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Artículo Original

In Vitro Screening for Antimicrobial and Antioxidant of a meditational plant: *Ziziphus jujube* leaves

Detección *in vitro* de actividades antimicrobianos y antioxidantes de una planta meditacional: Hojas de Ziziphus jujube

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Abstract

The current study explore was designed for the investigation of antimicrobial and antioxidant activity of crude methanol extract of the plant *Ziziphus jujuba.* The crude methanol extract and other extracts showed considerable total antioxidant activity and reducing capacity. In DPPH scavenging test, the crude extract showed 83.02% scavenging having IC_{50} of 22.23 $\mu g/ml$. Amongst the fractions, petroleum ether fraction demonstrated the highest DPPH scavenging activity with IC_{50} of 62.45 $\mu g/ml$, as long as, chloroform extract exhibited the lowest activity with IC_{50} of 32.13 $\mu g/ml$ and N-hexane fraction with IC_{50} of 58.45 $\mu g/ml$. The presences of endogenous substances in *Ziziphus jujuba* that may act as antioxidant were established by measuring the total content of phenolic and flavonoid activities. In antimicrobial activities, the petroleum ether and methanol extract showed significant activity of 15-18 mm and 11-16 mm zone of inhibition against *Bacillus cereus* and *micrococcus luteus* respectively.

Keywords: *Ziziphus jujube;* Antimicrobial activities; DPPH scavenging; Antioxidant.

Resumen

El estudio actual fué diseñado para la investigación de la actividad antimicrobiana y antioxidante del extracto de metanol crudo de la planta *Ziziphus jujuba*. El extracto metanólico crudo y otros extractos mostraron una considerable actividad antioxidante total y capacidad reductora. En la prueba de eliminación de DPPH, el extracto crudo mostró una eliminación del 83,02 % con una IC_{50} de 22,23 µg/ml. Entre las fracciones, la fracción de éter de petróleo demostró la mayor actividad de eliminación de DPPH con IC_{50} de 62,45 µg/ml, mientras que el extracto de cloroformo exhibió la actividad más baja con IC_{50} de 32,13 µg/ml y la fracción de N-hexano con IC_{50} de 58,45 µg/ml. . La presencia de sustancias endógenas en *Ziziphus jujuba* que pueden actuar como antioxidante se establecieron midiendo el contenido total de actividades fenólicas y flavonoides. En actividades antimicrobianas, el éter de petróleo y el extracto de metanol mostraron una actividad significativa de 15-18 mm y 11-16 mm de zona de inhibición contra *Bacillus cereus* y *micrococcus luteus* respectivamente.

Palabras clave: *Ziziphus jujube*; Actividad antimicrobiana; DPPH; Antioxidante.

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INTRODUCTION

In India, herbs have been considered as a natural form of medicine [1], plants have got remedial belongings to exist of different complicated chemical compounds of various substances that wear secondary metabolites such as saponin, terpenoids, flavonoids, phenolic, and alkaloids phytochemicals in various each of parts of the plants [2]. *Ziziphus jujuba* (Rhamnaceae) called as jujube [3], or Chinese date, or red date, as widely used as a food [4] and Today's, there is a widespread distribution of jujube-related products in the world. Ziziphus jujuba commonly known as Bor, it is traditionally used as tonic and aphrodisiac [1]. *Ziziphus jujube* has multiple pharmacological activities for these instances, Hypnotic-sedative [5], Sweetness inhibitors [6-7], Cancer (chemotherapy) [8-11], Antimicrobial activity [12-14], Antiulcer activity [15], Anti-inflammatory and Antispastic effect [16-17], Antiallergic [18], Permeability enhancement activity [19], Cognitive activities [20], Antifertility/contraceptive property [21], Hypotensive and Antinephritic effect [22], Cardiovascular activity [23], Immunostimulant effects [24], Antioxidant effects [25-26], and Wound healing activity [27-29].

