In vitro Hypoglycaemic Activity Of *Tinospora Cordifolia* Bark With Combination Of *Zizyphus Jujuba* Fruit

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Abstract:
In the worldwide 9% of deaths happed due to type 2 diabetes, which was an endocrine disease. So the main objective of this work is to evaluate the in vitro anti diabetic activity of ethanol and ethanol: water (70:30) extracts of *TINOSPORA CORDIFOLIA* bark and *ZIZYPHUS JUJUBA* fruit. *TINOSPORA CORDIFOLIA* belongs to family Menispermaceae while *ZIZYPHUS JUJUBA* belongs to Rhamnaceae. These plants have mostly same activities like antibacterial, anti-inflammatory, antioxidant, antidiabetic etc. Highest % yield in ethanol (68.88% Z.J., 65.28% T.C.) and water (72.24% Z.J., 85.32% T.C.) extract in both plants while maximum phytochemicals were present in ethanol extract of both plants. In this study to find out potenting effect of *TINOSPORA CORDIFOLIA* bark with *ZIZYPHUS JUJUBA* fruit in anti-diabetic activity. The present study was designed to assess the in vitro anti-diabetic activity as α-amylase and β- Glucosidase analysis of T.C. bark and Z.J. These plants were single and in a combine at 6:4 ratios and extracted with ethanol and ethanol: water (70:30) solvents then find out % of anti-diabetic activity with different concentrations. The results show totally different effect present in α-amylase and β- Glucosidase analysis. In α-amylase, Z.J. having more antidiabetic activity compared to *T.C.* and potentiating effect show in Z.Z. combinations while antagonist effect observe in T.C. combinations. While in β- Glucosidase, Z.J. having more antidiabetic activity compared to *T.C.* and potentiating effect show in Z.Z. combinations while antagonist effect observe in T.C. combinations. This means that Z.J. has more antidiabetic activity compare to T.C. So this study suggest that plant combine in different ratio shows different pharmacology effects and by using this plants can be reduce the side effect of some drugs.

Key words: *TINOSPORA CORDIFOLIA* bark, *ZIZYPHUS JUJUBA* fruit, anti-diabetic activity, α-amylase, β-Glucosidase, potentiating effect, antagonist effect.

Introduction:

Diabetes is one of the major causes of premature death in worldwide. Every ten seconds a person dies of diabetes or its related causes mainly due to cardiovascular failure[1]. Diabetes affects the metabolism of carbohydrates, proteins, fat, electrolytes and water and includes a group of metabolic diseases characterized by hyperglycaemia. So recently, there is a growing interest in herbal remedies due to the side effects associated with the oral hypoglycaemic agents for the treatment of daibetes[2]. The treatment for diabetes includes the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of the degradation of oligo and disaccharides [3,4]. Inhibition of α-amylase and α-glucosidase enzymes can be the most important strategy in the management of postprandial blood glucose level in type 2 daibetes[5]. The inhibition of the activity of α-amylase and α-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result, the reduction of postprandial blood glucose level elevation[6]. Apart from conventional diabetes therapy, it is observed in several studies that some plants used in traditional medicine have beneficial effects in daibetes[7]. Worldwide more than 400 plants have been documented as beneficial in the treatment of diabetes[8]. Multiple mechanisms, many phytoconstituents, etc. were documented for the antidiabetic activity in many medicinal plants. The medicinal plants were increased and their characterizations of phytochemical are focused on drug discovery programs to bring an effective molecule to treat daibetes[9]. The digestion of dietary starch was catalyzed by the pancreatic α-amylase, which then converts into a mixture of small oligosaccharides. After this step α-glucosidase further degrades the oligosaccharides and converts into glucose. This glucose then diffuses through the intestine wall into the bloodstream for increasing postprandial blood glucose levels in body[10]. Herbal drugs are prescribed due to their good effectiveness, less side effects in clinical experience and relatively low costs[11]. The evaluation of the antidiabetic activity of drugs by in-vitro tests is necessary as an initial screening tool, which might provide useful information about the mechanism of action of the therapeutic agents[12].

Tinospora cordifolia (Miers) is commonly known as Guduchi, Gully, etc. was a highly potent herb used in Ayurveda to cure diabetes and keep the function of various organs in harmony. Various dosage forms of Tinospora cordifolia (Miers) and a wide array of its derived products like active, natural principles and crude extracts have been used in traditional system of medicine and have reported anti-diabetic activity experimentally or clinically in numerous scientific journals. These different constituents directly or indirectly affect various metabolic cascades and influence the level of glucose. It has been reported that anti-diabetic potential of plants is due to the myriad of biologically active phytoconstituents isolated from *Tinospora cordifolia* (Miers) plant including alkaloids, tannins, cardiac glycosides, flavonoids, saponins, steroids, etc. [13,14].

The *Ziziphus Jujuba* belongs to family Rhamnaceae and it was used in folk medicine for the treatment of some diseases such as weakness, obesity, diabetes, digestive disorders, skin infections, fever, diarrhea, insomnia, urinary disorders, and liver complaints[15-17]. A survey of the literature revealed that a number of alkaloids, flavonoids, terpenoids and their glycosides have been found to occur in various amounts in *Ziziphus Jujuba*. Other reports also showed the beneficial effect of using fruit extracts infusion of other parts of the plants in diabetic patients[18]. The fruits of *Ziziphus Jujuba* generally found in Iran, India, China, etc. are used to treat diabetes mellitus[19]. Therefore, the aim of this study is to evaluate the anti-diabetic effect of *Ziziphus Jujuba* and *Tinospora Cordifolia* singly and in combination.

