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PHYTOCONSTITUENTS OF *ZIZIPHUS JUJUBA* FRUIT EXTRACTS AND THEIR ANTI-OXIDANT, ANTI-INFLAMMATORY AND ANTI-BACTERIAL POTENTIAL

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Abstract

This study evaluated the phytoconstituents of *Ziziphus jujuba* fruit extracts with reference to their anti-oxidant, anti-inflammatory and anti-bacterial potential. The phytochemical components of fruit extracts from *Ziziphus jujuba* showed the presence of all phytochemicals tested except tannins and glycosides. The greatest inhibitory zone was against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* by well diffusion method. The methanol extract of the *Ziziphus jujuba* fruit exhibited a higher level of free radical scavenging activity suggesting that it has a greater antioxidant potential. According to the DPPH assay, a decreased power potential was shown by an increase in absorbance with concentration. The methanol extract demonstrated strong reducing power used in the investigation; the reported inhibition percentage was 65%. The maximum denaturation inhibition of 93.33% was reached with 50 g of egg albumin-like denaturation inhibition concentration in *Ziziphus jujuba* fruit. At concentrations of 10–50 g/mL, diclofenac was found to have a denaturation-inhibiting effect on egg albumin, whereas *Ziziphus jujuba* fruit had an identical impact.

Keywords: *Ziziphus jujuba*, Anti-oxidant, Anti-inflammatory, Anti-bacterial, Diclofenac.

1. Introduction

The fruit of *Ziziphus jujuba* Mill, which is a member of the Rhamnaceae family, is known as *jujuba*, also known as a red date or a Chinese date. *Jujuba* is a plant that originated in China and has been used in traditional Chinese medicine and as a food supplement for thousands of years [1,2,3]. *Jujuba* plants are now readily available not only in China but also in other nations like Korea, India, Japan, Europe and the US. *Jujuba* was listed as one of the exceedingly beneficial fruits in the ancient Chinese medical literature *Huangdi Neijing* (475-221 BC). According to the literature, *jujuba*'s anti-oxidative action may be attributed to both flavonoids and polysaccharides. Triterpenic acids were thought to be the main components responsible for the anti-inflammatory and anticancer effects. Moreover, jujuboside B and betulinic acid may be the active ingredients with positive effects on the cardiovascular system [4,5,6].

The biological activity research has confirmed the *jujuba*'s health advantages as a food and a therapeutic plant. They are frequently used in alternative medicine to enhance sleep and lessen anxiety. They have trace amounts of many vitamins and minerals, but are especially high in vitamin C, an essential nutrient with immune-stimulating and antioxidant characteristics [7,8,9].

Antioxidants are substances that can stop and undo harm from too many free radicals. To enhance the quality of sleep and the functioning of the brain, *jujuba*s are commonly used in alternative medicine. New research reveals that these effects may be caused by their special antioxidants. *Jujuba* may improve immune function and inhibit the spread of cancerous cells. Reduced levels of free radicals and inflammation can aid in the prevention of chronic conditions like type 2 diabetes. The vitamin C-rich *jujuba* fruit is also known to have potent anticancer effects. *Jujuba* fruit is delicious and tiny. They taste like dates when dried and have a chewy texture. Although loquats are frequently grown from seeds, commercial plantings typically use grafted trees of superior types. Shield budding and cleft grafting are the two methods used to reproduce the tree; loquat seedlings or quince rootstocks grown from cuttings can be used, the latter if a miniature tree is required [10,11,12]. The blossoms are relatively vulnerable to fire blight, but the trees are resistant to the majority of illnesses and insect pests. Fruit production begins six to eight years after planting and continues to increase until the tree is fifteen to twenty years old. Indian custom dictates that after the hard seeds are removed and

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the fruits are dried in the sun, the dried flesh is crushed with tamarind, red peppers, salt, and jaggery. Fresh entire ripe fruit is crushed with the aforementioned ingredients in various regions of the Indian state of Tamil Nadu to create cakes known as ilanthai vadai or regi vadiyalu in Telugu. It is frequently eaten as a snack as well. The fruit is consumed fresh with salt and chilli flakes in Northern and Northeastern India. It is sometimes candied, jammed, or pickled with oil and spices. Hence, considering the above facts in view, this study evaluated the phytoconstituents of *Ziziphus jujuba* fruit extracts with reference to their antioxidant, anti-inflammatory and anti-bacterial potential.

