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Research Article

ANTI-DIABETIC AND ANTI-INFLAMMATORY ACTIVITIES OF WILD AND MICROPROPAGATED *CADABA FRUTICOSA* L.

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ABSTRACT

The present study was aimed to compare the anti-inflammatory and antidiabetic property of wild and micropropagated *Cadaba fruticosa* ethanol and methanol extracts. For albumin denaturation assay the highest inhibition percentage (60.82±0.54) was observed in WCFE followed by MCFE (57.28±0.92), WCFM (54.62±0.85) and MCFM (53.29±0.25) extracts at 1000 µg/ml concentration. The amount required for fifty percent inhibition (IC_{50}) was found to be 880.04, 750.42, 670.31 and 610.21 for WCFE, MCFE, WCFM and MCFM. For HRBC membrane stabilization assay WCFE with 62.15. MCFE with 58.28, WCFM with 57.24 and MCFM with 54.28 percentage inhibitions was observed at 1000 µg/ml concentration. The IC_{50} values were found to be 324.14, 880.15, 840.08 and 710.11 for WCFE, MCFE, WCFM and MCFM extracts. The highest percentage of α-amylase assay 60.29±0.64, 55.87±0.32, 55.45±0.43 and 54.87±0.22 was observed at 500 µg/ml concentration of WCFE, MCFE, WCFM and MCFM respectively. The IC_{50} value of the extracts were 383.43, 432.8, 422.5 and 458.61 µg/ml respectively. The result of the present study indicates that all the four extracts showed the maximum α-glucosidase inhibiting activity at 500 µg/ml concentration with IC_{50} value 417.61, 464.45, 451.55 and 471.44. *Cadaba fruitcosa* extracts could remarkably inhibit the activity of α-amylase and α-glucosidase. These results provide a rational for the use *cadaba fruticosa* to treat diabetics and inflammatory. The effect of wild and micropropagated plant on anti-inflammatory and anti-diabetic activity are almost similar, so instead of wild plant, the micropropagated plant can be used for further use.

Keywords: Micropropagated *Cadaba fruitcosa* (WCF), Wild *Cadaba fruitcosa* (WCF).

INTRODUCTION

Inflammation is a natural response of the mammalian body to a variety of hostile agents including parasites, pathogenic microorganism, toxic chemical substance and physical damage to tissue. Diabetics mellitus has very long history; it first points to the medical test of sexual ancient cultures over 2000 years ago. Worldwide around 135 million people were affected from diabetes in the year 1995 and it will increase to 300 million in the year 2025[1,2]. Due to changing in lifestyle, food habit and consumption of fast food leads to more number of diabetes cases Worldwide. Degeneration of carbohydrates, lipid and protein metabolism generates free radical formation and free radical generation due to the defects in insulin secretion insulin action or both. It leads to increase in blood glucose level and this causes oxidation stress and as a result there is increase in mitochondrial reaction oxygen species, non-enzymatic glycation of protein and glucose autoxidation[3]. Various grades of synthetic drugs and few herbal formulation are available in the market[4,5].

Cadaba fruticosa (L.) or the Indian *Cadaba* is a medicinally important, which belongs to Capparidaceae family commonly known as 'vizhuthi' in Tamil and 'Capper bush' in English. This species is endemic on Indian Subcontinent such as Bangladesh, India, Pakistan, SriLanka and Indo-

China (Myanmar) (https:// en. wikipedia. org/wiki/*Cadaba fruticosa*). *Cadaba fruticosa* is commonly used plant in indigenous traditional medicinal systems. The leaf juice is internally used in the case of general weakness, energetic during dysentery, diarrhoea, also to relieve general body pain, antidote against poisoning, stimulant, and antiscorbutic[6,7].

MATERIALS AND METHODS

In vitro anti-inflammatory assay of plant extracts

Inhibition of albumin denaturation assay

The anti-inflammatory assay of wild *Cadaba fruitcosa* ethanol (WCFE), micropropagated *Cadaba fruitcosa* ethanol (MCFE), wild *Cadaba fruitcosa* methanol (WCFM) and micropropagated *Cadaba fruitcosa* methanol (MCFM) extracts were examined by using inhibition of albumin denaturation technique[8, 9] followed with minor modifications. 1. 5% Bovine serum albumin, 1N HCl, and Phosphate buffer saline used as reagents. The reaction mixture (0.5 ml; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of distilled water. pH was adjusted at 6.3 using a small amount of 1 N HCl. Various concentrations (50-1000 µg/ml) of WCFE, MCFE, WCFM and MCFM extracts were added to the reaction mixture and were incubated at 37ºC for 20 min and then heated at 57º C for 5 min, after cooling the samples, 2.5 mL of phosphate buffer saline was added. Percentage Inhibition was measured spectrophotometrically at 600 nm. The inhibition percentage was calculated by using the formula.

