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Research Article

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *ZiziphusJujuba*(L.) FRUIT EXTRACT

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ABSTRACT

Ziziphusjujuba L. commonly known as Ber in Pakistan, belonged to the family *Rhamnaceae* that consists of 45 genera and 550 species is widely distributed in tropical and subtropical climates in the world. *Ziziphusjujuba L* is a hardy tree of arid region which can be grown successfully in saline soil under hot, arid environment. Its fruits are palatable and delicious with a good amount of vitamin A, C and B complexes and minerals. The present study was to evaluate *in-vitro* antiinflammatory activity of *Ziziphusjujuba* fruit. The aim of the present studies was carried out in the following objectives to determine the antioxidant and anti-inflammatory activity of PFZjF extract. The results showed that the DPPH and NO radical scavenging activity increased in concentration dependent manner of PFZjF extract when compared to standard ascorbic acid respectively. The PFZjF extract showed significant anti-inflammatory activity was observed at 30 µg/ml when compared to control 39 µg/ml of Diclofenac sodium respectively. Denaturation of proteins is a well-documented cause of inflammation. From the results of present study it can be stated that the extracts of PFZjF extract are effective in inhibiting heat induced albumin denaturation. The PFZjF extract can be recommended as a potent anti-inflammatory drug which can be used for treatment of numerous diseases.

INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of protein is a well-documented cause of inflammation. Inflammation is a group of natural comeback of vascular tissue to injurious stimulus, irritants and pathogens characterize through flush, heat, bulge as well as ache. Tenderness is moreover severe or constant tenderness. Sensitive inflammation could be an early reaction of the host to dangerous stimuli. Within constant irritation, the inflammatory reaction is a way of fraction ensuing inside spoil toward the body. Currently present is require for the novel secure, effective, non-poisonous or less poisonous anti-inflammatory remedy (Deepa *et al*., 2015).

Ziziphusjujuba be a plant of the relations *Rhamnaceae* conventionally cultured with in the Mediterranean constituency, Eastern Asia, Southern along with South China. At the present time jujubes could be extensively scattered in Asia, Europe furthermore Australia, together with Slovakia. Especially the inland regions of northern china Jujuba have a widespread narration of gathering as a fruit and therapy. This is a commonly known as a Indian jujube used to treat the syphilitic ulcers, diarrhoea, asthma, stomatitis, poutice, astringent as well as the can treat the gum bleeding (Eva *et al*., 2017).

MATERIAL AND METHODS

Collection of plant material

Fresh fruit of *Ziziphusjujuba* were collected in an area Tirupattur Super mark, Tirupattur. The collected leaves fruit thoroughly washed in running tap water to remove any impurities and used for the extract preparation.

Isolation of Plant Protein (Ni *et al.,* 1996) Ten gram of fresh fruit was taken in mortar and pestle placed in an ice pack. 20

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ml of plant protein extraction buffer (100 mM potassium phosphate buffer (pH 7.8), 1mM EDTA, 10% glycerol, 1mM DTT) was added and ground until no more chunks are visible. The ground fruit extract was centrifuged at 10000 rpm for 15 min in a refrigerated centrifuge (Remi, Mumbai, India) at 4°C. The clear liquid supernatant were taken in a new tube and kept at -20°C for further use.

Estimation of Protein using Bradford Reagent

The protein content in the extracted sample was estimated according to the dye binding method of Bradford (1976).

Reagents

Coomassie Brilliant Blue (CBB) G-250	- 100.0 mg
Ethanol (95 %)	- 50.0 ml
Orthophosphoric acid (85%)	- 100.0 ml

The dye CBB G- 250 was dissolved in ethanol and orthophosphoric acid. The mixture was diluted to 1000 ml with distilled water. The dye was checked for absorbance at 595 nm in a spectrophotometer and adjusted until to get the optical density (O.D) value 1.18 with dye or distilled water. The dye was filtered through Whatman No.1 filter paper and stored in an amber colored bottle at 4^{o}C.

