

# Evaluation of Antioxidant and Antifungal Activity of Local Species of *Ziziphus Xylopyrus*

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**Abstract:-** Traditional medicine, which is based on theories, beliefs and personal experiences, is a pool of knowledge skills and practices that are being used by various cultures for maintaining health and preventing, diagnosing, improving, and treating both physical and psychological illnesses. Objective of this study to evaluate the antioxidant and antifungal activity of local species of *Ziziphus Xylopyrus*. DPPH radical scavenging assay was employed for determination of antioxidant activity at 517nm in spectrophotometer. Antifungal activity was determined by Poisoned food technique. Methanolic extract of *Ziziphus Xylopyrus* showed comparable antioxidant effect when assessed with DPPH assay in comparison with ascorbic acid. Also, it showed comparable antifungal effect when compared with standard clotrimazole, itraconazole and amphotericin B. In Phytochemical investigation of fruits of *Ziziphus Xylopyrus* shows presence of alkaloids, flavanoids, sterods, terpenoids, glycosides which may be responsible for potential antioxidant and antifungal of this plant.

**Keywords:-** *Ziziphus xylopyrus*, DPPH, Antioxidant, Antifungal.

## I. INTRODUCTION

Traditional medicine, which is based on theories, beliefs and personal experiences, is a pool of knowledge skills and practices that are being used by various cultures for maintaining health and preventing, diagnosing, improving, and treating both physical and psychological illnesses [1]. Herbal medicine is an important component of traditional medicine [2]. Due to emerging interest the 80% of world's population is adopting traditional medicine A considerably small number of marketable drugs or phytochemical entities have entered on evidence-based therapeutics, but efforts are still needed to be established for bioactive molecules in herbal drugs.[3] *Ziziphus pylonus* (Retz.) wild (Family: Rhamnaceae) is distributed in North Western India, Uttar Pradesh, Bihar and Central South India. In Hindi it is known as katber, in Tamil -kottei, and in Telugu- Gotte[4]. *Ziziphus Xylopyrus* (Retz.) wild is a large straggling shrub of small tree, armed with spines and up to four meter in height. Its fruits are globose, 3, rarely 2 or 4-celled, with usually a seed in each cell. Fruits are very hard and woody and covered with short greyish tomentum [5]. This plant possesses several pharmacological activities like antimicrobial [6]. Antidepressant [7]. Antidiarrheal [8]. Antidiabetic [9]. Antiulcer [10]. Hepatoprotective [11]. Anticonvulsant and Antinociceptive [12]. Antibacterial [13]. Anti-inflammatory [14]. Wound healing [15]. Objective of this study to evaluate the antioxidant and antifungal activity of local species of *Ziziphus Xylopyrus*.

## II. MATERIAL AND METHOD

### A. Collection, identification and authentication of plant materials

Fresh fruits of the plant were collected in the month of April from Government nursery placed in Ghatkheda forest region District Amravati, Maharashtra, India. The specimen of *Z. xylopyrus* fruits with herbarium was deposited in botany department of Sant Gadge Baba Amravati University. It was identified and authenticated by Department of Botany, SGBAU, Amravati. The specimen of *Z. xylopyrus* (Retz.) Willd. With Rh/5/0/01/2022/I/SGBAU herbarium number was received from botany department of university.

### B. Chemicals

Methanol, hydrochloric acid Mayer's reagent, sodium hydroxide, concentrate sulphuric acid, chloroform, glacial acetic acid, ferric chloride, lead acetate, DPPH, ascorbic acid, dextrose and agar were procured from Ajanta pharma Mumbai.

### C. Extraction

The fruits were separated from the plant materials, washed well using clean water, dried under shade and powdered. Extraction was carried out by maceration process where known quantity of the powdered plant materials (10g) was immersed in 100ml of methanol in clean conical flasks. With occasional stirrings, the flasks were left at room temperature for 48 hours followed by filtering the contents of flasks through muslin cloth followed by Whatmann No. 1. The filtrates were evaporated to dryness at 40°C to get extracts and extract was stored at room temperature for further use [16].