$$\text{Percentage Inhibition (\%)} = \frac{\text{(Abs Control – Abs sample)}}{\text{Abs Control}} \times 100$$

HRBC membrane stabilization method

The WCFE, MCFE, WCFM and MCFM extracts at the concentration of 50, 100, 200, 500 and 1000 µg/ml respectively, were incubated separately with HRBC solution for membrane stabilization assay[10]. Blood was obtained (2 mL) from healthy volunteers and was mixed with equal volume of sterilized AL severs solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl in distilled water) and centrifuged at 3000 rpm. Isosaline solution was used for washing the packed cells. 50, 100, 200, 500 and 1000 µg/ml concentrations of extracts were prepared in normal saline, Aspirin was used as standard and control. All the assay mixtures were incubated at 37º C for 30 min and centrifuged at 3000 rpm for 20 min and haemoglobin content of the supernatant solution was estimated spectrophotometrially at 560 nm. The various extracts HRBC membrane stabilization or protection percentage was calculated by using the following formula:

$$\text{Percentage stabilization} = \frac{\text{(Abs Control -Abs sample)}}{\text{Abs Control}} \times 100$$

In vitro anti-diabetic assay

α-amylase inhibition assay[11]

Different concentrations of the WCFE, MCFE, WCFM and MCFM extracts and 0.02 M sodium phosphate buffer 500 µl (pH 6.9 with 0.006 M NaCl) with porcine pancreatic α-amylase enzyme (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25◦C for 10 min. After incubation, 500µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to the reaction mixture. Subsequently, the reaction mixture was incubated at 25° C for 10 min and 1.0 ml of dinitrosalicylic acid (DNSA) was added. The reaction was stopped by incubating the mixture in boiling water for 5 min and subsequently cooled at room temperature. With 10 ml of distilled water the reaction mixture was diluted, and the absorbance was measured at 540 nm in a spectrophotometer. Except the sample, the reagents mixture and the enzyme was used as a control. The inhibitory activity data was expressed in percentage. The IC50 value was calculated by fifty percent inhibition concentration of the sample extracts.

α-glucosidase inhibition assay[12]

Starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extracts (WCFE, MCFE, WCFM and MCFM) were incubated for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method.

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and As is the absorbance of the sample. The inhibitory activity was determined by incubating a solution of 100µl of α-glucosidase (0.5 mg/ml) in 0.1 M phosphate buffer (pH 6.9) solution and various amounts of plant extracts (200-1000 µg/ml)

were incubated at 25 ◦C for 10 min. After incubation, 50 µl of 5M p-nitrophenyl-α-D-glucopyranoside in 0.1M phosphate buffer (pH6.9) solution was added and incubated at 25◦C for 5 min. By using spectrophotometer the absorbance was taken at 405 nm. Except the sample, the reagents mixture and the enzyme was used as a control and the results of α-glucosidase inhibition activity were expressed in terms of inhibition percentage. The required concentration of the plant extracts to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at 100, 200, 300, 400 & 500 µg/ml of the extract.

RESULTS

Anti-inflammatory assay

The anti-inflammatory assay of WCFE, MCFE, WCFM and MCFM extracts were recorded through albumin denaturation and HRBC membrane stability activities.

Inhibition of albumin denaturation

The values obtained through anti-inflammatory assay of four extracts were evaluated through albumin denaturation assay by checking the ability of the extracts to inhibition protein denaturation and presented in the table 1 and figure 1. All the extracts were very effective in inhibiting heat induced albumin denaturation and percentage inhibition at 50-1000 µg/ml concentrations showed dose dependent increase. The inhibition percentage varied from 12.09±0.45 to 60.82±0.34, 10.11±0.37 to 57.28±0.92, 8.6±0.99 to 54.62±0.85, 9.48±0.11 to 53.29±0.25 for lowest concentration (50 µg/ml) to highest concentration (1000 µg/ml) of WCFE, MCFE, WCFM and MCFM extracts respectively. The control aspirin value also increased with increasing concentrations (17.25±0.56 to 62.57±0.39). The highest inhibition percentage (60.82±0.54) was observed in WCFEfollowed by MCFE (57.28±0.92), WCFM (54.62±0.85) and MCFM (53.29±0.25) extracts at 1000 µg/ml concentration. Whereas the inhibition percentage of control Aspirin was 62.57±0.39 at 1000 µg/ml concentration. The amount required for fifty percent inhibition (IC_{50}) was found to be 880.04, 750.42, 670.31 and 610.21 for WCFE, MCFE, WCFM and MCFM and IC_{50} value of control Aspirin was 570.32 (Fig. 3)