Procedure

One ml of the culture filtrate was added with 5 ml of the Bradford's reagent (CBB) and the intensity of the blue color that developed was read at 595 nm in a spectrophotometer. The amount of protein was determined using bovine serum albumin fraction V (Sigma, USA) as the standard.

Anti-inflammatory Activity

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess reaction (Marcocci *et al.,* 1994). The reaction mixture (3ml) containing sodium nitropruside (10mm) in phosphate buffer saline and the test protein sample was incubated at 25°C for 150min, after incubation 1.5ml of the reaction mixture was removed and 1.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% Napthylethylinediamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Percentage inhibition of nitric oxide scavenging was calculated using the formula. Percentage Inhibition = (A of Control - A of Sample)/A of Control× 100. A-absorbance.

Inhibition of albumin denaturation

Method of Mizushima and Kobayashi (1968) was followed with minor modifications. The reaction mixture was consisting of test extracts at different concentrations (10, 25, 50 and 100µl/ml) and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. Diclophenac sodium was taken as standard drug. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm.

The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

Percentage Inhibition = (A of Control - A of Sample)/A of Control x100 Concentration of proteins

Ammonium sulphate precipitation of protein

The extracted protein sample was precipitated with 85% ammonium sulphate for overnight at 4°C. The incubated sample was centrifuged using refrigerated centrifuge at 4°C for 15 min at 10000 rpm. The precipitated proteins sample was carefully taken in a 2 ml micro centrifuge tube. The precipitant sample was dissolved in 500 µl of 10 mM potassium phosphate buffer and dialyzed against same buffer for overnight. The dialyzed sample was again estimated of protein content, anti-oxidant activity.

Antioxidant activity of precipitated protein

DPPH Free radical activity

Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl). The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997). Two mL of 6 $\times 10^{-5}$ M methonolic solution of DPPH were added to 50 µl of a methonolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound [quercetin] were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration as follows:

All determinations were performed in triplicate.

The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

$$IP = [(AC(0)- AA(t)/ AC(0))] \times 100$$

Where AC (0) is the absorbance of the control at t = 0 min; and $AA(t)$ is the absorbance of the antioxidants at t = 16 min.

RESULTS AND DISCUSSION

Assessment of Protein Content of AmmouniumSulphate fraction of Ziziphusjujuba of fruit extract

Assessment of total protein content was carried out to identify the protein fraction having maximum protein content to be used for the antioxidative and anti-inflammatory studies. The maximum protein content was found to be present in 70 percent ammonium sulphate protein fraction and this protein fraction was selected for further studies.

Determination of Protein content

The protein content was determined in the extracted sample according to the dye binding method of Bradford. It was recorded that, the protein content of 1 gram was 28.2 mg. The Figure 1 showed the total protein content of the extracted sample liquid as below.

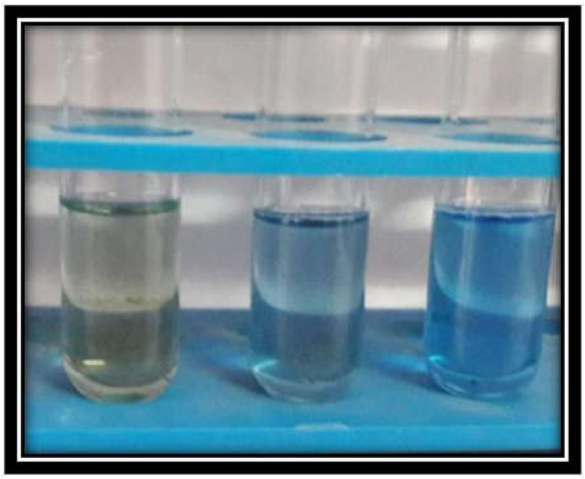

Figure 1 Protein estimation of extracted sample

Tube 1: Control; Tube 2: Protein extract; Tube 3: Dialyzed Protein Concentration of proteins

The extracted protein sample was precipitated with 70% ammonium sulphate for overnight and centrifuged to get precipitated protein. The precipitant sample was dialyzed and used for the other assays (Figure 2). The dialyzed sample was again estimated of protein content and anti-inflammatory activity. After concentration protein was estimated at 19.6 mg protein it was recovery of 70%.