Associated compounds include thiamine, ascorbic acid, riboflavin-bioflavonoids, and Pectin-A [30], as well as a variety of chemical agents. Amphibine-H; Mauritine-A; Mucronine-D; Nummularine-B; Jubanine-A; Jubanine-B; Sativanine-E [31-33], Frangufoline [34-35], Scutianine-C; Scutianine-D; Jubanine-C and Ziziphine-A to Q [36], Adouetine-X [37], 3-O-cis-p-coumaroylalphi 3-O-a-L-rhamnopyranosyl-3-O-a-L-rhamnopyranosyl-3-O-a-L-rham (1-2) jujubogenin, -aarabinopyranosyl-20-O-2,3-di-O-acetyl-a-L-rhamnopyranosyl 3-O- ((2-O- alpha – D – furopyranosyl – 3-O- beta – D –glucopyranosyl) – alpha – L – arabinopyranosyl) jujubogenin, as well as (6"'-sinapoylspinosin, 6"'-feruloylspinosin, and 6"'-p-coumaroylspinosin.

METHODS AND MATERIALS

Accumulation of sample/formulation of extract

Herb was selected for particular investigation, *Z. jujube* fresh leaves were collected during the month of February 2017, for the identification purposes of plant, and then the plant was taxonomically identified, help was taken from Bangladesh National Herberium, Mirpur, Dhaka for further reference.

Drying and Grinding of Plant Materials

Freshly leaves of *Z. jujube* were collected and cleaned entirely of dusty ingredients. Therefore, For a day's leaves were dried through directly sun and crushed to be made powder by the dried leaves, and was labeled for further use.

Solvent-solvent partitioning of crude extract

500 g powdered plant materials with 1.5 L methanol (98%) were mixed into bottle (amber-colored glass) for around 14 days and mixtures solvent was purified via cotton and by filter paper. At 50°C temperature, the extracts were subjected to the rotary evaporator to evaporate methanol. The methanolic extract was fractioned sequentially, fractioned at first n-hexane afterward chloroform, and lastly petroleum ether balanced according to Kupchan method with slightly modification [41]. Re-filtered the methanol extract and its fractions were evaporated to eliminate extra solvent and gained n-hexane (2. 95 g), chloroform (4.36 g), and petroleum ether (5.89 g).

Determination of total phenolic

By Singleton and Rossi, the method as described was used for determined the total phenolic content (TPC) of *Z. jujube* leaves extract and fractions [42]. Plant extract/fractions (with concentration of 1000 µg/mL and distilled water (diluted 1000-fold) were mixed with Folin-Ciocalteu reagent (0.75 mL). incubation period followed by 5 minutes, sodium carbonate (0.06%) solution was added, after that, at 22°C incubated followed by 90 minutes. Furthermore, to evaluate the absorbance of the solution by using a spectrophotometer (at 725 nm) against a reagent blank (excluding plant extract/fractions or standard). The resultant of dried sample were measured as mg of gallic acid equivalents (GAE)/g.

Determination of Total Flavonoids

Total flavonoids content of various extractives of *Z. jujuba* was evaluated by Dewanto *et al.* [43] and reference standard was used (Ascorbic acid). The 0.1 g/mL of herb extract or reference standard of different solution was taken into the test tube, 5 ml of methanol was added into the tube afterwards 200 µl of 10% $AlCl_3$ solution was added, and 5 mL of distilled water. At 25°C temperature, test tube was incubated to a 30 minute for completion of the reaction. Therefore, at 510 nm, the absorbance of the solution was measured by using a blank. The total flavonoid content was calculated below following the equation;

$$C = (c \times V)/m$$

Reducing Power activity

By Oyaizu with minor modifications [44], the reducing power activity (RPA) of *Z. jujube* leaves extracts and its fractions was described. Plant extract/fractions or standard (ACA) of 1.0 mL of solution of different concentrations was taken into the test tubes and 0.5 mL of 200mM/L trisodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide was mixed. The reaction was incubated to 20 minutes at 50 ^{0}C and then2.5 mL of trichloroacetic acid (10%) was added to the mixture to complete the reaction which was centrifuged for 10 min at 650 rpm. Then was mixed of 0.5 mL distilled water and 0.1 mL of ferric chloride, off the upper layer of the solution. After that the solution was measured at 700 nm for the absorbance by using a spectrophotometer against a reagent blank that excluding plant extract/fractions or standard.