HRBC membrane stability activity

The extracts WCFE, MCFE, WCFM and MCFM reveled significant anti-inflammatory activities by assessing through membrane stabilization assay, which are shown in table 2 and figure 2. Notable activities (68.55%) was recorded by control Aspirin at 1000 µg/ml concentration. All the plant extracts exhibited excellent anti-inflammatory activities at highest concentrations (200-500 µg/ml). Noteworthy activities were recorded for WCFE, MCFE, WCFM and MCFM with 62.15, 58.28, 57.24 and 54.28 respectively at 1000 µg/ml concentration. The extracts at the concentration of 50, 100, 200, 500 and 1000 µg/ml were incubated with HRBC solution individually and the hemolysis data was compared with control Aspirin.

Among these extracts the WCFE with 62.15. MCFE with 58.28, WCFM with 57.24 and MCFM with 54.28 percentage inhibition was observed at 1000 µg/ml concentration respectively. Both wild and micropropagated plant sample extracts activity was almost similar. The IC_{50} values were found to be 324.14, 880.15, 840.08 and 710.11 for WCFE, MCFE, WCFM and MCFM extracts. When compared with Aspirin IC_{50} value 356.11 (Fig. 3).

Anti-diabetic assay

α-amylase inhibitory assay

A significant inhibitory action on α-amylase enzyme was observed from the WCFE, MCFE, WCFM and MCFM extracts. The different concentrations of the extracts and the activity of the extracts were presented in table 3 and figure 4.

It clearly shows that the extracts exhibited concentration dependent increase in inhibition percentage. The highest percentage 60.29±0.64, 55.87±0.32, 55.45±0.43 and 54.87±0.22 was observed at 500 µg/ml concentration of WCFE, MCFE, WCFM and MCFM respectively against the control with 81.57±1.14. The IC_{50} value of the extracts were 383.43, 432.8, 422.5 and 458.61 µg/ml respectively against the control Acarbose 162.04 µg/ml (Fig. 6).

α- Glycosidase inhibitory assay

Table 4 and figure 5 exhibited the inhibition percentage of various concentrations of WCFE, MCFE, WCFM and MCFM extracts against α-Glycosidase enzyme. The inhibition percentage was more at higher concentration of extracts. The highest inhibition percentage of WCFE (63.22±0.15), MCFE (60.26±0.40), WCFM (58.59±0.77), MCFM (57.29±0.62) and control acarbose (85.12±2.01) was observed at 500 µg/ml concentration. The second best concentration in term of inhibition was at 400 µg/ml. Among the five concentration tried the lowest percentage of inhibition was observed at 100 µg/ml concentration in all the four extracts. Inhibition percentage was more in ethanol extract when compared to methanol extract. The value of control acarbose was also increasing

with increase in concentration from 100-500 µg/ml. The lowest inhibition percentage was 45.21±0.61 at 100 µg/ml concentration and highest 85.12±2.01 at 500 µg/ml concentration. The IC50 value of standard acarbose was 130.2, where as 417.61, 464.45, 451.55, 471.44 against WCFE, MCFE, WCFM and MCFM extracts respectively (Fig. 6).

DISCUSSION

The inflammation related disorders affected majority of the human population world wide. Due to the unwanted secondary effects of present pain killer are not useful in all the cases[13, 14]. Generally the folk and traditional medicine practitioners used many medicinal plants for inflammation. Such medicinal plants are having active principles or compounds for inflammation[15,14]. In the present study WCFE, MCFE, WCFM and MCFM extracts were used for anti-inflammatory studies by albumin denaturation assay and membrane stabilization assay. A number of non-steroidal anti-inflammatory agents inhibit heat induced lysis of erythrocytes, probably by stabilizing the cell membrane. This is the basic for HRBC membrane stability test[16]. Some of the herbal preparation were capable of stabilizing the RBC membrane and this may be evidence for their potential to extract anti-inflammatory activity[17]. The extracts or drugs may be attached with the erythrocytes membranes and alter the surface charges of the cells. This may arrest physical relation with aggregating agents or encourage dispersal by repulsion of similar charges which are involved in the red blood cells haemolysis. Reports says that some of the secondary metabolite exerted extensive stabilizing effect on lysosomal membrane and also possess ability to bind caution leads to erythrocyte and other biological macromolecules stabilization[18]. It was noted that WCFE, MCFE, WCFM and MCFM extracts exhibited higher membrane stabilizing activities of 62, 58, 57 and 54 percentage compared to that of standard aspirin, which displayed 68% at 1000 µg/ml concentration respectively. All the extracts has rich source of plant chemicals and exhibited good anti-inflammatory activity with IC_{50} values 880.04, 750.42, 670.31 and 610.21 against the standard 570.32 respectively. Similar activities were observed in both wild and micropropagated plant extracts. This may be due to the presence of almost similar phytochemicals in both the cases.