Figure 2 Dialysis of ammonium sulphate precipitated protein from *Ziziphusjujuba* of fruit extract

DPPH radical scavenging activity of selected protein fraction of Ziziphusjujuba of fruit extract

DPPH is a stable free radical that accepts an electron or hydrogen radical to become stable diamagnetic molecules. It involves the reaction of specific antioxidants with a stable 2,2diphenyl -1- picrylhydrazyl (DPPH). The percentage of DPPH radical scavenging efficacy of PFZjF was exhibited a maximum dose dependent DPPH activities when compared to control ascorbic acid were respectively. The Figure 3 shows the percentage free radical scavenging activity of selected protein fraction of *Ziziphus jujube* of fruit extract.

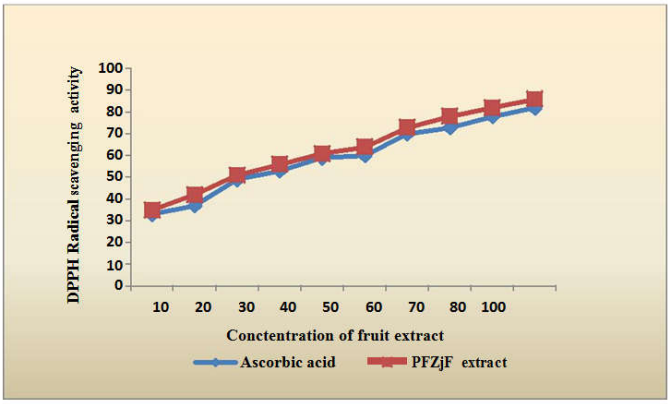

Figure 3 Percentage Free Radical Scavenging Activity of Selected protein fraction of *Ziziphus jujube* of fruit extract

The protein fraction of *Ziziphusjujuba* of fruit extract showed the dose dependent DPPH radical scavenging activity. From the graph, the IC_{50} effective concentrations was found to be 29µg and 34µg and used in the further studies. The revealed that the radicals and their scavenging systems play important role in the healing of normal and delayed types of wounds. The dose response of DPPH radical scavenging activity of the extract and standards showed the maximum concentration.

Nitric oxide scavenging activity

Nitric oxide is a very unstable species under aerobic condition it reacts with O2 to produce stable products, nitrate and nitrite through intermediates. The percentage of inhibitions was increased with increased concentrations of PFZjF extract. The IC_{50} value of scavenging of nitric oxide of PFZjF extract was found to be 28µg and 35 µg of control ascorbic acid respectively. The result of the nitric oxide scavenging ability is shown in Figure 4.

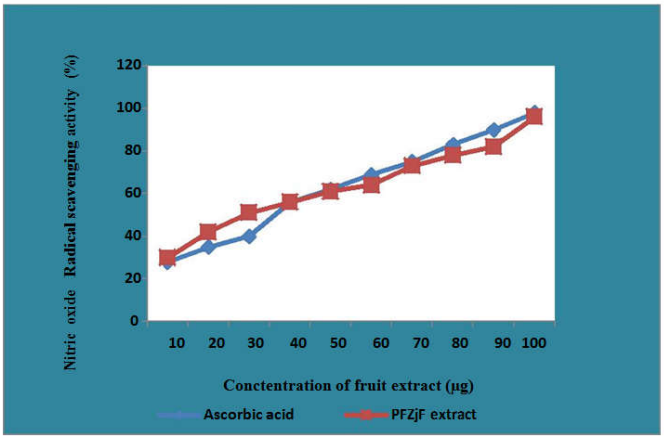

Figure 4 Nitric oxide scavenging activity of Protein Fraction of *Ziziphusjujuba* of fruit extract

Nitric oxide plays an essential role in various inflammatory processes but the overproduction of nitric oxide contributes to various diseases. The toxicity of NO increases significantly when it responds with superoxide radical, forming the highly reactive peroxynitrite anion (Biswas *et al.,* 2016).

