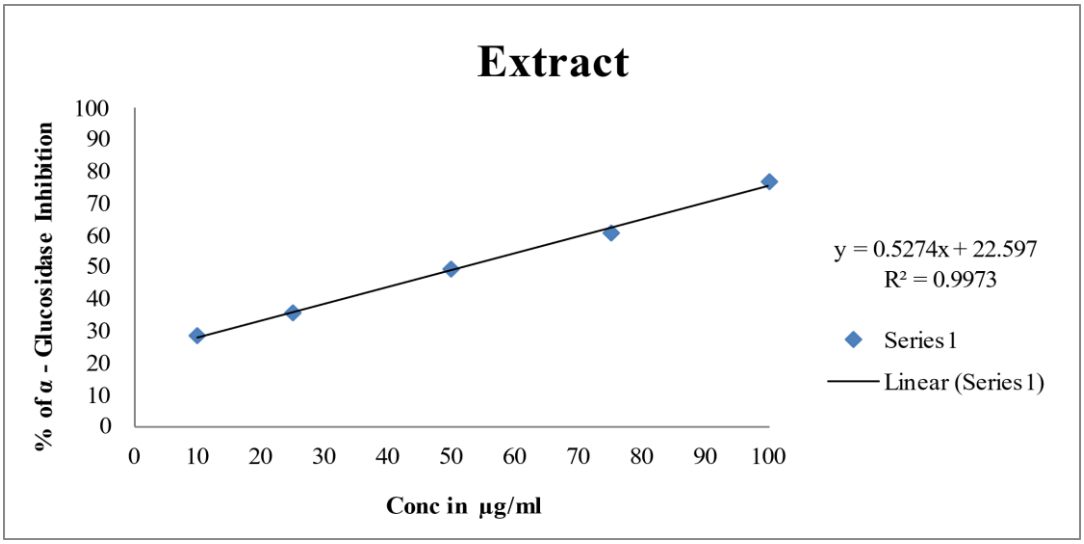
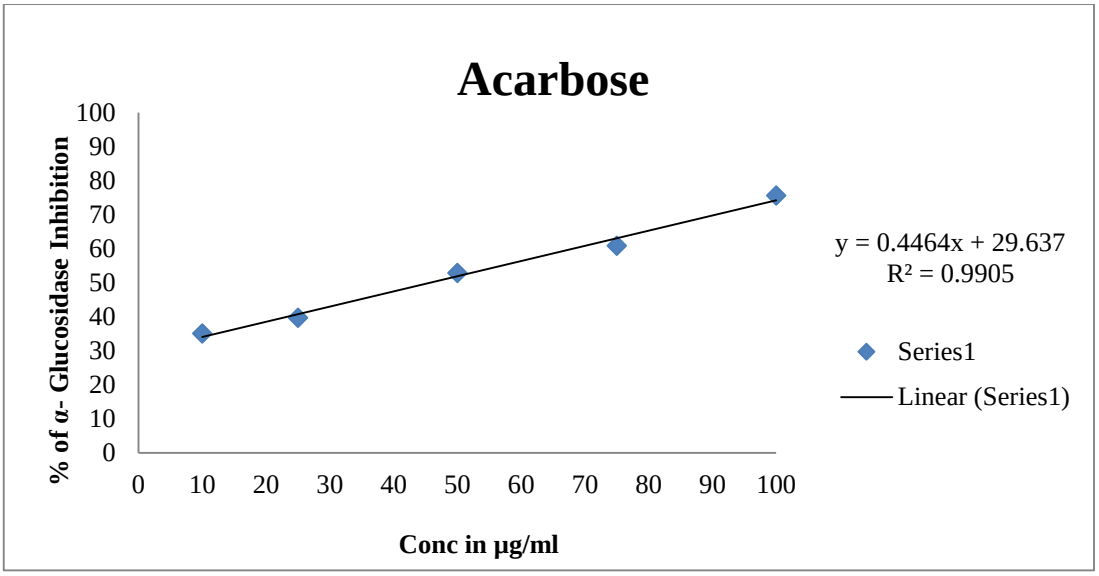


Figure 7: Plot depicting the percent inhibition of α – glucosidase inhibition by ethanolic extracts of seeds at varying concentrations



(A)



(B)

Figure 8: Graphs representing the % of α – Glucosidase inhibition treated with different concentrations of (A) sample (extract) and (B) standard control (Acarbose).

4. Discussions

Herbs and spices like fennel, ajwain, and cumin have long been utilised in herbal medicines and a variety of culinary applications. In addition to being well-liked for their ability to enhance taste, these seed spices are also thought to provide various health advantages.

These seed spices and lemon extract combinedly shows anti-obesity effect. Individually these spices have anti-obesity activity but, in this study, we have combined all these seed spices and perform MTT assay. In the MTT assay when the concentration increases the cell viability of 3T3-L1 cells decreases. The standard was treated with the concentration approximately 9.2µg/mL and the extract were treated with 5,10,25,50 and 100µg/mL The treatment of 3T3-L1 cells with the extract increased early and later decreased in dose dependent manner. When compared to standard the extract shows decrease in the viability of cells providing a basis to expect anti-obesity activity.

According to the experiment results, after 24 hours of incubation, the test chemical (Extract) demonstrated dose-dependent cytotoxicity against the cell line 3T3-L1, with an IC50 value of 7.55µg/ml. From the results of MTT assay, we can conclude that these seed spice and lemon extract exhibiting anti-obesity activity.

Alpha glucosidase inhibitory test, which demonstrates an anti-diabetic activity, has also been carried out using the same plant extract. According to the findings, these extracts have anti-alpha glucosidase action. The percentage inhibitory activity of the seed extracts against alpha glucosidase inhibition increased in a dose-dependent manner. The extract exhibits good anti-alpha glucosidase inhibitory efficacy when compared to the standard. Accordingly, our study's combination of these seed spice extracts and lemon extract demonstrated both anti-obesity and anti-diabetic effects.

5. Conclusion

The results of our present study clearly showed that ethanolic extract of seeds contain different types of active constituents like phenols, tannins ,glycosides, flavonoids, alkaloids and carbohydrates due to the presence of this phytoconstituents this extracts has shown different pharmacological activities like Anti-obesity ,Anti diabetic, Anti-oxidant, Anti-bacterial, Anti inflammation .In this study MTT assay was performed and the extract treated with 3T3-L1 cell lines demonstrates dose dependent cytotoxicity ,exhibiting anti-obesity effect. *In-vitro* method of anti-diabetic activity of α- glucosidase inhibition method with the extract showed the efficacy on diabetes. As the concentration of the compound increases, the percentage of inhibition also increases. This suggests that while the extract has significant inhibitory effects, Acarbose is slightly more potent.

Results of our work suggest that the extract has strong anti-obesity activity and moderate anti-diabetic activity suggesting potential for further investigation into the mechanisms of action and potential therapeutic applications.

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