The protein denaturation as one of the well documented causes of inflammatory. Dose dependent activity of inflammatory drugs was observed against thermally induced protein denaturation[19]. Many literature related to plant extracts showed similar effects[20]. The heat denaturation is a reaction between protein and water. The denaturation of protein leads to the production of auto antigens and this is the cause of rheumatoid arthritis[21,19]. The denaturation mechanism is involved in alternation of electrostatic force, hydrogen, hydrophobic and disulphide bond[22]. In the present study also the extracts WCFE, MCFE, WCFM and MCFM showed higher protein denaturation inhibition 60.82, 57.28, 54.62 and 53.29 percentage at 1000 µg/ml concentration respectively..

Phytochemicals present in both wild and micropropagated plant extracts revealed a better anti-inflammatory activity with IC_{50} values 321.14, 880.15, 840.08 and 710.11 for control aspirin356.11. Almost similar results were observed on both wild and micropropagated plant extracts. It shows that both the plants have similar secondary metabolites and the micropropagated plant can be used for medicinal purpose. In general the anti-inflammatory activities may be due to the presence of phenolic compounds such as N-Hexadecanoic acid, Octadecanoic acid, Dodecanoic acid, Trans-13-octadecenoic acid, Lupeol and1,6,10,14,18,22-Tetracosahexaen-3-OL, 2,6,10,15,19,23-hexamethyl-, (all-e)[22-25].

Diabetics is a non-infectious or non transmissible disease generally genetic in nature but it can develop due to the life style also. The present day medicines are not constructive to treat diabetes and also until now no successful treatments discovered globally[26,27]. The safer and economical antidiabetic drug can be obtained from many medicinal plants. Reviewed and listed around 47 species belonging to 29 families with alpha Glucosidase inhibitors[28]. Biological activities of the plants are mainly based on the chemical constituents like phenolic, alkaloids, flavonoids, terpenoids, coumanins glycosides. The conventional drugs used to treat diabetes such as metformin are obtained from plants[29]. In the present research investigation WCFE, WCFM, MCFE and MCFM extracts of *C. fruticosa* are evaluated for their anti-diabetic activity through α-amylase and α-glucose inhibitory activity.

α-amylase is an intestinal enzyme, play a vital role in digestion of carbohydrates and absorbance of glucose. Pharmacological active compound present in the plant suppress the effect of α-amylase and various direct and indirect effects of several blood parameters responsible for the occurrence of diabetes[30]. All the four extracts WCFE, MCFE, WCFM and MCFM exhibited higher percentage of inhibition 60.29±0.64, 55.87±0.32, 55.45±0.45 and

54.87±0.22 at 500 µg/ml concentration. The significant antidiabetic activity was comparable to that of standard drug inhibition. This is in agreement with the other reports on *Ceropegia juncea*[14]. The IC_{50} value of WCFE, MCFE, WCFM and MCFM are 383.43, 432.8, 422.5 and 458.61 µg/ml against control acarbose162.04 µg/ml respectively.

α-glucosidase enzyme present in the intestine breaks disaccharides into absorbable monosaccharaides. Whereas α-glucosidase inhibits the disaccharide digestion and impedes the postprandial glucose excursion to enable overall smooth glucose profile[31]. The medicinal plants are good source of such inhibitory therapy motivating to explore biologically active compounds from the plants. The result of the present study indicates that all the four extracts (both wild and micropropagated ethanol and methanol) showed the maximum α-glucosidase inhibiting activity at 500 µg/ml concentration with IC_{50} value 417.61, 464.45, 451.55 and 471.44 against control 130.12. The findings related to antidiabetic activity of *C. fruticosa* confirmed by the previous findings in this species by using Wistar rats with ethanol and aqueous leaf extracts[32]. The percentage of inhibition was more in ethanol extract when compared to methanol extract in wild and micropropagated samples. Whereas the inhibition percentage was more in wild plant extracts when compared to micropropagated plant extract. Similar results were also observed in anti-diabetic activity of *Ceropegia juncea in vivo* and *in vitro* plant ethanol and methanol extracts[14]. Further studies are needed to isolate and characterize the compounds responsible for the activity.

The wild and micropropagated plant extracts inhibit effectively both α-amylose and α-glucosidase in dose dependent manner. All the extracts exhibited almost similar activity invariably. The effect of wild and micropropagated plant on antidiabetic activity are almost similar, so instead of wild plant, the micropropagated plant can be used for further use.