Antioxidant Activity

Through using 98% methanol at 1mg/mL concentration, a solution of each obtained plant extract/fractions were made in order to antioxidant activity. 0.5 mL of *Z. jujube* leaves

Total Antioxidant Activity

According to Prieto *et al.*, with slightly adjustments, was evaluated the total antioxidant activity (TAA) of different concentration of extractives of *Z. jujube* leaves extract and its fractions [45]. 0.5 mL of *Z. jujube* extract/fractions or standard [(+)-catechin, CTC] mixture of different concentration was taken into a test tubes with 3 mL of reagent solution containing 0.6 M sulfuric acid, afterwards 28 mM sodium phosphate, 4 mM ammonium molybdate was appended into the test tube. A spectrophotometer (at 695 nm), the absorbance solution was estimated against a reagent blank that excluding plant extract/fractions or standard.

DPPH Radical Scavenging Activity

The DPPH (1, 1- diphenyl-2-picrylhydrazyl) free radicals scavenging activity of different concentration of extractives of *Z. jujube* was described Fresin *et al.* [46], with minor adjustment. Plant extract/fractions or standard of 2 mL solution of different concentration was added to 3 mL 0.02% of methanol solution of DPPH which was mixed very well, and it was incubated in a dark place followed by 30 min. Eventually, at 517 nm the absorbance of the solution was evaluated by using a spectrophotometer against a blank reagent that excluding plant extract/fractions or standard. % of DPPH free radical scavenging following equation was used:

$$(\%) = [1 - (A/A_o)] \times 100$$

Antimicrobial Screening

Rios *et al.*, [47] used the disc diffusion technique to test the antibacterial efficacy of Z. jujube extract against sic bacteria. Bacterial strains for the experiment were obtained from the University of Jahangirnagar in Bangladesh. Each of the four extracts was dissolved in the appropriate solvent before being applied to sterile 400g/disc filter paper and dried to remove the leftover solvent. As a positive control, standard antibiotic kanamycin (30 mg/disc) was utilized.

All extracts were tested against three Gram-positive bacteria (*Micrococcus luteus, Bacillus cereus, Staphylococcus aureus*) and three Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*). The antimicrobial activities were calculated by the respective zone of inhibition in millimeters.

Statistical Analysis

The tests were done in three times or triplicate and whole resultant were denoted as mean by ± SD. Every experiments (whole data) were explored followed by Student's supervisor and student's test. SPSS 14.0 (Chicago, IL, USA) and Microsoft Excel 2010 (Roselle, IL, USA) were used for the statistical analysis and graphical evaluations. The data of significance was thought about at $P < 0.05$.

RESULTS

Evaluation of Total Phenolic Content

On the basis of the standard curve for gallic acid (Y= 0.0003x + 0.0317; R^2 = 0.9985) were evaluated the *Z. jujube* extract and its fractions which is explained in mg of GAE/g of dried sample demonstrated of the following figure-1. The crude methanolic extract of *Z. jujube* extract shown the maximum value (75.14±0.95 mg of GAE/g), of total phenolics. Others, CLF (Chloroform fraction) , NHF (N-hexane fraction) and PEF (Petroleum ether fraction) that were exhibited 68± 0.002, 55.12±0.25 and31.08 ± 0.39 mg of GAE/g of dried extract, respectively.