2.Experimental Section

2.1.Preparation of aqueous and solvent Ziziphus jujuba fruit extracts

The stored fruit powder of *Ziziphus jujuba* (10 g) was extracted with 100 ml of respective solvents namely aqueous, ethanol, chloroform, and methanol. After the extraction process, the solvents were removed by soxhlet method and evaporated by open air at 40°C to obtain crude extract and stored in beaker.

2.2. Phytochemical screening aqueous and solvent fruit extracts

Extracts Phytochemical screening of *Ziziphus jujuba* fruit extracts were assessed by standard method as described by Savithramma *et al.* (13,14).

Test for Tannins: One ml. of the fruit extract was added to 1 ml. 5% ferric Chloride Formation of dark blue or greenish black indicates the presence of tannins.

Test for Quinones: One ml. of the fruit extract was added to I ml. conc. Sulphuric acid. Formation of red colour indicates the presence of quinones.

Test for Flavonoids: One ml of the fruit extract was added to 1 ml. 2N sodium hydroxide. Formation of yellow colour indicates the presence of Flavonoids.

Test for Alkaloids: One ml of the fruit extract was added to 2 ml conc. HCI. Then, few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides: One ml of the fruit extract was added to 3 ml Chloroform and 10% ammonium solution. Formation of pink colour indicates the presence of glycosides.

Test for Terpenoids: One ml of the fruit extract was added to 2 ml Chloroform along with cone, sulphuric acid. Formation of red brown Colour at the interface indicates the presence of terpenoids.

Test for Phenols: One ml of the fruit extract was added to 2 ml. distilled Water followed by few drops of 10% $FeCl_3$. Formation of blue/green colour indicates the presence of phenols.

Test for Steroids: One ml of the fruit extract was added to 2 ml. chloroform and 1 ml sulphuric acid. Formation of reddish brown ring at interface indicates the presence of steroids.

2.3.Antibacterial activity of aqueous and solvent extracts

Aqueous and solvent extracts of *Ziziphus jujuba* fruit were tested against pathogenic bacterial strains namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* The bacterial cultures were grown in Mueller Hinton Agar and Broth (Himedia) (15). Antibacterial activity was measured using diffusion disc plates on agar, About 0.1 ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth (Hi media) for 24 hours at 37°C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper discs (6 mm in dia were placed on the agar medium to load leaf extracts (100 µL) of *Ziziphus jujuba*. Inhibition diameters were measured after incubation for 24 to 48 hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

2.4.Antioxidant activity of fruit extracts

The antioxidant activity of aqueous and solvent extracts of *Ziziphus jujuba* was determined by following and Shi *et al.* (16). About 100 µL of fruit extracts of *Z. jujuba* were taken in the microtiter plate, 100 µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

2.4.1.Free radical scavenging activity of fruit extract

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Fruit extract of 100 µL were mixed with 2.7 ml methanol and then 200 µL of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (16). Subsequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan at 517 nm. Free radical scavenging activity was calculated by the following formula:

% DPPH radical scavenging =[(Abs.of control-Abs.of test Sample)/(Abs. of control)] x 100.

2.5.Anti-inflammatory activity by egg albumin denaturation assay

Inhibition of egg albumin denaturation was determined using the method prescribed by Chandra *et al.* (17). Phosphate buffer saline (pH 6.4), 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 800 ml of distilled water was prepared. The pH was adjusted to 6.4 using 1N hydrochloric acid (HCl)

and made the volume to 1000 ml with distilled water. About 2.8 ml of phosphate buffer (pH 6.4) and 0.2 ml of egg albumin were incubated with various concentrations (10, 20, 30, 40 and 50 µg/ml) of test samples and standard drug Diclofenac sodium (10, 20, 30, 40 and 50 µg/ml) and the samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultraviolet visible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula.

Percentage inhibition = (Abs control − Abs sample)/ Abs control × 100

Table 1. Phytochemical screening of *Ziziphus jujuba* fruit extract.

S.No.	Phytochemicals	Methanol	Chloroform	Ethanol	Aqueous
1.	Alkaloids	+	+	+	+
2.	Saponins	+	+	+	+
3.	Tannins	-	-	+	+
4.	Glycosides	-	-	+	+
5.	Flavonoids	+	+	+	+
6.	Phenols	+	+	-	-
7.	Steroids	+	-	-	-
8.	Terpenoids	+	+	+	-
9.	Quinones	+	-	-	-
10.	Carbohydrate	+	+	+	+

’ + Present; ‘-’- Absent.