MATERIALS AND METHODS:

Chemicals and Reagents:

The chemicals α-amylase, α-glucosidase, soluble starch, para-nitrophenyl glucopyranoside and dinitrosalicylic acid (DNSA), were purchased from Hi-Media Laboratories, Mumbai, India. The solvents were of AR grade and were purchased from SRL Pvt. Ltd., Mumbai, India. All other chemicals and reagents used in this study were purchased from local dealers.

Collection of plant materials:

Zizyphus Jujuba fruit (Z.J.) material was collected in the month of January to February from the local vegetable market of ADIPUR, KACHCHH, GUJARAT, INDIA and Tinospora Cordifolia bark (T.C.) was collected in August to September month from GOVERNMENT AYURVEDIC CENTER, RELDI VILLAGE (KUKMA), KACHCHH, GUJARAT, INDIA. Both these plants were taxonomically identified by the Gujarat Institute of Desert Ecology (GUIDE) in BHUJ, KACHCHH (GUJARAT).

Preparation of plant powder and Sample details:

Firstly, collected plants were washed twice with tap water and then with distilled water and dried in shade at ambient temperature (25±1ºC) and packed in paper bags. Then both the plants were crushed in the mechanical grinding machine to get a fine powder. The powdered material was sieved (< 1mm) and stored in airtight bag[20].

Preparation of extracts:

The aqueous and organic extracts (petroleum ether, benzene, chloroform, acetone, diethyl ether and ethanol) of fine powder of T.C. & Z.J were prepared by taking the weighed amount separately in distilled water and organic solvents in 1:10 ratio of powder and solvents for 72hrs with intermittent shaking, and then filtered through Whatman filter paper No.1. Subsequently, by using rotary evaporator the solvents were removed and the dried crude mass was weighed. The dried yield was stored in the refrigerator at 4°C until further use[21].

Sample details and Solvent extraction:

Dried powdered materials of T.C & Z.J. plants were taken individually and in 6:4 mixture form. Solvent extracts of the plants were prepared by successive continuous extraction by using Soxhlet extractor apparatus with ethanol and ethanol: water (70:30) solvents. All the extracts were filtered by Whatman paper no. 1 and evaporated to dryness under reduced pressure using a rotary evaporator and stored at 4ºC[22]. These semisolid extracts were preserved in a tightly closed glass container and used for different analysis.

Table 1. Sample details of combination of *Tinospora Cordifolia* bark with *Zizyphus Jujuba* fruit in 6:4 ratios.

Sr. No	Sample ID	Combinations	Ratio
Ethanol			
1	A_1	Z.J.	-
2	A_2	T.C.	-
3	A_3	Z.J. + T.C.	6:4
4	A_4	T.C. + Z.J.	6:4
Ethanol: Water (70:30)			
5	A_5	Z.J.	-
6	A_6	T.C.	-
7	A_7	Z.J. + T.C.	6:4
8	A_8	T.C. + Z.J.	6:4

Standard Sample (AC)

Standard Acarbose drug IP 50mg tablets of the brand "Glucobay 50" was purchased from Jamnagar city, having batch no. P16141 and manufactured by Bayer Pharmaceutical Pvt. Ltd.

α- Amylase inhibitory activity:

The α-amylase inhibitory activity was determined according to the method described by Miller with slight modification. A total of 500 µl of test samples and standard drug (50-400µg/ml) were added to the 500µl solution of 0.20mM phosphate buffer (pH 6.9) with 500 µl (0.5mg/ml) α-amylase solution and were incubated at 25°C for 10 min. After this, 500 µl of a 1% starch solution added by 0.02 M sodium phosphate buffer 500 µl (pH 6.9) was added to each tube. The reaction mixtures were incubated at 25°C for 10 min. The reaction was stopped with adding 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. Then further the test tubes were incubated in a boiling water bath for 5 min and cooled at room temperature for 5 min. The reaction mixture was diluted by adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and was conducted in a similar way by replacing extract with vehicle[23, 24].

α- Glucosidase inhibitory activity:

The α-Glucosidase inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 500 µl with 1000 µl (0.2 M) tris buffer pH 8.0 and various concentration of plant extract and standard drug (50-400µg/ml), keep it for 5 min at 37°C in incubator. The reaction was initiated by adding 1 ml of the α-glucosidase enzyme (1U/ml) to it and incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in a boiling water bath to stop the reaction and then added 250µl standard glucose reagent in each test tube. Calculate, the amount of liberated glucose is measured by glucose oxidase-peroxidase method and absorbance was measured at 510 nm in spectrophotometer[25-27].

The results of both were expressed as % inhibition calculated using the formula:

$$\text{Inhibitory activity of } \alpha - \text{amylase enzyme } = \left(\frac{\text{Abs. of Test } - \text{ Abs. of control}}{Abs.\,of\ Test}\right) * 100$$

Results:

Data analysis:

Results were expressed as mean ±SD. In all cases, the antidiabetic activity was based on at least three independent experiments performed in triplicate. The analysis of regression was followed to check out the linearity for the mean absorbance for all concentration.

Calculating % of yield with different solvents:
The calculation of % yield has been reported in different organic solvents determined by standard procedures[28]. The % of yield in both plants with different solvents is shown in table 2.

Table 2: The % of yield in T.C. & Z.J. with different solvents.

SOLVENTS	% OF YIELD	
	T.C.	Z.J.
Benzene	2.06	1.64
Petroleum ether	2.92	2.44
Diethyl ether	6.28	3.30
Chloroform	10.04	5.56
Acetone	28.68	22.61
Ethanol	66.83	63.21
Distilled Water	86.67	72.24

By the calculation of % of yield, in the T.C. the highest yield present in water extract (86.67%) and the lowest yield present in benzene extract (2.06%). While in the Z.J. results shows that highest yield present in distilled water (72.24%) and low yield present in benzene extract (1.64%). This result indicates that distilled water was efficient in extracting phytochemicals from the T.C. & Z.J. plants more than other extraction solvents[29].