### D. Phytochemical testing

For the detection of phytoconstituents different test was performed like Mayer's test for alkaloids, froth test for Saponin, Alkaline reagent test for flavonoids, test for steroids, test for glycosides, test for steroid, gelatine test for tannins. Also, the evaluation of Total ash value, acid insoluble ash value, water insoluble ash value, extractive values and loss on drying were performed.[17]

### E. In Vitro Anti-Oxidant Study

#### (2, 2-Diphenyl-1-picrylhydrazyl) DPPH Radical Scavenging Activity:

Fruit extracts and ascorbic acid (6.25- 200Jg/ml) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical solution (0.004%) were prepared in methanol. 1ml of different concentrations extracts was mixed with 3ml of DPPH radical solution in clean and labelled tubes. The tubes were kept in dark for 30 minutes at room temperature.

The absorbance of each tube was measured in spectrophotometer at 517nm. Methanol replacing the extract/ascorbic acid served as control. Scavenging activity (%) of each concentration of extracts/ascorbic acid was calculated using the formula:

$$\text{Scavenging of DPPH radicals (\%)} = (C - T / C) \times 100$$

Where, 'C' and 'T' denotes to absorbance of DPPH control and absorbance of DPPH in presence of extract/ascorbic acid. The IC50 (Inhibitory Concentration) value was calculated which indicates the concentration of extract/ascorbic acid required to scavenge 50% of free radicals [18].

#### F. In Vitro Anti Fungal Study

It was determined by Poisoned food technique. Test fungi were inoculated aseptically at the centre of control (without extract) and poisoned (0.5mg extract/ml of medium) potato dextrose agar (PDA) plates followed by

incubating the plates at  $28 \pm 2^\circ\text{C}$  for 72 hours. The diameter of fungal colonies in mutual perpendicular directions was measured using a ruler. Antifungal activity, in terms of inhibition of mycelial growth of test fungi, was determined using the formula:

$$\text{Inhibition of mycelial growth (\%)} = (C - T / C) \times 100$$

Where, C and T denote the diameter of colonies in control and poisoned plates respectively [186].

### III. RESULTS

#### A. Preliminary Phytochemical Screening

The phytochemical screening carried out of *Ziziphus xylopyrus* reveals the of saponins, Tannins, Anthraquinone, phenolics were not detected. The secondary metabolites like alkaloids, flavonoids, steroids, terpenoids, glycosides are presents (Table No.1).

NAME OF THE TEST	INFERENCE
Test for alkaloids	+
Test for saponin	-
Test for flavonoids	+
Test for steroids	+
Test for terpenoids	+
Test for glycoside	+
Test for tannis and phenol	-

Table 1: Preliminary Phytochemical Screening of *Ziziphus Xylopyrus* fruits

#### B. Physicochemical Investigations

Ash values and extractive values can be used as a reliable aid to detect adulteration. These studies help in the identification of the plant material. Percentage extractives and ash analysis were carried out and results showed that total ash value of fruits is higher than other ash values and

water-soluble extractive value of fruit was higher than alcohol soluble extractive value. In *Ziziphus Xylopyrus* have total ash value is 14.2% w/w, extractive value is 11.38% w/w, loss on drying is 11.21% w/w and foaming index is greater than 100.

