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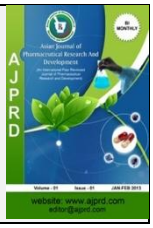

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Research Article

Anti Inflammatory and Antioxidant Activity of Ziziphus Jujuba Extract

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A B S T R A C T

The antioxidant activity of the leaves and fruits of *Ziziphus jujuba* and contribution in some phytochemical characteristics was obtained. Phytochemical analysis was found that *Ziziphus jujuba* leaves and Fruits were loss on drying 0.85%, 0.65%, total ash values were 5.3%, 7.6% , Acid insoluble ash value were 2.9%, 4.4%, Water soluble ash value were 0.7%, 1.1% and Foaming index were 13ml, 12ml. Phytochemical screening was found that carbohydrate present in JFE and JFA, alkaloids were present in JLE, JLA and JFE, glycosides were present in JFA, flavonoids were present in all type of extracts, steroids were present in only JLE and protein and amino acids were present in JFE and JFA. JFE possessed higher antioxidant activity with IC_{50} value of < 300 µg/ml among all extracts for both methods and higher inhibition found in DPPH method 96.32% and in ABTS method it is 98.68 %. Aqueous extract of *Ziziphus Jujuba* fruit (JFA) was exhibited excellent anti-inflammatory activity against COX-1 & Cox-2 with IC_{50} value less than <0.2mg/ml. Ethanolic extract of leaf (JLE) & Ethanolic extract of fruit (JFE) possessed moderate activity (< 0.3) against both COX-1 & Cox-2. Aqueous extract of leaf (JLA) exhibited poor activity against both COX-1 & Cox-2. Overall conclusion of anti-inflammatory study of different extracts of *Ziziphus Jujuba* leaves and fruit were possessed anti inflammatory activity and inhibition was increased with concentration of samples increased.

KEYWORDS: *Ziziphus jujuba,* Phytochemical, antioxidant activity, anti-inflammatory, COX, DPPH, ABTS

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INTRODUCTION

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood[1,2]. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration[3,4]. Numerous inflammatory mediators are synthetized and secreted during inflammatory responses of different types. Inflammatory substances are usually divided to two main categories: pro- and anti-inflammatory mediators[5]. Nevertheless, some mediators such as interleukin (IL)-12 possess both pro- and anti-inflammatory properties[6]. Among the inflammatory mediators and cellular pathways that have been extensively studied in association with human pathological conditions are cytokines, chemokines and the potent inflammation-modulating transcription factor nuclear factor κ B[7]. The practice of using plants, their parts or extracts as anti-inflammatory compounds is known since antiquity[8]. The use of plants or plant products for medicinal purposes was mostly documented in books and, lately, in an enormous number of websites (where the reliability of some of these websites must be examined carefully)[9]. In the last decades, hundreds of research and review articles were published regarding the anti-inflammatory activities of plants[10].

Free radicals are produced continuously in the cells as part of normal cellular function however excess production might play a role in pathophysiology of many disease conditions[11]. As earlier studies, reported that oxidation and free radical generation are the key risk factor of several diseases[12]. So, in case of oxidative stressed conditions the thing is clear that, free radicals developed several diseases including cancer, Alzheimer's disease, atherosclerosis, diabetes and some of the drug-induced toxicity[13]. Many

basic research studies and observational epidemiologic studies in human suggest that antioxidants can prevent oxidative damage[14]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplement[15].

In this research work, the mechanisms of catabolism of free radicals, examines their beneficial and deleterious effects on cellular activities, also highlights the potential role of the antioxidants in preventing and repairing damages caused by oxidative stress in health maintenance also anti-inflammatory activity was determined.

MATERIALS AND METHODS

Collection and Authentication of Plant: The leaves and fruits of *Ziziphus jujuba* was collected in the month of March from the local territory, Bhopal (M.P.). Collected material was authenticated by Dr. Z. U. Hasan (Professor, Department of Botany), Safia College Bhopal.

Drying and Size Reduction of Plant Material : The leaves and fruits of *Ziziphus jujuba* was dried under shade then hard seeds were removed and only dried pulp and dried leaves were pulverized to coarse powder. The coarse powder of leaves was passed through sieve No.16 to maintain uniformity and stored in cool and dry place.

Physiochemical Screening of Powders

Loss on Drying: About 10 gm. of the powdered drug was weighed in a tarred Petridish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined from calculating the initial and final weight.