Protein Denaturation inhibition assay

The protein denaturation inhibition assay was carried out for the anti-inflammation effect of the extracted proteins was showed greatest activity when concentration increased The

highest concentration of fruit extract showed 32.42% whilst lowest concentration showed 10.74%.. The results of the Protein denaturation inhibition ability are shown Figure 5.

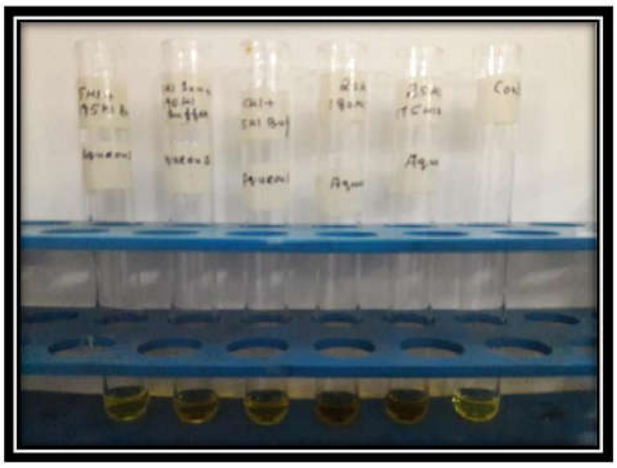

Figure 5 Protein Denaturation inhibition

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by use of external stress known as strong acid or strong base, a focused inorganic salt, an organic solvent or heat. Maximum biological proteins lose their biological purpose denatured. Denaturation of proteins is a well-documented cause of inflammation. The investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced protein (albumin) denaturation

Anti- inflammatory activity of Protein fraction of Ziziphusjujuba of fruit extract

The PFZjF extract showed significant anti-inflammatory activity was observed 30µg/ml when compared to control 39 µg/ml of Diclofenac sodium respectively. Denaturation of proteins is a well-documented cause of inflammation. From the results of present study it can be stated that the extracts of PFZjF extract are effective in inhibiting heat induced albumin denaturation. The results are shown in (Figure 6).

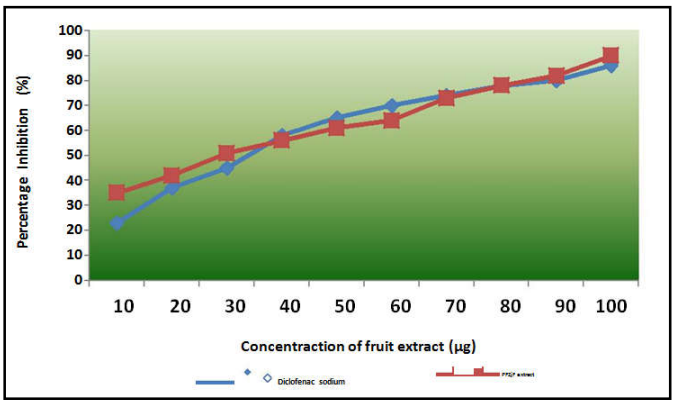

Figure 6 Anti-inflammatory activity of *Ziziphus jujube* of fruit extract

Anoop and Bindu (2015) reported that the good anti-inflammatory activity of *Syzygiumzeylanicum*(L.) The inflammatory reaction of the host is life-threatening for interruption and resolution of the transferable process but also is often responsible for the signs and symptoms of disease. It involves an intricate series of crowd responses, such as the complement, kinin, and coagulation pathways. An inability to kill or contain the microbe usually results in further damage due to progression of inflammation and infection.

CONCLUSION

It concluded that, the highest protein content (19.6 mg/g fruit) was recorded in 70 percentage saturation of recrystallised ammonium sulphate. The DPPH and NO radical scavenging activity increased in concentration dependent manner of PFZjF extract showed 29µg and 28 µg when compared to standard ascorbic acid (34 µg and 35 µg) were respectively. The anti-inflammatory activity of PFZjF extract showed effective inhibition heat induced albumin denaturation. The PFZjF extract can be recommended as a potent antioxidant and anti-inflammatory drug which can be used for treatment of numerous diseases for instance cancer, neurological disorder, aging and inflammation.

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