Calculation of the potentiating effects of antidiabetic activity in T.C. & Z.J. mixtures:
The experimental antidiabetic capacity of the T.C & Z.J single and mixture was calculated by using the absorption of the extracts and standard curve of acarbose drug. The theoretical yield of antidiabetic activity was calculated as the sum of the separate values of each extract. If the experimental value was greater than the theoretical value of the antidiabetic activity, it was considered as potentiating effect and if the theoretical value was greater than the experimental value of antidiabetic activity it was interpreted as antagonist effect, also when experimental value and the theoretical value was same addictive effect may be present[30].

Antidiabetic activity in T.C. and Z.J.:
In this study the in vitro α–amylase inhibitory activities of the ethanol and ethanol: water (70:30) extract of Tinospora Cordifolia (T.C.) and Zizyphus Jujuba (Z.J.) singly and in combination was investigated. The results of this experiment showed a dose-dependent increase in percentage inhibitory activity against α–amylase and α-glucosidase enzyme. The plant extract might be used as starch blockers since it prevents or slows the absorption of starch in the body mainly by blocking the hydrolysis of 1, 4-glycosidic linkages of starch and other oligosaccharides into maltose and other simple sugars[31].
In this work, different combination extracts obtained from these two plants were tested for their antidiabetic capacity by α-amylase and α-glucosidase enzyme analysis. The results of this study show Potentiating and Antagonist effect present in combine extract T.C. & Z.J. of ethanol and ethanol: water (70:30). The bark of Tinospora Cordifolia & fruits of Zizyphus Jujuba singly and in combination with each other were tested for antidiabetic activity. It shows that through overlapping or complementary effects, the complex mixture of phytochemicals in selected herbs provides a better potentiating effect on health than single phytoconstituent32. There was a dose-dependent inhibitory effect of Zizyphus Jujuba fruit (Z.J.) and Tinospora Cordifolia bark (T.C.) on α-amylase and α-glucosidase enzyme. Table 3 shows % of α-amylase and α-glucosidase enzyme analysis of Acorbose-std. drug and Table 4 shows % of α-amylase and α-glucosidase enzyme analysis of T.C. & Z.J. ethanol and ethanol: water (70:30) extract in single and combination of plants.

Table 3: α-amylase and α-glucosidase enzyme analysis of Acorbose-std

α-amylase				α-Glucosidase			
Sample ID	Constration	% of Amylase	IC50	Sample ID	Constration	% of Amylase	IC50
Acorbose-std.	50 µg/ml	5.61±0.39	432.6	Acorbose-std..	50 µg/ml	54.84±0.27	39.88
	100 µg/ml	11.48±0.17			100 µg/ml	64.37±0.17	
	200 µg/ml	26.63±0.35			200 µg/ml	72.13±0.10	
	300 µg/ml	35.63±0.14			300 µg/ml	77.15±0.07	
	400 µg/ml	44.67±0.37			400 µg/ml	82.87±0.04	

Table 4: α-amylase and α-glucosidase enzyme analysis of T.C. & Z.J. individually and combination extracts in ethanol and ethanol: water (70:30) solvent

Sample ID	Constration	% of Amylase	IC50	% of α-Glucosidase	IC50
ETHANOL				**ETHANOL**	
A1 Z.J.	50 µg/ml	7.39±0.38	637.32	19.49±0.42	328.07
	100 µg/ml	17.53±0.15		27.70±0.03	
	200 µg/ml	24.94±0.39		31.18±0.05	
	300 µg/ml	29.60±0.27		33.55±0.04	
	400 µg/ml	32.61±0.49		37.51±0.11	
A2 T.C.	50 µg/ml	1.74±0.23	917.91	38.17±0.01	596.37
	100 µg/ml	10.81±0.42		43.59±0.04	
	200 µg/ml	17.19±0.53		48.46±0.06	
	300 µg/ml	20.78±0.07		50.12±0.04	
	400 µg/ml	25.28±0.34		53.24±0.05	
A3 Z.J. + T.C.	50 µg/ml	22.92±0.51	288.81	63.41±0.12	37.51
	100 µg/ml	36.69±0.25		77.20±0.07	
	200 µg/ml	43.57±0.10		83.45±0.06	
	300 µg/ml	51.73±0.10		84.80±0.03	
	400 µg/ml	59.26±0.21		91.52±0.05	
A4 T.C. +Z.J.	50 µg/ml	8.40±0.46	378.72	48.35±0.35	35.16
	100 µg/ml	18.45±0.59		67.42±0.18	
	200 µg/ml	30.55±0.26		76.19±0.05	
	300 µg/ml	40.60±0.12		80.70±0.05	
	400 µg/ml	51.52±0.38		88.44±0.04	
ETHANOL:WATER (70:30)				**ETHANOL:WATER (70:30)**	
A5 Z.J.	50 µg/ml	7.14±0.44	1019.74	34.55±0.09	799.34
	100 µg/ml	11.48±0.42		39.30±0.10	
	200 µg/ml	17.15±0.42		42.76±0.13	
	300 µg/ml	20.87±0.20		44.60±0.18	
	400 µg/ml	23.93±0.44		46.66±0.08	
A6 T.C.	50 µg/ml	4.84±0.48	1137.27	22.45±0.23	529.37
	100 µg/ml	10.81±0.42		28.17±0.12	
	200 µg/ml	16.86±0.36		30.20±0.14	
	300 µg/ml	19.58±0.14		35.16±0.03	
	400 µg/ml	22.19±0.24		37.06±0.05	
A7 Z.J. + T.C.	50 µg/ml	13.40±0.11	323.58	59.51±0.14	40.04
	100 µg/ml	27.37±0.23		70.89±0.07	
	200 µg/ml	40.95±0.33		78.18±0.08	
	300 µg/ml	49.65±0.08		85.89±0.03	
	400 µg/ml	57.18±0.53		90.80±0.04	
A8 T.C. +Z.J.	50 µg/ml	4.28±0.31	735.49	55.53±0.32	449.02
	100 µg/ml	10.33±0.49		63.26±0.09	
	200 µg/ml	19.13±0.33		67.51±0.09	
	300 µg/ml	23.42±0.19		72.51±0.09	
	400 µg/ml	26.77±0.32		75.59±0.06	