Total Ash Value: About 5 gm. accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The % *w/w* of ash with reference to the air-dried drug was calculated.

Acid Insoluble Ash Value: Weighed accurately 1 gm. ash was boiled for 5 minute with 25ml hydrochloric acid by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with hydrochloric acid (5ml) and the liquid was added in to the crucible. Then the content was filtered on a previously weighed Whattman filter paper and filtrate was dried and weighed. Acid insoluble ash value was determined by calculating the % content remaining after deducting the weight of filter paper.

Water Soluble Ash Value: Weighed accurately 1 gm and boiled the ash for 5 minute with 25ml distilled water by covering the crucible with a watch-glass on water bath and then cooled. The watch-glass was rinsed with distilled water (5ml) & this liquid was added in to the crucible. The % of remaining content was reduced from initial % of ash taken (i.e. 100%) to determine the water soluble ash value.

Foaming Index: About 1 gm of coarse powder was weighed and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minutes on water bath. It was cool and filtered in to a 100 ml volumetric flask. Volume was diluted by adding required amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index.

Extraction procedure: Extraction of leaves and fruit powder of *Ziziphus jujuba* was done by Soxhlet extraction method. The procedure followed with both powders. Prepared extracts were listed below: (a) Ethanolic extract of leaves (JLE) (b) Aqueous extract of leaves (JLA) (c) Ethanolic extract of fruits (JFE) (d) Aqueous extract of fruits (JFA). Macroscopic characters e.g. color, odor, test, apperence etc. was observed

Qualitative Phytochemical Analysis of Crude Extracts

The crude extract obtained by solvent extraction was subjected to various qualitative tests to detect presence of common chemical constituents as: Alkaloids, Glycosides, Carbohydrates, Phytosterols, Saponins, Tannins, Flavonoids Proteins etc.

Antioxidant activity

Free radical scavenging by DPPH scavenging method: Free radical scavenging activity of samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 1.0 ml of sample solution with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml) was added to a 4 ml of 0.004% methanolic solution of DPPH. The absorbance was read at 517 nm after 30 min incubation at room temperature in the dark. Ascorbic acid was used as a standard. The DPPH radical-scavenging activity was calculated according to the following equation:

$$\text{DPPH scavenging activity}\,(\%) = 1 - \frac{Ai - Aj}{Ac} \text{ X } 100$$

Where, Ac was the absorbance of DPPH solution without sample (2 ml DPPH + 2 ml of 95% methanol); Ai was the absorbance of the test sample mixed with DPPH solution (2 ml sample + 2ml DPPH) and Aj was the absorbance of the sample without DPPH solution (2 ml sample + 2 ml of 95% ethanol).

Reducing power by ABTS radical scavenging method: The ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity was carried out based on the method of Gan and Latiff with some modifications. Briefly, $ABTS^{+}$ was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 16 h at room temperature in the dark. Prior to beginning the assay, the ABTS solution was diluted with methanol. One milliliter of sample solution with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml) was added to 2 ml of the ABTS solution mixed solution was observed at 734 nm. The sample absorbance was read at 734 nm after 30 min incubation at room temperature. Ascorbic acid was used as a standard. The ABTS radical-scavenging activity was calculated according to the following equation:

$$\text{ABTS} + \text{ scavenging activity}(\%) = 1 - \frac{A2 - A1}{A0} \text{ X } 100$$

Where, A0 was defined as the absorbance of control at 734 nm, and A1 and A2 were defined as the absorbance of the sample without the $ABTS^+$ solution and with added $ABTS^+$ solution, respectively.

Method of *in-vitro* Anti-Inflammatory Evaluation

COX Inhibition assay Kit: The assay was performed by using Colorimetric COX (human ovine) inhibitor Screening assay kit. Contains assay buffer, heme, enzyme COX-1and COX-2.

Preparation of Test Solutions: Accurately weighed amount of standard drug (aspirin) and novel synthesized semicarbazone derivatives was dissolved in some quantity of methanol and final volume was make up with water to produced 0.1mM, 1mM and 10mM.

COX Inhibition assay:

Principle: The COX Inhibition assay utilized the peroxidase component of the COX catalytic domain. The peroxidase activity was assayed colorimetric method by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm.

General Procedure: mixed well 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2) then added 10 µl of different concentration (0.1 mM, 1 mM and 10 mM) of each synthesized derivatives as sample or standard drug. Aspirin (acetylsalicylic acid) was used as a standard drug.