CONCLUSION

The outcome of this research work reaffirms the *cadaba fruticosa* to treat diabetics and inflammatory. Similar observation of anti-inflammatory and anti-diabetic activity was observed in both wild and micropropagated plants. So the micropropagated plants can be used instead of wild plants.

Table 1: Anti-inflammatory activity (Albumin denaturation assay) of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

Concentration (µg/ml)	WCFE	MCFE	WCFM	MCFM	Aspirin
50	12.09 ± 0.45	10.11± 0.37	8.56 ± 0.99	9.48 ± 0.11	17.25±0.56
100	21.87 ± 0.83	17.28± 0.48	17.35± 0.30	15.29± 0.20	25.28± 0.27
200	34.45 ± 0.12	28.28± 0.37	26.40± 0.44	24.50L±0.29	36.48± 0.57
500	46.21 ± 0.98	42.38± 0.28	41.87± 0.78	38.39 ± 0.37	47.52± 0.46
1000	60.82 ± 0.34	57.28± 0.92	54.62± 0.85	53.29 ± 0.25	62.57± 0.39

Values are expressed as mean ± SD (n=3)

Table 2: Anti-inflammatory activity (Membrane stabilization assay) of WCFE, WCFM, MCFE and MCFM extracts of *Cadaba fruticosa*

Concentration (µg/ml)	WCFE	MCFE	WCFM	MCFM	Aspirin
50	17.34±0.95	12.59±0.37	11.43±0.13	10.36±0.26	25.78±0.84
100	26.87±0.63	26.38±0.27	24.62±0.95	22.58±0.27	37.49±0.58
200	38.41±0.30	35.28±0.26	35.88±0.47	33.59±0.16	47.11±0.24
500	51.04±0.84	48.20±0.44	46.31±0.55	43.73±0.38	59.34±0.52
1000	62.15±0.21	58.28±0.73	57.24±0.20	54.28±0.40	68.55±0.56

Values are expressed as mean ± SD (n=3)

Table 3: Antidiabetic activity (Inhibition of α-amylase inhibitory activity) of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

Concentration (µg/ml)	WCFE	MCFE	WCFM	MCFM	Acarbose
100	12.30±0.22	9.11±0.10	9.12±0.82	8.45±0.34	41.21±0.41
200	25.38±0.72	18.20±0.45	22.14±0.66	17.63±0.32	53.63±0.81
300	39.28±0.73	32.54±0.60	35.10±0.50	28.50±0.65	62.39±0.95
400	47.80±0.20	41.20±0.50	44.20±0.33	39.21±0.20	74.62±1.31
500	60.29±0.64	55.87±0.32	55.45±0.43	54.87±0.22	81.57±1.14

Values are expressed as mean ± SD (n=3)

Table 4: Antidiabetic activity (α- Glycosidase inhibitory activity) of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

Concentration (µg/ml)	WCFE	MCFE	WCFM	MCFM	Acarbose
100	18.34±0.20	10.22±0.45	15.78±0.29	9.75±0.45	45.21±0.61
200	29.23±0.56	22.49±0.51	26.40±0.76	21.34±0.48	57.15±0.81
300	40.53±0.77	38.41±0.94	38.17±0.52	32.20±0.32	64.33±0.75
400	51.88±0.55	46.25±0.45	47.50±0.60	41.30±0.20	78.45±1.31
500	63.22±0.15	60.26±0.40	58.59±0.77	57.29±0.62	85.12±2.01

Values are expressed as mean ± SD (n=3)