Table 3 shows that comparatively more inhibitory activity on α-amylase enzyme was demonstrated by standard drug Acarbose while α-glucosidase showed less inhibitory activity as compared to standard drug. Antidiabetic potential of ethanol extract was higher than ethanol: water (70:30) extract (Table- 4). Water is a common solvent used in the extraction process, but ethanol has a greater polarity than water, so it can dissolve more polar compounds contained in the sample than water. The single extract of Z.J. & T.C. with ethanol and ethanol: water (70:30) solvents shows sensitive results in α-amylase and α-glucosidase analysis. In α-amylase analysis, Z.J. has more capacity as compared to T.C and in α-glucosidase analysis the result shows Z.J. has more capacity as compared to T.C. While combination samples, Z.J. combined with T.C. shows higher antidiabetic activity as compared to T.C. combined with Z.J.

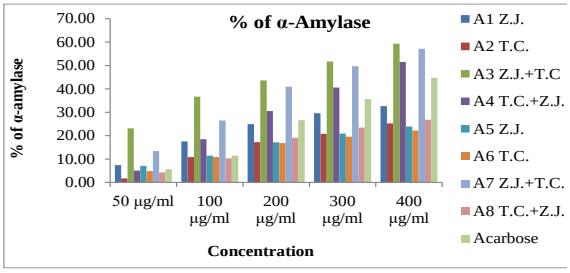

Fig.1 α-amylase, individually and combination of T.C. & Z.J. ethanol & ethanol: water (70:30) extract

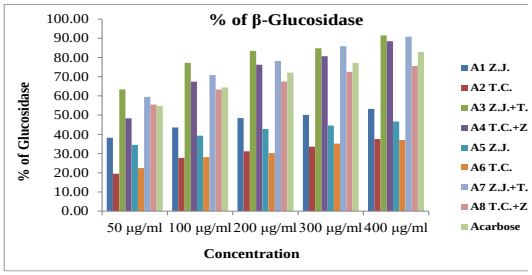

Fig.2 α- glucosidase, individually and combination of T.C. & Z.J. ethanol & ethanol: water (70:30) extract

From both the extracts tested, the ethanol extract of the bark of T.C. and fruit of Z.J. displayed an excellent activity compared to ethanol: water (70:30) extract against the antidiabetic activity. The ethanol extract showed the highest α-amylase enzyme activity (59.29±0.21%) at 400µg/ml in extract A3 (Z.J + T.C.) and lowest (1.74±0.23 %) at 50µg/ml in extract A2 (T.C) while the highest α- glucosidase enzyme activity (91.52±0.05 %) at 400µg/ml was in extract A3 (Z.J. + T.C) and lowest (19.49±0.42 %) at 50µg/ml in extract A2 (T.C.). The ethanol: water (70:30) extract showed maximum α-amylase enzyme activity (57.18±0.53 %) at 400µg/ml in extract A7 (Z.J + T.C.) and lowest (1.74±0.23%) at 50µg/ml in extract A6 (T.C.). IC50 value highest was shown in A6 (T.C) extract (1137.27) and lowest was shown in A4 (T.C. + Z.J.) extract (35.16).

This study includes a different mode of combination. The individual T.C. & Z.J. extract was combined and different concentration solution was prepared for analysis of antidiabetic activity as α-amylase and α-glucosidase. Table 5 indicates the results of a single extract combination of Z.J and T.C. in an ethanol solvent.

Table 5. Antidiabetic activity of combination of individual T.C. & Z.J. in ethanol extract

Sample ID	Constration	α-amylase	α-Glucosidase
		% of Amylase	% of Glucosidase
A1+2	50+50=100	4.50±0.30	42.79±0.28
	100+50=150	15.29±0.47	81.85±0.07
	100+100=200	20.37±0.34	63.34±0.30
	200+100=300	29.48±0.16	89.34±0.09
	200+200=400	38.56±0.21	76.26±0.07
A2+1	50+50=100	4.50±0.30	42.79±0.28
	100+50=150	27.29±0.29	59.22±0.14
	100+100=200	20.37±0.34	63.34±0.30
	200+100=300	47.41±0.27	67.27±0.14
	200+200=400	38.56±0.21	76.26±0.07

From both the extracts tested, the ethanol extract of the bark of T.C. and fruit of Z.J. displayed an excellent activity compared to ethanol: water (70:30) extract against the antidiabetic activity. The ethanol extract showed the highest α-amylase enzyme activity (59.29±0.21%) at 400µg/ml in extract A3 (Z.J + T.C.) and lowest (1.74±0.23 %) at 50µg/ml in extract A2 (T.C) while the highest α- glucosidase enzyme activity (91.52±0.05 %) at 400µg/ml was in extract A3 (Z.J. + T.C) and lowest (19.49±0.42 %) at 50µg/ml in extract A2 (T.C.). The ethanol: water (70:30) extract showed maximum α-amylase enzyme activity (57.18±0.53 %) at 400µg/ml in extract A7 (Z.J + T.C.) and lowest (1.74±0.23%) at 50µg/ml in extract A6 (T.C.). IC50 value highest was shown in A6 (T.C) extract (1137.27) and lowest was shown in A4 (T.C. + Z.J.) extract (35.16).