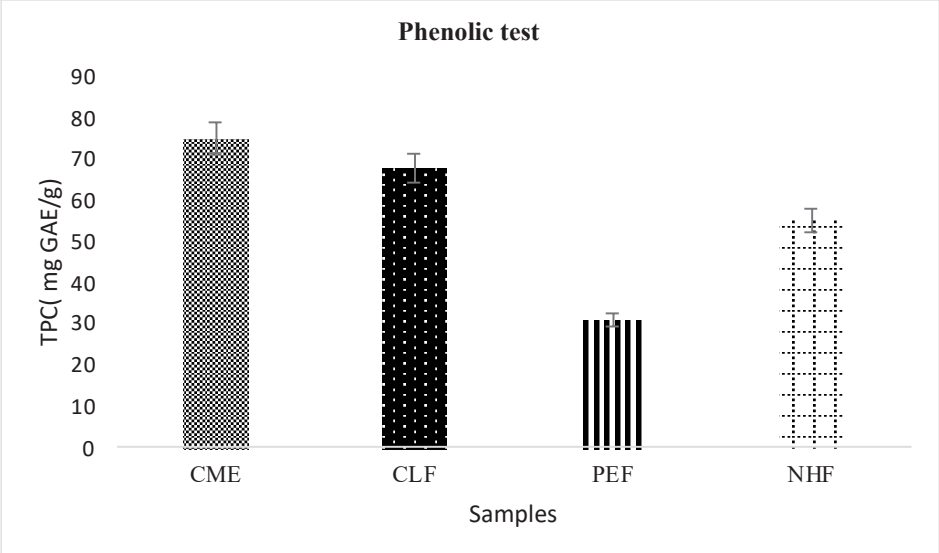

Figure-1. TPC of *Z. jujube* leaves extract and its fractions.

Determination of TF (Total Flavonoids)

The TF of leaves extract of *Z. jujube* and their fractions were demonstrated as per standard curve for ascorbic acid (y= 0.0019x + 0.0576; R^2 = 0.9895) which is expressed in mg of ACE/g of dried sample demonstrated of the following figure-2. Furthermore, the petroleum ether fraction of *Z. jujube* leaves shown the highest value (80.14±0.95 mg of ACE/g of dried extract) of total flavonoids, and CLF, NHF and PEF that were exhibited 53.08± 0.39, 51.12±0.25 mg of ACE/g of dried extract, respectively.

Values were demonstrated as per same procedure that evaluated of Total Phenolic Content.

Determination of DPPH Radical Scavenging Activity

The crude methanolic extract and their fractions of *Z. jujube* leaves, the highest potent activity was resulted in crude methanolic extract, IC_{50} value of 46.55±2.25 mg/mL that was shown the following figure-3. CLF, NHF, and PEF were activity found such as 32.13±1.95, 58.45±2.45 and 62.45±2.95 mg/mL respectively.

Determination of Total Antioxidant Activity

At 400 µg/mL (the maximum concentration), the absorbance of CME and PEF were 2.263±0.0045 and 1.842±0.0025 respectively that is demonstrated in figure-4 and CME exhibited the highest, total antioxidant activity (TAA) followed by CLF, and NHF which were 1.69±0.478, and 1.324±0.001 respectively at maximum concentration.

Determination of RPA

The reducing power activity of methanolic extract of *Z. jujube* leaves and its fractions activity is given in figure-5. The increased concentration of the extract and fractions with the increased the reducing power activity of extract and fractions. PEF exhibited the highest iron reducing power activity was 2.025±0.008, reducing power activity (RPA) followed by CME, CLF, and