3. Results and Discussion

3.1. Phytochemical screening of fruit extract

For the sake of human health, secondary metabolites provide essential medicinal qualities. In particular, some of these compounds appear to be capable of preventing and suppressing many types of cancer. Compounds belonging to the carbohydrate, alkaloids, quinones, and steroid families are utilized as medications or dietary supplements to treat or prevent various disorders. *Ziziphus* jujuba fruit were gathered from Tirupattur for this investigation. The fruits were thoroughly cleaned in distilled water after being washed with running tap water, and they were then allowed to dry in the open air for about a month at room temperature. In order to be used later, the dried fruit material was thoroughly pulverized into powder and stored in a sterile container. *Ziziphus jujuba* fruit powder that had been stored was extracted using 100 ml of each of the two solvents, methanol and chloroform. To get crude extract, the solvents were eliminated following the extraction procedure using air drying and an evaporator set at 40°C. *Ziziphus jujuba* fruit extract phytochemical screening was evaluated using a conventional procedure as described by Savithramma *et al.* (13,14). The phytochemical components of fruit extracts from *Ziziphus jujuba* are listed in Table 1. Except for tannins and glycosides, all of the phytochemical components tested were found in the methanol extract compared to all other solvent and aqueous extracts.

3.2. Antibacterial activity of methanolic fruit extract

Throughout the beginning of human civilization, people have employed plants as medicine. The use of plants to treat diseases was inevitable, as is clear from the problems with synthetic antibiotics. Many researchers studied *Ziziphus jujuba* leaves using a variety of polar chemical solvents, including high (Methanol). Qualitative analysis and antimicrobial activity were examined. The greatest
inhibitory zone was against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* were 19 mm, 18 mm, 16 mm, and 13 mm when methanol extracts were compared to the well diffusion method.

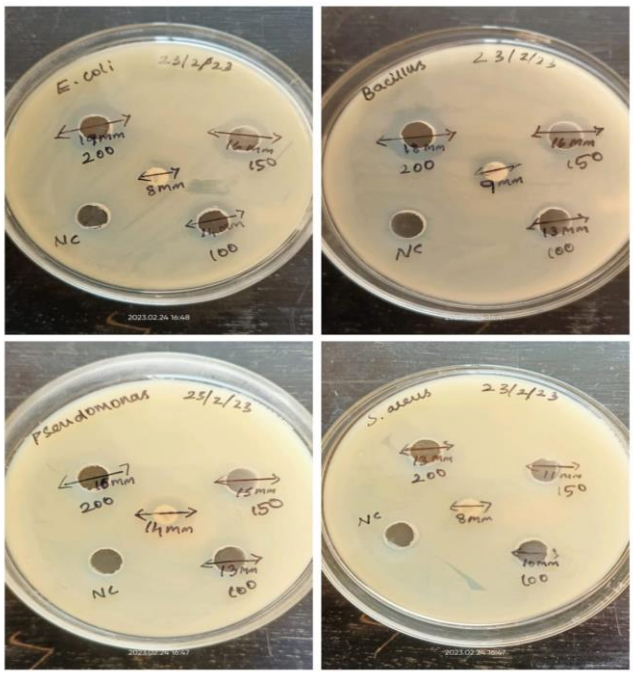

Fig. 1. Antibacterial activity of methanolic *Ziziphus jujuba* fruit extract.

3.3. Antioxidant activity of fruit extract

Antioxidants are substances that can stop the chain reactions caused by free radicals. Recently, increased focus has been placed on the therapeutic potential of medicinal plants as antioxidants and re-antioxidants in avoiding tissue damage brought on by oxidative stress. It

has been demonstrated that they can bind heavy metal ions, remove free radicals and active oxygen species, and stop lipid peroxidation by inhibiting lipoxygenase. Recently, increased focus has been placed on the therapeu tic potential of medicinal plants as antioxidants and re-antioxidants in avoiding tissue damage brought on by oxidative stress. The antioxidant activity was assessed using the DPPH assay. The methanol extract of the *Ziziphus jujuba* fruit was shown to have a higher level of free radical scavenging activity suggesting that it has a greater antioxidant potential.