Calculation: The percent COX inhibition was calculated using following equation:

$$\text{COX inhibition Activity } (\%) = 1 - \frac{T}{C} X\ 100$$

Where, **T** = Absorbance of the inhibitor well at 590 nm. **C** = Absorbance of the 100 % initial activity without inhibitor well at 590 nm.

Statistical Analysis

Results were expressed as the mean SD (n = 3) for each analysis. Differences were estimated by analysis of variance (ANOVA), statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Morphological parameter of jujuba fruit and leaves

Table 1: Morphological parameter of jujuba fruit and leaves

Plant part	Parameter	No. of sample (n)	Average value
Jujuba Leaf	Leaf length (mm)	15	46.3
	Leaf width (mm)	15	21.2
	Leaf shape	15	Ovate
	Color	15	Green
	Odor	15	Characterstic
	Taste	15	Acrid
Jujuba Fruit	Fruit length (mm)	15	22.7
	Fruit width (mm)	15	13.4
	Fruit weight (g)	15	8.4
	Pulp weight (g)	15	6.2
	Seed weight (g)	15	2.2
	Color	15	Red
	Odor	15	Odorless
	Taste	15	Sweet

Phytochemical Screening of Powders (leaf and fruit)

Table 2:Physiochemical analysis of powders of *Ziziphus jujuba* leaves and Fruits

S. no.	Parameters	*Ziziphus jujuba* Leaves	*Ziziphus jujuba* Fruits
1	Loss on drying (%)	0.85	0.65
2	Total ash value (%)	5.3	7.6
3	Acid insoluble ash value (%)	2.9	4.4
4	Water soluble ash value (%)	0.7	1.1
5	Foaming index	13(ml)	20 (ml)

Macroscopic character of extracts of *Ziziphus Jujuba*

Table 3: Macroscopic character of extracts of *Ziziphus Jujuba*

S. No.	Parameters	Ethanolic extract of leaf	Aqueous extract of leaf	Ethanolic extract of fruit	Aqueous extract of fruit
1	Color	Green	Dark green	Brown	Dark brown
2	Odour	Characteristics	Characteristics	Characteristics	Characteristics
3	Test	Acrid	Acrid	Sweet	Sweetish
4	Physical Appearance	Semisolid	Semisolid	Semisolid	Brittle cake
5	Yield (%)	57.3 %	23.1%	48.8%	19.2%

Phytochemical screening of extracts of *Ziziphus Jujuba*

Table 4 :- Phytochemical screening of extracts of *Ziziphus Jujuba*

S. No.	Chemical Tests	Ethanolic extract of leaf	Aqueous extract of leaf	Ethanolic extract of fruit	Aqueous extract of fruit
1	**Carbohydrates**				
	i) Molisch's Test	(-)	(-)	(+)	(+)
	ii) Fehling's Test	(-)	(+)	(+)	(+)
	iii) Benedict's test	(+)	(+)	(+)	(+)
2	**Tannins**				
	i) with 5%ferric chloride solution	(-)	(-)	(-)	(-)
	ii) with 10% aqueous Potassium dichromate solution	(-)	(-)	(-)	(-)
	iii) with 10% lead acetate solution	(-)	(-)	(+)	(+)
3	**Alkaloids**				
	i)Dragendorff's Test	(+)	(+)	(+)	(-)
	ii) Mayer's Test	(+)	(+)	(+)	(-)
	iii) Hager's Test	(+)	(+)	(+)	(-)
4	**Glycosides**				
	i) Borntrager's Test	(+)	(+)	(+)	(+)
	ii) Legal Test	(-)	(-)	(–)	(+)
	iii) Baljet Test	(-)	(-)	(–)	(+)
5	**Flavonoids**				
	i) Shinoda's Test	(+)	(+)	(+)	(+)
	ii) Alkaline reagent test	(+)	(+)	(+)	(+)
	i) Iii) Lead test	(+)	(+)	(+)	(+)
6	**Steroids and Sterols**				
	i) Libermann-Burchard Test	(+)	(-)	(-)	(-)
	ii) Salkowski Test	(+)	(-)	(–)	(–)
7	**Proteins and Amino Acids**				
	i) Biuret Test				
	ii) Ninhydrin Test	(-)	(-)	(+)	(+)
	iv) Millon's Test	(–)	(–)	(+)	(+)
		(–)	(–)	(+)	(+)