This study includes a different mode of combination. The individual T.C. & Z.J. extract was combined and different concentration solution was prepared for analysis of antidiabetic activity as α-amylase and α-glucosidase. Table 5 indicates the results of a single extract combination of Z.J and T.C. in an ethanol solvent. The table-5 result indicates

that when both the plants were extracted together it showed better antidiabetic activity. It indicates that when the plants were mixed with each other and extracted by solvents, maximum phytochemicals were isolated so it gives good activity. Literature survey suggested the reaction mechanisms involved in the inhibition of α-amylase enzymes by plant protein inhibitors are not clearly understood. But some suggestions are obtained that the plant protein (flavanols) might cause conformational changes in structure[33]. Inhibitory activity of such type of enzyme-like α-amylase, α-glucosidase, etc. in the form of dealing carbohydrate digestion, reduction of absorption blood glucose level[34].

The effect present in Antidiabetic activity of the extract in T.C & Z.J:

T.C. & Z.J. have almost resembling medicinal activity like anti-inflammatory, anti-carcinogenic, antidiabetic, antioxidant, anti-inflammatory and anti-atherosclerotic 35. For the antidiabetic activity, the combined extract was prepared in 6:4 ratios of T.C. & Z.J. and vice-versa with using ethanol and ethanol: water (70:30) solvents. This result indicates that the combined sample shows potentiating and antagonist effect in ethanol and ethanol: water (70:30) solvents. Fig 3a & 3b indicates the effect present in ethanol & ethanol: water (70:30) extracts of T.C. & Z.J as α-amylase analysis and fig 3c shows effect present in combine extract. Fig 4a & 4b indicates the effect present in ethanol & ethanol: water (70:30) extracts of T.C. & Z.J as α- glucosidase analysis and fig 4c shows effect present in combine extract.

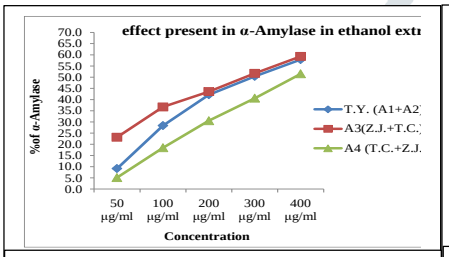

Fig.3a in α- amylase, effect present in combination of T.C. & Z.J. with ethanol solvent.

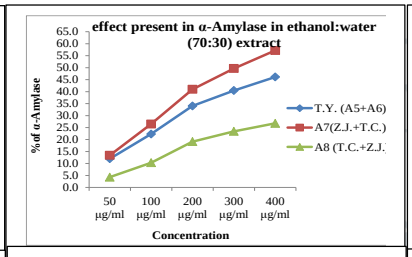

Fig.3b in α- amylase, effect present in combination of T.C. & Z.J. with ethanol:water (70:30) solvent.

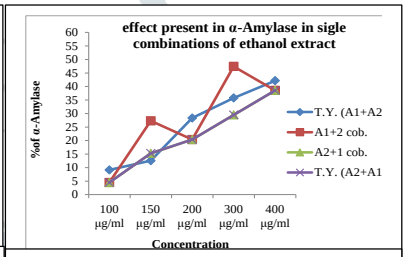

Fig.3c in α- amylase, effect present in single combination of T.C. & Z.J. with ethanol solvent

Whenever the theoretical yield was more than antidiabetic activity, it showed a potentiating effect and when the theoretical yield was less than antidiabetic activity show antagonist effect[36]. Theoretical yield was calculated as the sum of both single antidiabetic activity T.C. & Z.J. Potentiating effect and antagonist effect in α-amylase of T.C. and Z.J. with ethanol and ethanol:water (70:30) is shown in table 6 and potentiating effect and antagonist effect in α-glucosidase of T.C. and Z.J. with ethanol and ethanol: water (70:30) is shown in table 7.

Table 6: Potentiating effect and antagonist effect in α-amylase of T.C. and Z.J. with ethanol and ethanol: water (70:30)

α-amylase					
Sample ID	**Constration**	**% of Amylase**	**Theo.yield**	**Effect**	**%effect**
ETHANOL					
A1 Z.J.	50 µg/ml	7.39±0.38	9.13±0.30		
	100 µg/ml	17.53±0.15	28.342±0.29		
	200 µg/ml	24.94±0.39	42.132±0.46		
	300 µg/ml	29.60±0.27	50.38±0.17		
	400 µg/ml	32.61±0.49	57.884±0.41		
A2 T.C.	50 µg/ml	1.74±0.23	9.13±0.30		
	100 µg/ml	10.81±0.42	28.342±0.29		
	200 µg/ml	17.19±0.53	42.132±0.46		
	300 µg/ml	20.78±0.07	50.38±0.17		
	400 µg/ml	25.28±0.34	57.884±0.41		
A3 Z.J. + T.C.	50 µg/ml	22.92±0.51		Potentiating	153.40
	100 µg/ml	36.69±0.25		Potentiating	29.45