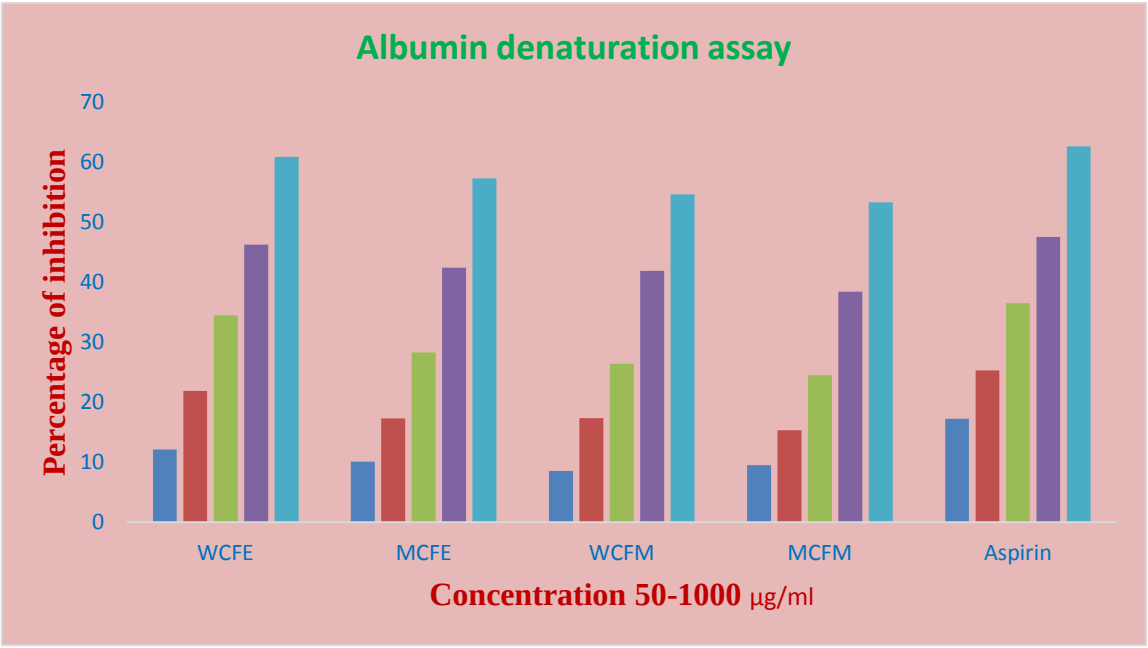

Fig. 1: Anti-inflammatory activity (Albumin denaturation assay) of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

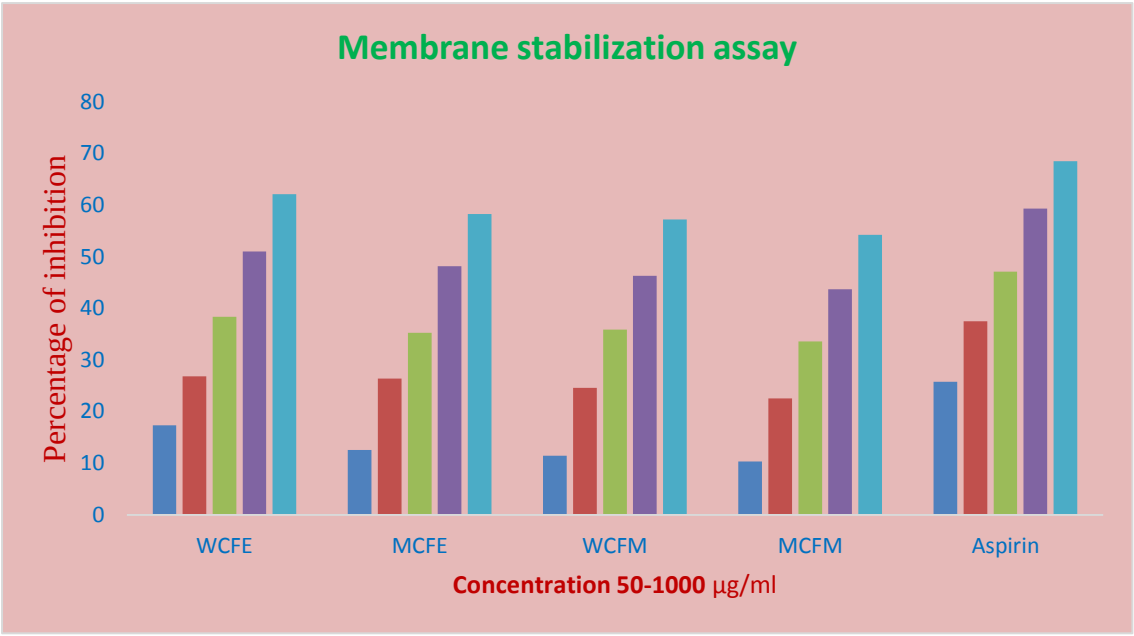

Fig. 2: Anti-inflammatory activity (Membrane stabilization assay) of WCFE, WCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