S. N	PARAMETER	VALUES
1)	Ash values	
	Total Ash	14.2% w/w
	Acid Insoluble Ash	0.52% w/w
	Sulphated ash value	1.16% w/w
2)	Extractive Values	
	Alcohol Soluble Extractive	
3)	Water soluble Extractive	20% w/w
	Loss on drying	11.21% w/w

Table 2: Physico-chemical parameters of powdered *Ziziphus Xylopyrus* fruits

#### C. In-Vitro Antioxidant Study

In DPPH, the odd electron of nitrogen atom is reduced when it receives a hydrogen atom from antioxidants leading to the formation of corresponding hydrazine. DPPH assay is widely used method to measure the antioxidant capacity of

plant extracts. On DPPH assay, the extract of *Ziziphus Xylopyrus* fruits shows anti-oxidant activity while comparing with standard as ascorbic acid (Table No.3 and Figure No.3).

Concentration (µl/ml)	% Activity of Fruit Extract of Ascorbic Acid	% Activity of Fruit Extract of ZX
20	29.5	8.1
60	32.5	18.2
100	41.6	30.5
150	49.5	40.1
200	75.6	69.5
250	84.5	81
300	98.5	90.2

Table 3: DPPH radical scavenging activity of *Ziziphus Xylopyrus* fruit extracts

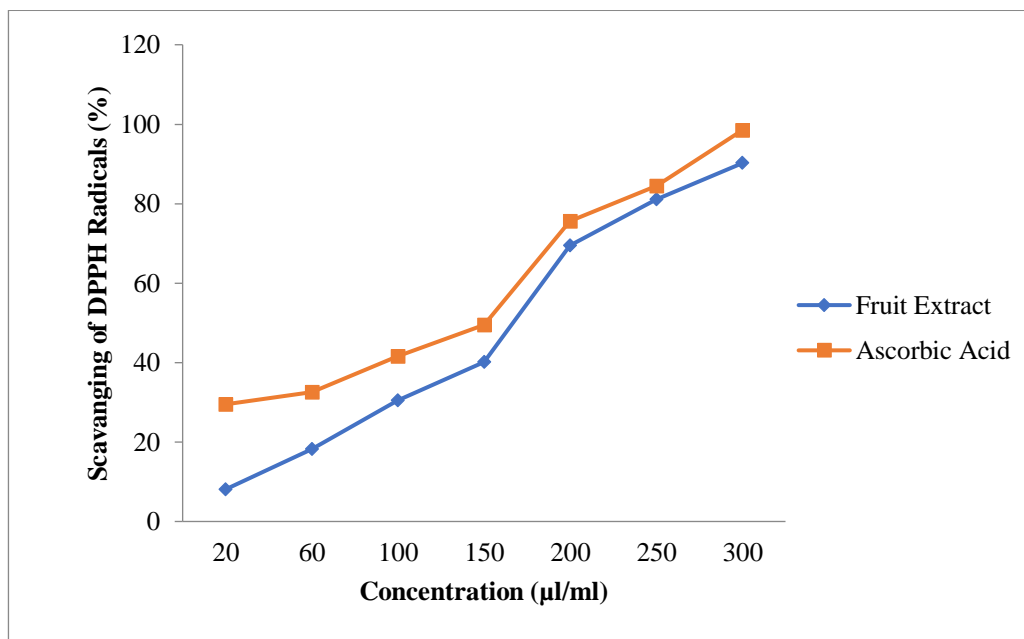


Fig. 1: DPPH radical scavenging activity of fruit extract.

D. 4. In Vitro Antifungal Study

For detection of anti-fungal activity std. Itraconazole (0.8), std. Amphotericin B (0.8), std. clotrimazole (0.6), std. Nystatin (0.7) are used and sample of *Ziziphus Xylopyrus* are used at different concentrations and gives zone of inhibition in mm after 36 hrs.