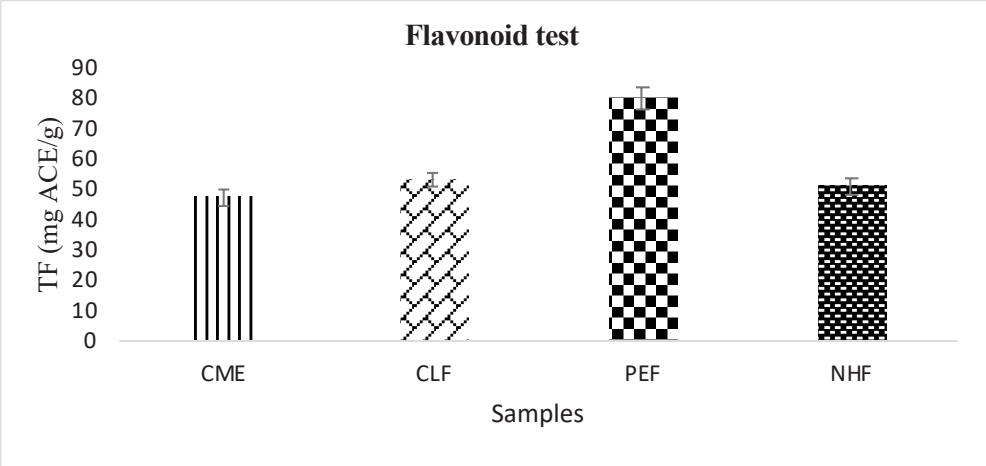

Figure-2. TF of *Z. jujube* leaves extract and its fractions.

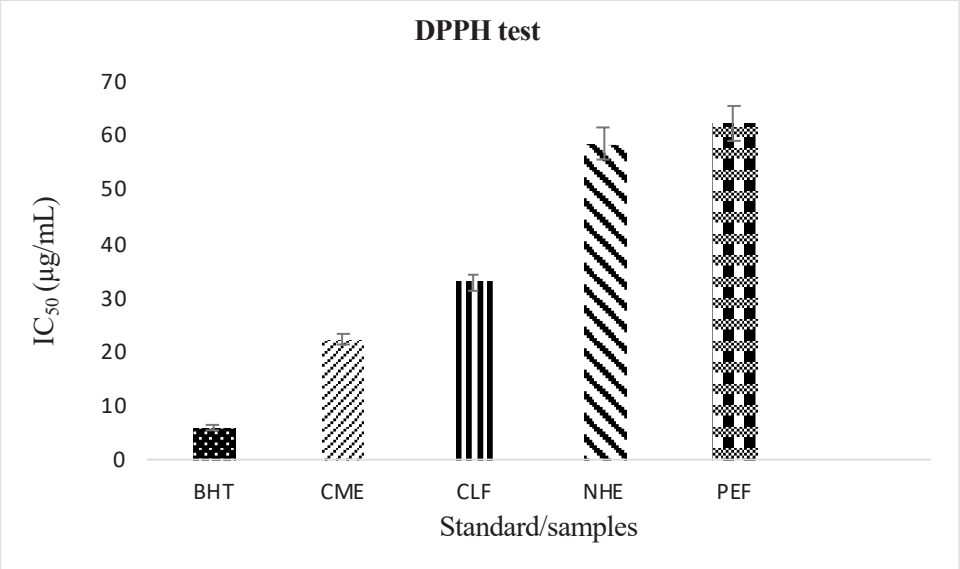

Figure-3. IC_{50} values of *Z. jujube* leaves and its fractions.