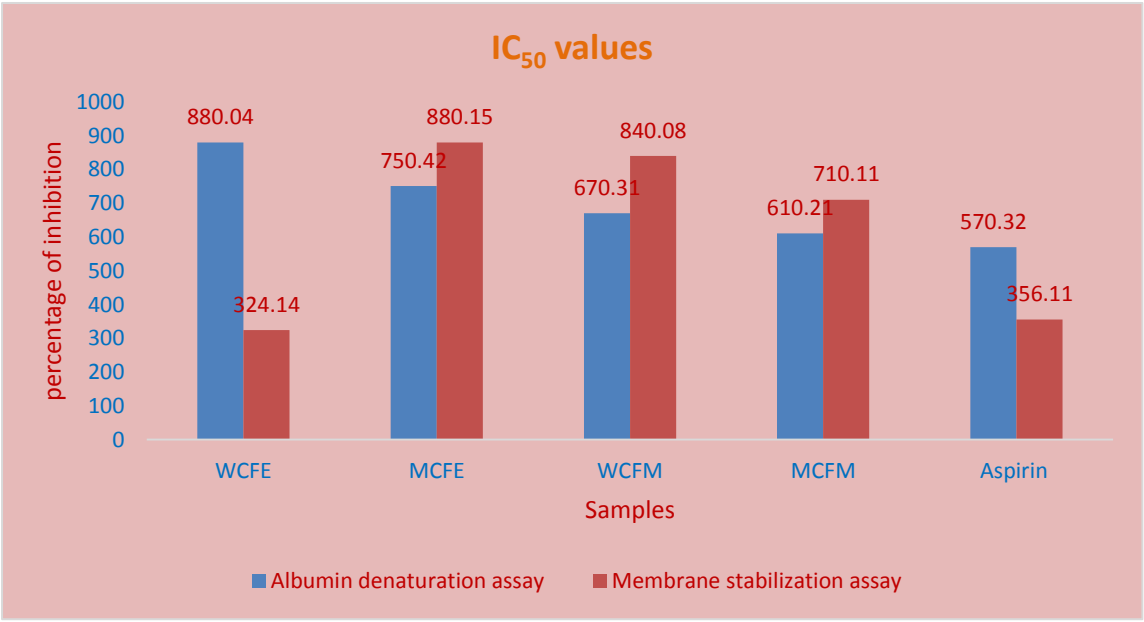

Fig. 3: Anti-inflammatory IC_{50} values of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

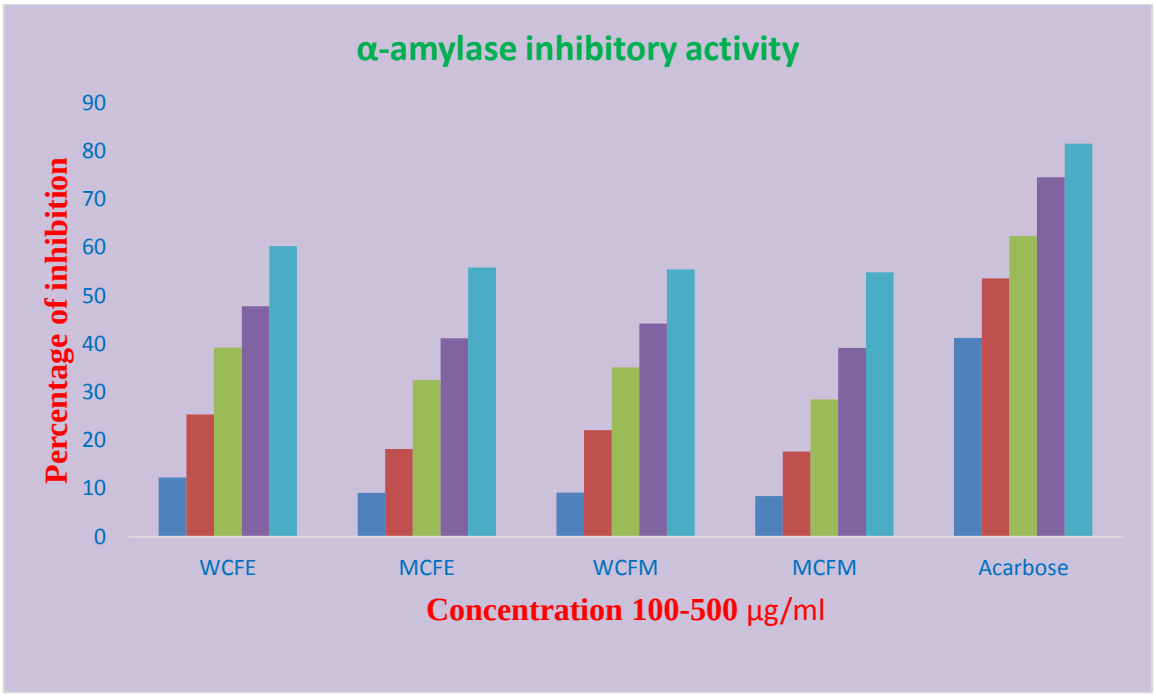

Fig. 4: Antidiabetic activity (α-amylase inhibition activity) of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