	200 µg/ml	43.57±0.10		Potentiating	3.41
	300 µg/ml	51.73±0.10		Potentiating	2.69
	400 µg/ml	59.26±0.21		Potentiating	2.42
A4 T.C. +Z.J.	50 µg/ml	8.40±0.46		Antagonist	44.04
	100 µg/ml	18.45±0.59		Antagonist	34.91
	200 µg/ml	30.55±0.26		Antagonist	27.50
	300 µg/ml	40.60±0.12		Antagonist	19.40
	400 µg/ml	51.52±0.38		Antagonist	11.01
A1+2	50+50=100	4.50±0.30	9.13±0.30	Antagonist	20.93
	100+50=150	15.29±0.47	18.2±0.19	Potentiating	34.44
	100+100=200	20.37±0.34	28.34±0.29	Antagonist	8.17
	200+100=300	29.48±0.16	34.72±0.34	Potentiating	19.02
	200+200=400	38.56±0.21	42.13±0.46	Antagonist	2.41
A2+1	50+50=100	4.50±0.30	9.13±0.30	Antagonist	20.93
	100+50=150	15.29±0.47	18.2±0.19	Potentiating	34.44
	100+100=200	20.37±0.34	28.34±0.29	Antagonist	8.17
	200+100=300	29.48±0.16	34.72±0.34	Potentiating	19.02
	200+200=400	38.56±0.21	42.13±0.46	Antagonist	2.41
ETHANOL:WATER (70:30)					
A5 Z.J.	50 µg/ml	7.14±0.44	11.98±0.46		
	100 µg/ml	11.48±0.42	22.28±0.42		
	200 µg/ml	17.15±0.42	34.01±0.39		
	300 µg/ml	20.87±0.20	34.01±0.17		
	400 µg/ml	23.93±0.44	46.11±0.34		
A6 T.C.	50 µg/ml	4.24±0.48	11.98±0.46		
	100 µg/ml	10.81±0.42	22.28±0.42		
	200 µg/ml	16.86±0.36	34.01±0.39		
	300 µg/ml	19.58±0.14	34.01±0.17		
	400 µg/ml	22.19±0.24	46.11±0.34		
A7 Z.J. + T.C.	50 µg/ml	13.40±0.11		Potentiating	11.88
	100 µg/ml	27.37±0.23		Potentiating	18.79
	200 µg/ml	40.95±0.33		Potentiating	20.40
	300 µg/ml	49.65±0.08		Potentiating	22.74
	400 µg/ml	57.18±0.53		Potentiating	24.00
A8 T.C. +Z.J.	50 µg/ml	4.84±0.31		Antagonist	64.27
	100 µg/ml	10.33±0.49		Antagonist	53.62
	200 µg/ml	19.13±0.33		Antagonist	43.76
	300 µg/ml	23.42±0.19		Antagonist	42.10
	400 µg/ml	26.77±0.32		Antagonist	41.95

Table 6 shows the result of potentiating effect and antagonist effect in α-amylase of T.C. and Z.J. with ethanol and ethanol: water (70:30). In this fig. 3(a, b & c), the red line shows the presence of potentiating effect and green line shows antagonist effect while the blue line shows a theoretical yield of T.C. & Z.Z. In the α-amylase analysis, maximum potentiating effect (153.40%) is shown in ethanol extract of A3 (Z.J. + T.C.) at 50µg/ml and minimum effect (2.42%) is shown in ethanol extract of A3 (Z.J. + T.C.) at 400µg/ml. While the antagonist effect is shown highest (64.27%) in ethanol: water (70:30) of A8 (T.C. +Z.J.) at 50µg/ml and lowest (11.01%) in ethanol extract A4 (T.C. + Z.J.) at 400µg/ml. But when singly extract of plants combine and making different contraction show different effect in results. When the high amount of T.C. extract mixed with Z.J. extract in concentration solution, it shows antagonist effect and results obtained are 20.91, 4.79, 8.16, 10.39 and 2.41 % at concentration 100, 150, 200, 300 and

400µg/ml. Whenever the high amount of Z.J. combine with T.C., it shows potentiating effects are 34.44 and 19.02 % at 150 and 300µg/ml and antagonist effect are 20.93, 8.16 and 2.41 at 100, 200 and 400µg/ml.

Table 7: Potentiating effect and antagonist effect in α-glucosidase of T.C. and Z.J. with ethanol and ethanol: water (70:30)