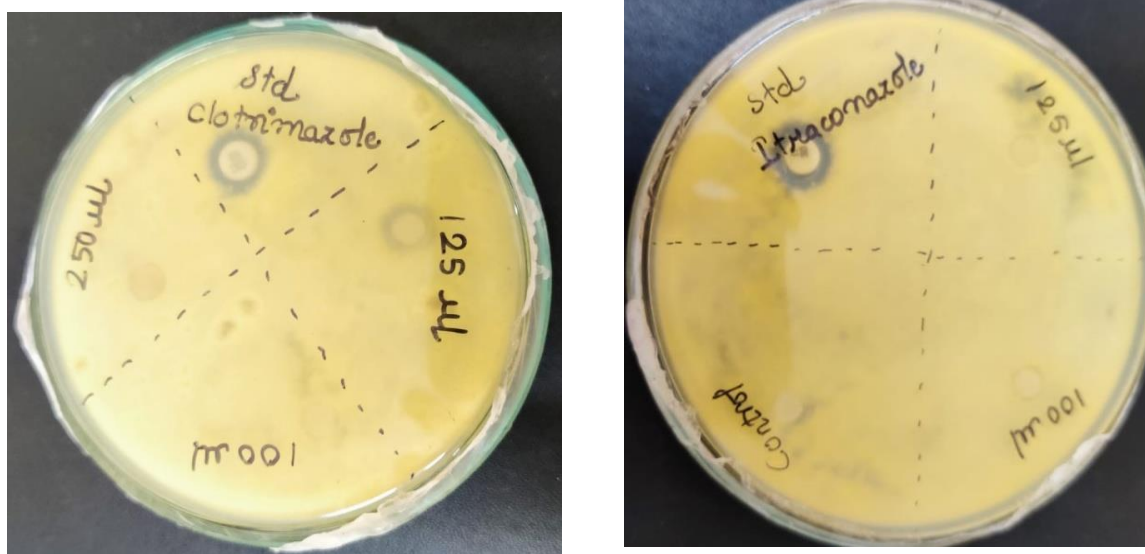


Fig. 2: Plates show anti fungal activity of methanolic extract of *Ziziphus Xylopyrus* fruits.

In our results Methanolic extract of *Ziziphus Xylopyrus* fruits shows promising antifungal action when compared with the effects of synthetic drugs available in market.

*Ziziphus Xylopyrus* fruits extract in dose of *Ziziphus Xylopyrus* (750  $\mu$ l/ml) shows maximum inhibition (Table No. 4)

S.N	Anti-fungal activity of samples	Zone of inhibition In mm (after 36 hrs)
1	Control Methanol	0.6
2	Std Itraconazole	0.8
3	Std Amphotericin B	0.8
4	Std Clotrimazole	0.6
5	Std Nistatin	0.7
6	<i>Ziziphus Xylopyrus</i> (100 $\mu$ l/ml )	0.5
7	<i>Ziziphus Xylopyrus</i> (125 $\mu$ l/ml )	0.5
8	<i>Ziziphus Xylopyrus</i> (250 $\mu$ l/ml )	0.5
9	<i>Ziziphus Xylopyrus</i> (500 $\mu$ l/ml )	0.6
10	<i>Ziziphus Xylopyrus</i> (750 $\mu$ l/ml )	1