(+) = Present, (–) = Absent

Antioxidant Activity

DPPH scavenging activity

Table 5: DPPH scavenging activity in the form of % inhibition

Conc. (µg/ml)	ASCO % inhibition	JLE % inhibition	JLA % inhibition	JFE % inhibition	JFA % inhibition
0	0	0	0	0	0
100	54. 53	31.12	28.32	31.11	21.74
200	62.43	40.43	30.65	44.23	34.42
300	78.65	49.52	41.94	57.83	45.86
400	87.98	71.41	59.23	71.02	57.31
500	98.32	85.32	69.43	83.92	68.37
600	99.81	86.32	71.02	96.32	81.85

ABTS radical Reducing method

Table 6.: ABTS scavenging activity in the form of % inhibition

Conc. (µg/ml)	ASCO % inhibition	JLE % inhibition	JLA % inhibition	JFE % inhibition	JFA % inhibition
0	0	0	0	0	0
100	51. 84	35.53	29.00	34.64	21.41
200	64.15	43.38	41.03	47.32	33.21
300	77.21	50.12	53.21	60.04	45.06
400	89.42	59.32	65.23	73.96	57.84
500	95.32	61.81	77.74	86.96	69.58
600	99.81	78.32	89.74	98.68	82.74

ASCO = ascorbic acid, JLE = Ethanolic extract of Jujuba Leaf, JLA = Aqueous extract of Jujuba Leaf, JFE = Ethanolic extract of Jujuba Fruit and JFA = Aqueous extract of Jujuba Fruit

Statistical Analysis of anti oxidant activity

DPPH scavenging activity

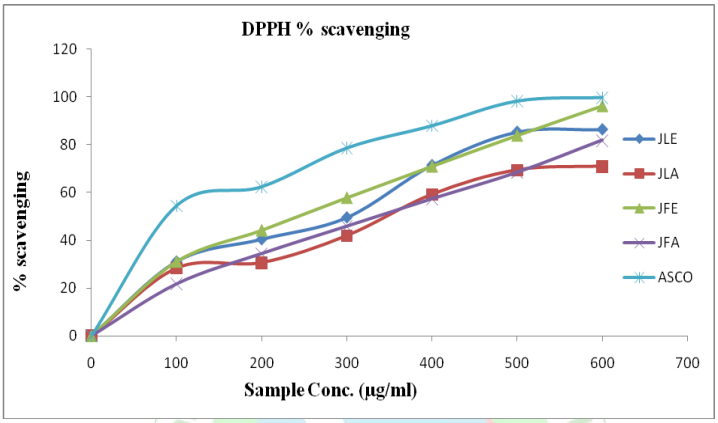

Figure 1: DPPH scavenging activity in the form of % inhibition

ABTS radical Reducing method

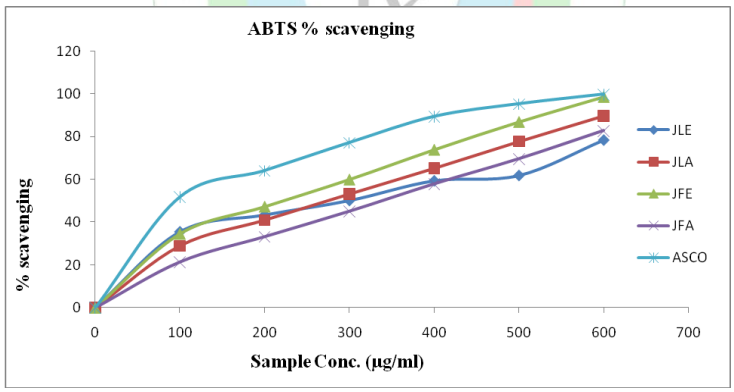

Figure 2: ABTS scavenging activity in the form of % inhibition

***In-vitro* Anti-Inflammatory Evaluation by COX- Inhibition assay**

COX- 1 Inhibition Assay

Table 7: Effect of different extracts of *Ziziphus Jujuba* on COX-1

S. No.	Code of Sample	% Inhibition in different Concentrations			IC $_{50}$ Value (mg/ml)
		0.1 mg/ml	0.2 mg/ml	0.3 mg/ml	
1	**Ethanolic extract of leaf (JLE)**	28.75 ± 0.33	36.73 ± 0.14	51.86 ± 1.75	< 0.3
2	**Aqueous extract of leaf (JLA)**	23.52 ± 1.32	35.46 ± 0.63	46.11 ± 0.75	> 0.3
3	**Ethanolic extract of fruit (JFE)**	34.43 ± 0.92	42.53 ± 0.72	56.48 ± 1.43	< 0.3
4	**Aqueous extract of fruit (JFA)**	39.97 ± 0.23	**58.46 ± 0.69**	**69.37 ± 0.12**	**< 0.2**
5	**Diclofenac sodium**	47.72 ± 0.23	76.43 ± 0.43	93.52 ± 1.23	> 0.1