α-Glucosidase					
Sample ID	**Constration**	**% of Glucosidase**	**Theo. yield**	**Effect**	**%effect**
ETHANOL					
A1 Z.J.	50 µg/ml	19.49±0.42	57.65±0.42		
	100 µg/ml	27.70±0.03	71.28±0.02		
	200 µg/ml	31.18±0.05	79.69±0.01		
	300 µg/ml	33.55±0.04	83.66±0.04		
	400 µg/ml	37.51±0.11	90.75±0.05		
A2 T.C.	50 µg/ml	38.17±0.01	57.65±0.42		
	100 µg/ml	43.59±0.04	71.28±0.02		
	200 µg/ml	48.46±0.06	79.69±0.01		
	300 µg/ml	50.12±0.04	83.66±0.04		
	400 µg/ml	53.24±0.05	90.75±0.05		
A3 Z.J. + T.C.	50 µg/ml	63.41±0.12		Potentiating	9.09
	100 µg/ml	77.20±0.07		Potentiating	7.66
	200 µg/ml	83.45±0.06		Potentiating	4.51
	300 µg/ml	84.80±0.03		Potentiating	1.34
	400 µg/ml	91.52±0.05		Potentiating	0.84
A4 T.C. +Z.J.	50 µg/ml	48.35±0.35		Antagonist	16.12
	100 µg/ml	67.42±0.18		Antagonist	5.42
	200 µg/ml	76.19±0.05		Antagonist	4.39
	300 µg/ml	80.70±0.05		Antagonist	3.54
	400 µg/ml	88.44±0.04		Antagonist	2.55
A1+2	50+50=100	42.79±0.28	54.11±0.37	Antagonist	20.93
	100+50=150	81.85±0.07	63.39±0.35	Potentiating	34.44
	100+100=200	63.34±0.30	71.48±0.03	Antagonist	11.39
	200+100=300	89.34±0.09	75.07±0.30	Potentiating	19.02
	200+200=400	76.26±0.07	79.57±0.22	Antagonist	4.17
A2+1	50+50=100	42.79±0.28	54.11±0.37	Antagonist	20.93
	100+50=150	59.22±0.14	62.20±0.34	Antagonist	4.80
	100+100=200	63.34±0.30	71.48±0.03	Antagonist	11.39
	200+100=300	67.27±0.14	75.99±0.24	Antagonist	11.47
	200+200=400	76.26±0.07	79.57±0.22	Antagonist	4.17
ETHANOL:WATER (70:30)					
A5 Z.J.	50 µg/ml	34.55±0.09	57.00±0.14		
	100 µg/ml	39.30±0.10	67.47±0.01		
	200 µg/ml	42.76±0.13	72.96±0.01		
	300 µg/ml	44.60±0.18	79.76±0.16		
	400 µg/ml	46.66±0.08	83.72±0.03		
A6 T.C.	50 µg/ml	22.45±0.23	57.00±0.14		
	100 µg/ml	28.17±0.12	67.47±0.01		

	200 µg/ml	30.20±0.14	72.96±0.01		
	300 µg/ml	35.16±0.03	79.76±0.16		
	400 µg/ml	37.06±0.05	83.72±0.03		
A7 Z.J. + T.C.	50 µg/ml	59.51±0.14		Potentiating	4.22
	100 µg/ml	70.89±0.07		Potentiating	4.82
	200 µg/ml	78.18±0.08		Potentiating	6.67
	300 µg/ml	85.89±0.03		Potentiating	7.13
	400 µg/ml	90.80±0.04		Potentiating	7.80
A8 T.C. +Z.J.	50 µg/ml	55.53±0.32		Antagonist	2.58
	100 µg/ml	63.26±0.09		Antagonist	6.24
	200 µg/ml	67.51±0.09		Antagonist	7.47
	300 µg/ml	72.51±0.09		Antagonist	9.09
	400 µg/ml	75.59±0.06		Antagonist	9.71

Table 7 indicates the potentiating effect and antagonist effect in α-glucosidase of T.C. and Z.J. with ethanol and ethanol: water (70:30). In the α-glucosidase analysis, maximum (9.09%) potentiating effect is shown by ethanol extract of A3 (Z.J. + T.C.) at 50µg/ml and minimum (0.84%) effect is shown by ethanol extract of A3 (Z.J. + T.C.) at 400µg/ml. While the antagonist effect is highest (16.12%) in ethanol extract of A4 (T.C. + Z.J.) at 50µg/ml and lowest (2.55%) in ethanol extract of A4 (T.C. + Z.J.) at 400µg/ml. In the single plant extract combination, when a high amount of T.C. extract was mixed with the lower amount of Z.J. extract were taken and making the different concentration, the results showed an antagonist effect in all concentration 20.93, 4.80, 11.39, 11.47 and 4.17 % at 100, 150, 200, 300 and 400µg/ml. The reverse combination proportion shows the potenting effect with values as 34.44 and 19.02 % at 150 and 300µg/ml and antagonist effect are 20.93, 11.39 and 4.17 at 100, 200 and 400 µg/ml concentration. Fig 5a, 5b, and 5c indicate the effect present in α-amylase in ethanol extract of T.C. & Z.J. and the fig. 6a, 6b, and 6c show the effect present in α-glucosidase of T.C. and Z.J.

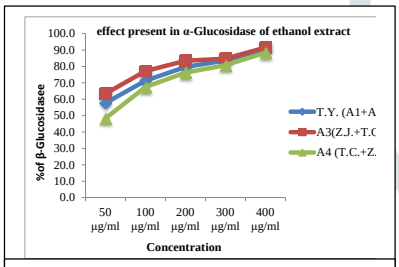

Fig.4a in α-glucosidase, % effect present in combination of T.C. & Z.J.

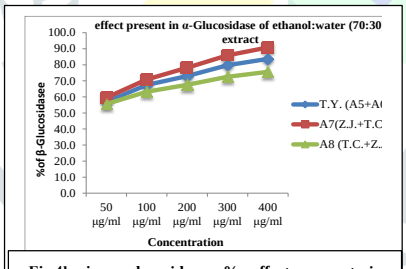

Fig.4b in α-glucosidase, % effect present in combination of T.C. & Z.J. with ethanol: water (70:30) solvent.

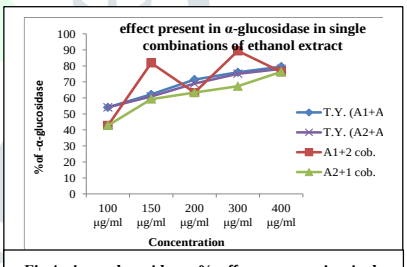

Fig.4c in α-glucosidase, % effect present in single combination of T.C. & Z.J. with ethanol solvent.