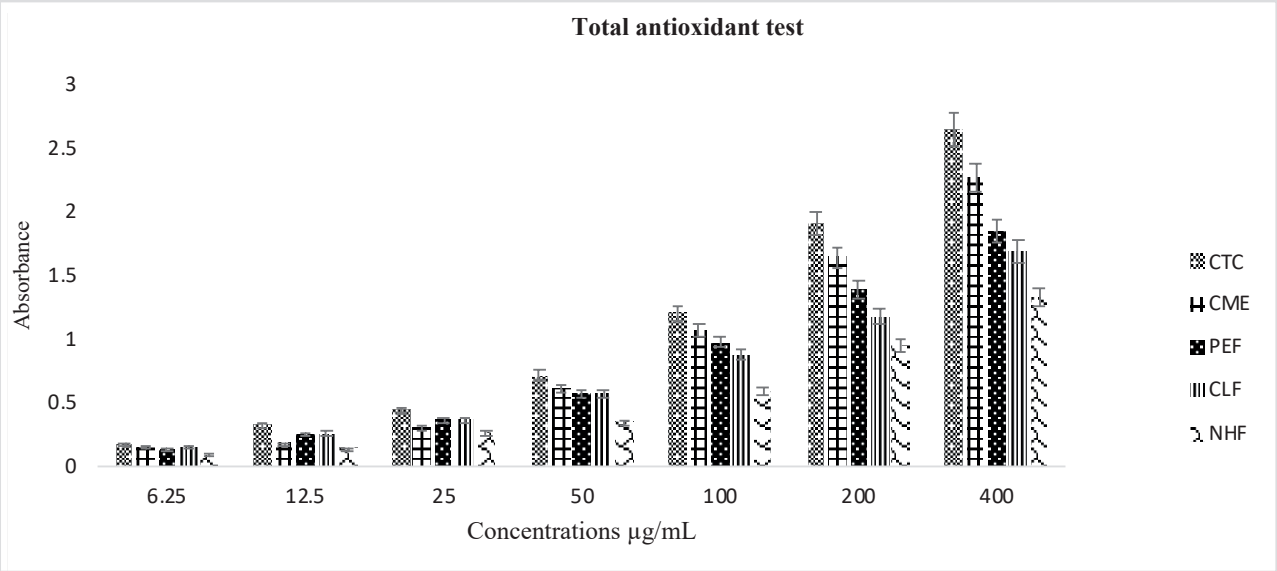

Figure-4. At various concentrations of Total Antioxidant Activity of crude extract and their fractions of *Z. jujube*.

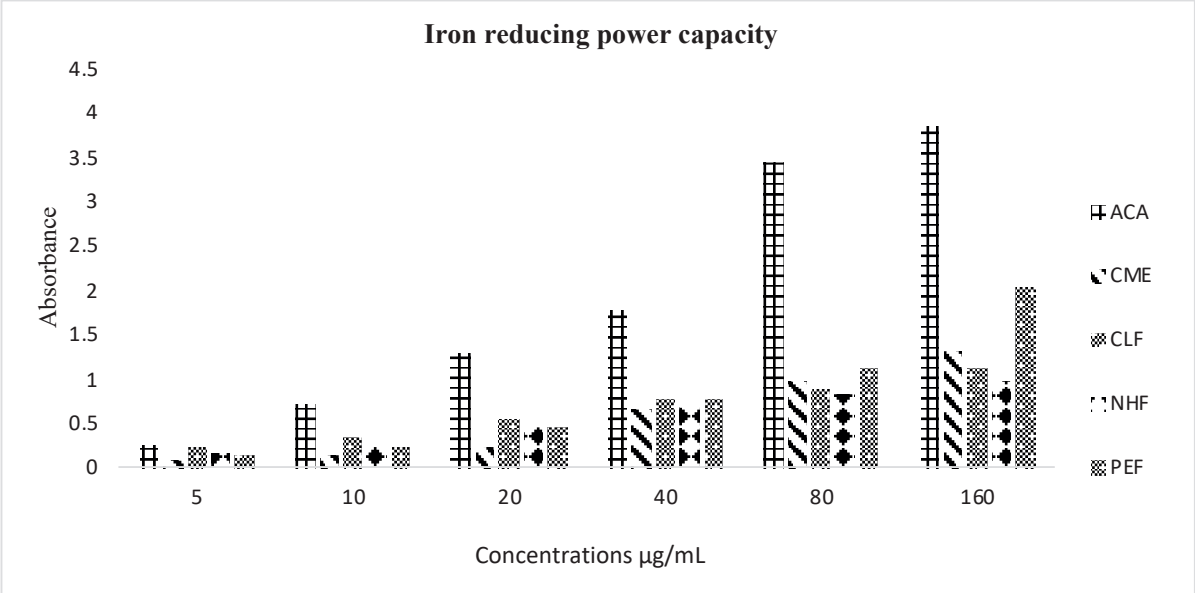

Figure-5. *Z. jujube* extract and their fractions at various concentrations RPA.

NHF which were 1.302±0.256, 1.102±0.001, and 0.964±0.001 respectively at maximum concentration (160 µg/mL).