Results summarized are the mean values of n = 3 ± S.D

COX- 2 Inhibition Assay

Table 8: Effect of different extracts of *Ziziphus Jujuba* on COX-2

S. No.	Code of Sample	% Inhibition in different Concentrations			IC $_{50}$ Value (mg/ml)
		0.1 mg/ml	0.2 mg/ml	0.3 mg/ml	
1	**Ethanolic extract of leaf (JLE)**	19.32 ± 0.12	25.63 ± 1.44	51.22 ± 0.74	< 0.3
2	**Aqueous extract of leaf (JLA)**	22.85 ± 0.43	32.32 ± 1.11	45.74 ± 1.85	> 0.3
3	**Ethanolic extract of fruit (JFE)**	25.86 ± 0.84	38.12 ± 0.53	53.78 ± 0.65	< 0.3
4	**Aqueous extract of fruit (JFA)**	41.63 ± 0.64	**51.23 ± 0.24**	**78.86 ± 1.43**	**< 0.2**
5	**Diclofenac sodium**	48.43 ± 1.45	64.74 ± 0.13	83.32 ± 0.22	> 0.1

Results summarized are the mean values of n = 3 ± S.D

Statistical Analysis of anti-inflammatory activity

COX- 1 Inhibition Assay

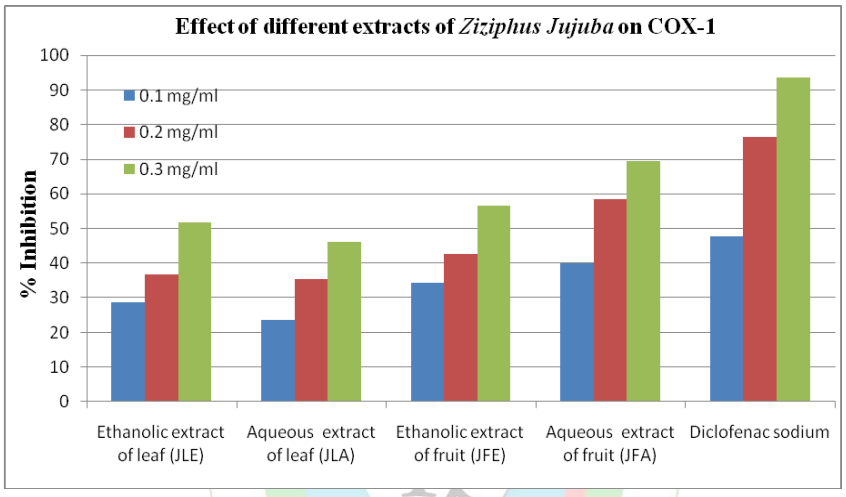

Figure 3: Effect of different extracts of *Ziziphus Jujuba* on COX-1

COX- 2 Inhibition Assay

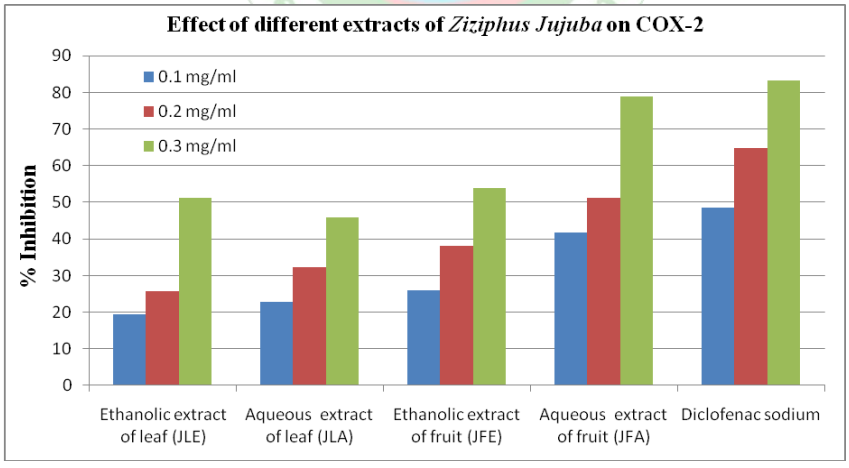

Figure 4: Effect of different extracts of *Ziziphus Jujuba* on COX-2

DISCUSSION

The leaves and fruits of *Ziziphus jujuba* was collected locally from Bhopal. All plant parts were dried under shade and hard seeds were removed from fruits. All dried parts of plant were pulverized in to coarse powder and sieved through no16 sieve.