Table 2. Antibacterial activity of methanolic *Ziziphus jujuba* fruit extract.

S.No.	Organisms	DMSO	Gentamycin	Zone of inhibition		
				100 µg/mL	150 µg/mL	200 µg/mL
1.	*Pseudomonas aeruginosa*	-	14 mm	14 mm	13 mm	15 mm
2.	*Escherichia coli*	-	8 mm	8 mm	14 mm	10 mm
3.	*Bacillus subtilis*	-	9 mm	9 mm	13 mm	16 mm
4.	*Staphylococcus aureus*	-	8 mm	8 mm	10 mm	11 mm

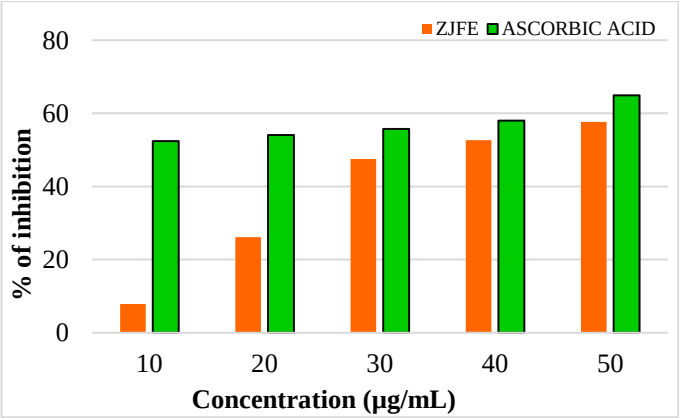

Fig. 2. Antioxidant activity of *Ziziphus jujuba* fruit extract.

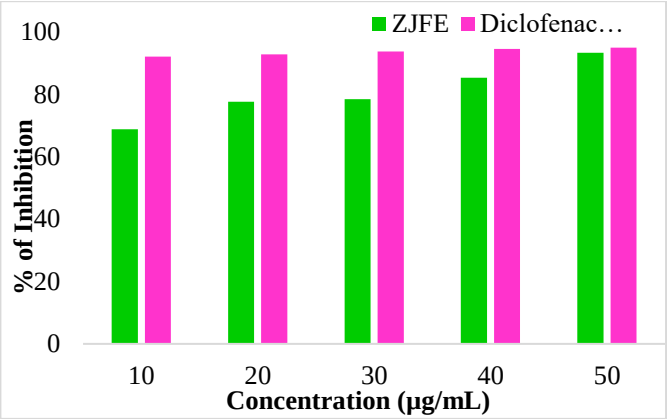

Fig. 3. Anti-inflammatory activity of *Ziziphus jujuba* fruit extract.

According to the DPPH assay, a decreased power potential was shown by an increase in absorbance with concentration. The methanol extract demonstrated strong reducing power used in the investigation; the reported inhibition percentage was 65% (Fig. 2).

3.4. Anti-inflammatory activity of fruit extract

The inhibition of egg albumin denaturation was assessed spectrophotometrically at 660 nm for *Ziziphus jujuba* concentrations between 10 and 50 g/ml, Diclofenac, and their interactions. *Ziziphus jujuba*'s lowest dose of 10 g reduced denaturation of egg albumin protein. There was a progressively rising percentage of denaturation inhibition as *Ziziphus jujuba* concentration increased. The maximum denaturation inhibition of 93.33% was reached with 50 g of egg albumin-like denaturation inhibition concentration in *Ziziphus jujuba* fruit. At concentrations of 10–50 g/ml, diclofenac was found to have a denaturation-inhibiting effect on egg albumin, whereas *Ziziphus jujuba* fruit had an identical impact (Fig. 3).

4. Conclusions

The Methanolic *Ziziphus jujuba* fruit Extract (ZJFE) showed the presence of phytochemical constituents like alkaloids, saponins, flavonoids, phenols, steroids, terpenoids, quinones and proteins except tannins and glycosides. Different concentrations (100, 150 and 200 µg/ml) of the methanolic ZJFE were tested against the pathogenic bacterial strains and it exhibited good antibacterial activity. The methanolic ZJFE extract showed significant antioxidant activity in the DPPH assay.

The methanolic ZJFE showed good anti-inflammatory activity by Albumin denaturation method compared to the standard drug Diclofenac.

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Conflict of Interest: Nil

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