Antimicrobial Screening

The antibacterial activity of *Z. jujuba* crude methanol extract and its fractions against Gram-positive and Gram-negative bacteria was found to be moderate. In a disc sensitivity test, the zone of inhibition ranged from 6 to 18 mm, while conventional kanamycin (30 g/disc) exhibited 19 to 28 mm. Despite this, the maximum activity was discovered against *Bacillus cereus* and *E.coli*, with an 18mm zone of inhibition and a 500 g/disc concentration per disc. In addition, *Micrococcus luteus* and *Pseudomonas aeruginosa*, as well as *Staphylococcus aureus*, were discovered to have zones of inhibition of 13mm and 16mm, respectively, and *Staphylococcus aureus* was shown to have a zone of inhibition of 12 mm. The petroleum ether fraction of *Z. jujube* leaves was tested for antibacterial activity against a variety of microorganisms (see Table 1).

DISCUSSION

The importance of medicinal values of different plants have been traditionally received, over the last few decades [48]. Wide range of secondary metabolites produced by medicinal plants that are known to have antioxidant effects, several diseases of pathogenesis are blocked by secondary metabolites of medicinal plants and the activity of matureness involving through the free radicals.

Table-1. Antibacterial activity of *Z. jujube* leaves.

Gram positive bacteria					
Name of microorganism	**Conc. (30µg\disc)**	**Zone of inhibition (mm)**			
		CME	**NHF**	**PEF**	**Kanamycin(30µg\disc)**
Micrococcus luteus	250	16	6	11	24
	500	13	7	16	
Bacillus cereus	250	6	7	18	23
	500	7	9	15	
Stapylococcus aureus	250	7	8	13	28
	500	9	9	11	

Gram negative bacteria					
Name of microorganism	**Conc. (30µg\disc)**	**Zone of inhibition (mm)**			
		CME	**NHF**	**PEF**	**Kanamycin (30µg\disc)**
Escherichia coli	250	8	6	9	19
	500	11	8	11	
Pseudomonas aeruginosa	250	6	9	8	25
	500	10	7	10	
Klebsiella pneumoniae	250	12	6	7	21
	500	9	7	9	

In this article, antioxidant activities of *Z. jujube* leaves carried out followed by using different tests. In various medicinal plants found abundantly antioxidant agents and the most significance of phenolic compounds, phenolic compounds have various fundamental activities appending sticking material contributing phenolic polymers to cell wall polysaccharides, regulation of cell growth, cell division, protection against pathogens [49]. In this study, crude methanolic extract of *Z. jujube* leaves remarked highest TPC as well as its fractions, phenolic compounds insufficient in medicinal plants following to the antioxidant properties [50]. Flavonoids, phenolic compounds, and reducing agents of combined effects in the plant extracts are well narrated through TAA [51]. Crude methanolic extract of *Z. jujube* leaves illustrated highest TAA. An earlier study of antioxidant activity of *A bunius* L. leaf et al., remarked rising outcome [52]. Herein, highest iron reducing capacity in petroleum ether fraction to crude methanolic extract of *Z. jujube,* the higher absorbance of petroleum fraction mentions the possible potent reducing power capacity, and the extracts reducing power is caused probably to the biologically active compounds in the extract that carry out strong electron donating capabilities. The study of antioxidant profile of *U. littoralis* provided good action [53]. In DPPH study, the highest radical scavenging activity found in crude methanolic extract of *Z. jujube* leaves. Ibrahim et al., antioxidant activity of *Z. jujube* leaves was given the information comparable finding almost [54]. All the being said, substitute natural and synthetic antioxidants maybe benefits. In addition, the extracts showed moderate antibacterial activity where range of zone inhibition was from 6-18 mm.

CONCLUSION

The present study revealed antioxidant activity of methanol extracts, petroleum ether, chloroform, N-hexane extracts of leaves of *Z. jujuba*. Here, extracts should mild to moderate antibacterial activity. Furthermore, potent antioxidant activity of the extracts was confirmed by the scavenging DPPH radical, where petroleum ether extract was found with highest antioxidant property. As a result, *Z. jujuba* can be studied further to isolate and explain the bioactive components responsible for the improved activity.

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