In all figures, the red line indicates the potentiating effect, the green line shows the antagonist effect and the blue line indicates the theoretical yield of T.C. & Z.Z. The most effective and useful method to control diabetes was to inhibit the activity of the α-amylase enzyme which was responsible for the collapse of starch to more simple sugars (dextrin, maltotriose, maltose, and glucose)[37]. These results indicate that the plant material extracted with ethanol shows highest antidiabetic activity as compared to other solvents. When Z.J. combines more with T.C. we get potentiating effect in antidiabetic activity analysis of ethanol and ethanol: water (70:30) extract. But whenever more T.C. combines with Z.J. it shows antagonist effect in ethanol and ethanol: water (70:30) extract in both combines.

Comparison of Effect in Antidiabetic activity of the extract in T.C & Z.J:

In the antidiabetic activity, % of the effect is calculated as potentiating an antagonist effect. The % of potentiating and antagonist effect is shown in table 6. T.C. plant was used in various herbal preparations for the treatment of different diseases for its anti-periodic, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic properties[38]. Fig.7 indicates the comparison between potentiating an antagonist effect of combination between T.C. and Z.J. ethanol, ethanol: water (70:30) and single combination of Z.J. & T.C. in α- amylase and fig.8 indicates the comparison between potentiating an antagonist effect of combination between T.C. and Z.J. ethanol, ethanol: water (70:30) and single combination of Z.J. & T.C. in α- glucosidase.

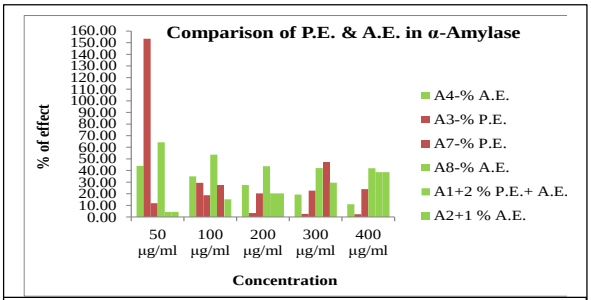

Fig.5 in α-Amylase, comparison of effect present in combination of T.C. & Z.J.

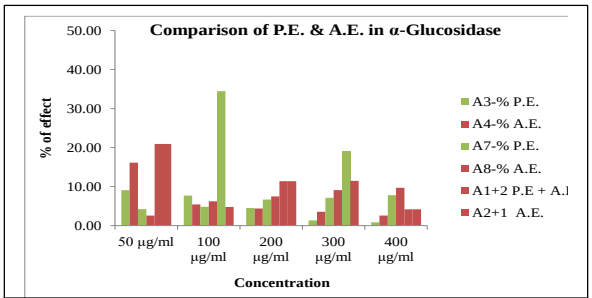

Fig.6 in α-Glucosidase, comparison of effect present in combination of T.C. & Z.J.

In Fig. 5 & 6, red column indicated the potentiating effect and green column indicated antagonist effect. The results of comparison in potentiating and antagonist effects of T.C. and Z.J. in ethanol, ethanol: water (70:30) extract and separate combination of both plants in ethanol extracts shows that in α-amylase highest (153.40%) effect is found in A3 (Z.J. + T.C.) in ethanol extract at 50µg/ml as potentiating effect and lowest (2.42%) effect also in A3 (Z.J. + T.C.) in ethanol extract at 400µg/ml as potentiating effect. In antagonist effect results shows that maximum (64.27%) effect is found in A8 (T.C. + Z.J.) at 50µg/ml in ethanol: water (70:30) extract and minimum (2.41%) effect in A(2+1) and A(1+2) single combination of T.C. & Z.J. in ethanol extract at 400µg/ml.

In the α-Glucosidase analysis, maximum (34.44%) potentiating effect present at singly plants extracts combination sample A(1+2) at 150µg/ml of ethanol extract and minimum (0.84%) potentiating effect shown in A3 (Z.J. + T.C.) of ethanol extract at 400µg/ml concentration. While in the antagonist effect, highest (20.93%) effect present at 50µg/ml of singly plant extract combination sample in ethanol extract and lowest (2.55%) effect at 400µg/ml in A4 (T.C. + Z.J.) of ethanol extract. In anti-diabetic activity analysis, when more Z.J. amount is present in combinations, the sample shows a potentiating effect and for more amount of T.C. present in combinations, the sample shows an antagonist effect. Also, the combinations extract in ethanol solvents shows good and effective results of potentiating effect. In ethanol extract, the % of effect increase with a decrease in the concentration of extract but in ethanol: water (70:30) extract show opposite results compare to ethanol extract. It shows % of effect increase with the increase in the concentration of extract.

CONCLUSION:

The present study shows the highest potentiating effect of antidiabetic activity in sample A3 (Z.J. + T.C.) in ethanol extract and the antagonist effect in sample A(2+1) in ethanol extract. Ethanol extract shows more and effective anti-diabetic activity compare to ethanol: water (70:30) extract. So this study suggests that Zizyphus Jujuba fruit shows a potentiating effect in combination with Tinospora Cordifolia bark, and Tinospora Cordifolia bark indicates antagonist effect in combination with Zizyphus Jujuba fruit in antidiabetic activity determination. Hence it can be said that this study supports the traditional usage as antidiabetic agents in new drugs for the therapy of many diseases. The most active extracts can be subjected to isolation of the active compound and carry out the further pharmacological evaluation.

ACKNOWLEDGMENT:

The authors are thankful to the authorities of Krantiguru Shyamji Krishna Verma Kachchh University, Bhuj (Kachchh) for providing necessary facilities to carry out this work. We are thankful to Dr. Mukesh Nariya, Head, Pharmacology, IPGT & RA, Gujarat Ayurveda University, Jamnagar, for providing necessary facilities to carry out the required testing. One of the authors Jagruti P. Matang is thankful to UGC, New Delhi for providing RGNF-fellowship as financial support.

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