Morphological parameters were revealed that *Ziziphus jujuba* leaves are ovate, green acrid in taste, 46.3mm in length, 21.2mm in width and fruits are red, sweet, 22.7 in length, 13.4 in width, average weight was 8.4g and pulp weight was 6.2g.

Phytochemical analysis was found that *Ziziphus jujuba* leaves and Fruits were loss on drying 0.85%, 0.65%, total ash values were 5.3%, 7.6% , Acid insoluble ash value were 2.9%, 4.4%, Water soluble ash value were 0.7%, 1.1% and Foaming index were 13ml, 12ml. Macroscopically, all four type of extracts, Ethanolic extract of leaf (JLE) was green, acrid, semisolid with yield of

57.3%, Aqueous extract of leaf (JLA) was dark green color, acrid, semisolid with yield of 23.1%, Ethanolic extract of fruit (JFE) was brown in color, sweet in taste, semisolid consistency with yield of 48.8% and Aqueous extract of fruit (JFA) was dark brown in color sweetish in taste, form brittle cake with 19.2% yield.

Phytochemical screening was found that carbohydrate present in JFE and JFA, alkaloids were present in JLE, JLA and JFE, glycosides were present in JFA, flavonoids were present in all type of extracts, steroids were present in only JLE and protein and amino acids were present in JFE and JFA.

Antioxidant activity was obtained by DPPH and ABTS scavenging method in the form of % inhibition using ascorbic acid as standard antioxidant. DPPH scavenging activity, 50% inhibition of DPPH (IC_{50} value) for ascorbic acid was found < 100 µg/ml, for JLE > 300 µg/ml, for JLA > 300 µg/ml, for JFE < 300 µg/ml and for JFA < 400 µg/ml. ABTS scavenging activity, 50% inhibition of DPPH (IC_{50} value) for ascorbic acid was found < 100 µg/ml, for JLE < 300 µg/ml, for JLA < 300 µg/ml, for JFE < 300 µg/ml and for JFA < 400 µg/ml. Overall, conclusion was that JFE possessed higher antioxidant activity with IC_{50} value of < 300 µg/ml among all extracts for both methods and higher inhibition found in DPPH method 96.32% and in ABTS method it is 98.68 %. All extracts showed % of inhibition in increasing order. Statistical analysis was also proved sentences that written above numerically.

Different extracts of *Ziziphus jujuba* leaves and Fruits were evaluated *in-vitro* for anti-inflammatory activity by kit available in market estimate the inhibition of Cox-1 and Cox-2 activity.

Aqueous extract of *Ziziphus Jujuba* fruit (JFA) was exhibited excellent anti-inflammatory activity against COX-1 & Cox-2 with IC_{50} value less than <0.2mg/ml. Ethanolic extract of leaf (JLE) & Ethanolic extract of fruit (JFE) possessed moderate activity (< 0.3) against both COX-1 & Cox-2. Aqueous extract of leaf (JLA) exhibited poor activity against both COX-1 & Cox-2. But over all conclusion of anti-inflammatory study of different extracts of *Ziziphus Jujuba* leaves and fruit were possessed anti inflammatory activity and inhibition was increased with concentration of samples increased.

CONCLUSION

This study showed the antioxidant activity of the leaves and fruits of *Ziziphus jujuba* and contributed to reveal some phytochemical characteristics of this species. The ethanolic Jujuba fruit extract showed the better antioxidant activity by the DPPH and ABTS scavenging assay, which can be attributed to its high content of total flavonoids. In addition, the JFE extract that presented high values of flavonoids, tannins and alkaloids, was also shown to be able to remove reactive species by reducing oxidative stress, lipid peroxidation and damage to proteins. Additionally, our results indicated that this plant has antioxidant potential and can be a promising source of natural antioxidants.

Thus, antioxidant activity and anti inflammatory activity property of the extracts definitely attributed to the phytoconstituents they contain, which may be either due to their individual or additive effect that fastens the process of these activities. At this stage, it is difficult to say which component(s) of the extract are responsible for the above activities. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities. Further investigations also needed for evaluation these actions.

CONFLICTS OF INTERESTS

There are no Conflicts of interests

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