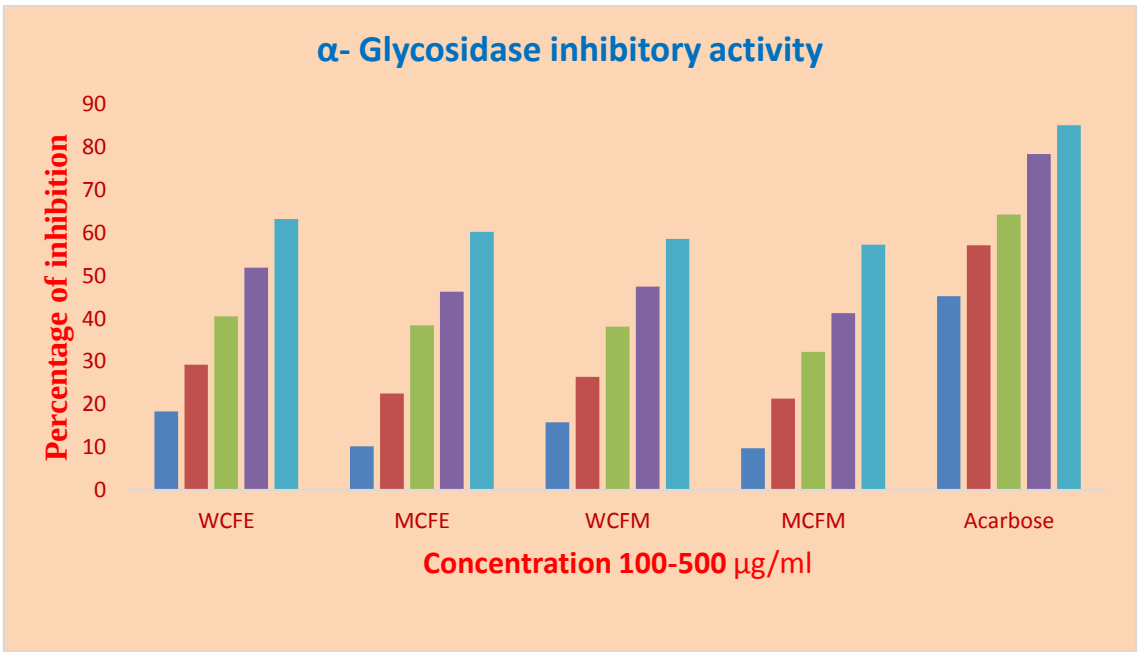

Fig. 5: Antidiabetic activity (α- Glycosidase inhibitory activity) of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

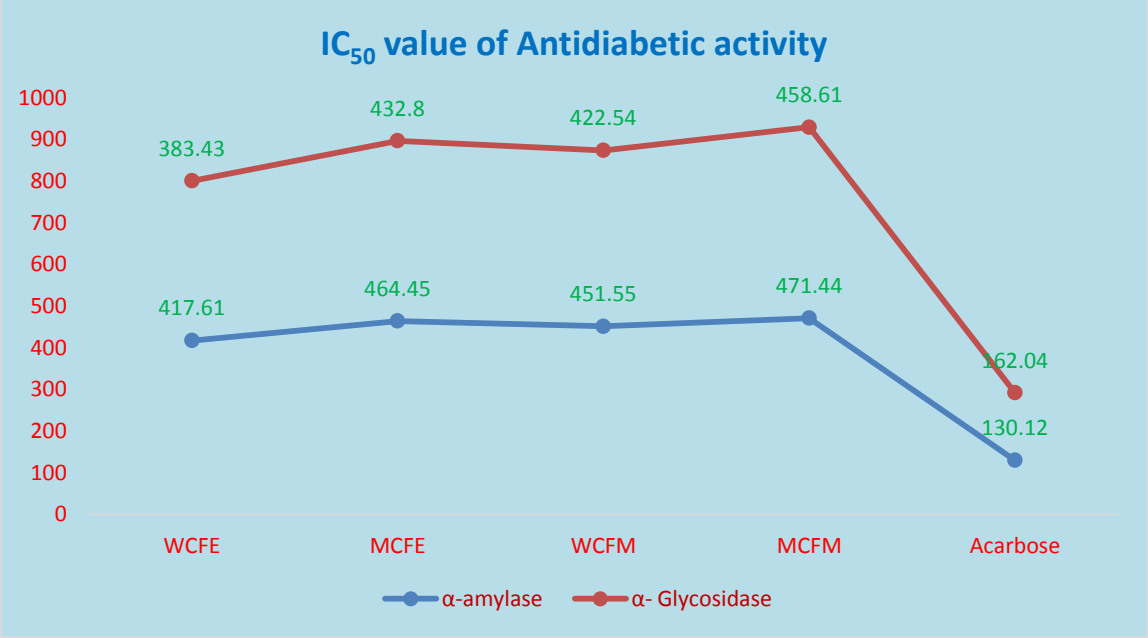

Fig. 6: IC_{50} value of Inhibition of α-amylase, α- Glycosidase inhibitory assays of WCFE, MCFE, WCFM and MCFM extracts of *Cadaba fruticosa*

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