Table 4: Antifungal Activity of *Ziziphus Xylopyrus* fruit extracts

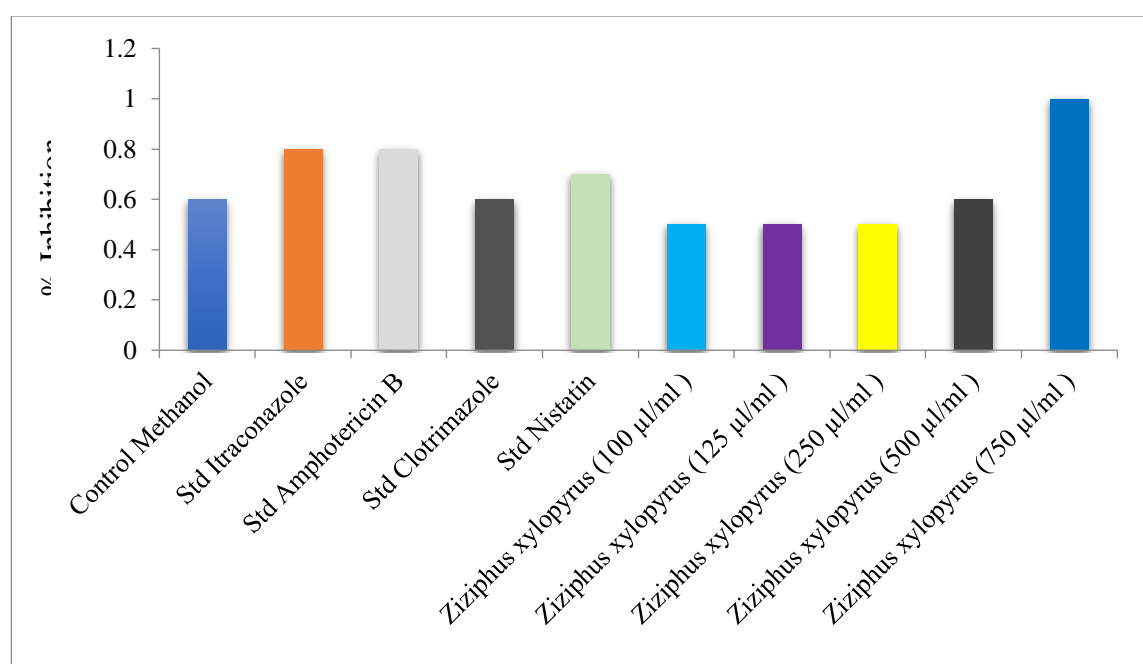


Fig. 3: Antifungal Activity of *Ziziphus Xylopyrus* (retz.) wild Fruit extract

Methanolic extract of *Ziziphus Xylopyrus* showed comparable antioxidant effect when assessed with DPPH assay in comparison with ascorbic acid. Also, it showed comparable antifungal effect when compared with standard clotrimazole, itraconazole and amphotericin B. In Phytochemical investigation of fruits of *Ziziphus Xylopyrus* shows presence of alkaloids, flavanoids, sterods, terpenoids, glycosides which may be responsible for potential antioxidant and antifungal of this plant.

In conclusion, results of study suggested that further detail investigation is needed, which may developed this plant a potential antifungal agents and can be an alternative to synthetic drugs.

## REFERENCES

- [1.] Mansoori MH et al, *Ziziphus Xylopyrus* (Retz) Wild: A Plant of Biomedical Potential, International Journal of Green Pharmacy, 2018; 12(2): S322- S329.
- [2.] Shaikh H, Shaikh S, A Comprehensive Review on *Ziziphus Xylopyrus* (Retz.) Wild: It's Phytoconstituents and Pharmacological Activities, World Journal of Advanced Scientific Research, 2020; 3(3): 91- 100.
- [3.] Modi A et al, *Ziziphus Xylopyrus* (Retz.) Wild: A Review of its Folkloric, Phytochemical and Pharmacological perspectives, Asian Pacific Journal of Tropical Disease, 2014; 4(1): 51- 56.
- [4.] Singhal U et al, Pharmacognostical Study on Fruit of *Ziziphus Xylopyrus* (Retz.) Wild, International Journal of Drug Development and Research, 2012; 4(3): 263- 267.
- [5.] Singhal U, Senthilkumar K, Antidiarrhoeal Activity of Fruit extract of *Ziziphus Xylopyrus* (Retz.) Wild in

- Rats, International Journal of Pharmaceutical Erudition, 2011; 1(2): 57- 61.
- [6.] El Maaaiden E et al, Genus *Ziziphus*: A Comprehensive Review on Ethnopharmacological, Phytochemical and Ethnopharmacological Properties, Journal of Ethnopharmacology, 2020; 259: 112950.
- [7.] Sharma VK et al, Antidepressant Activity of *Ziziphus Xylopyrus*, International Journal of Phytomedicine, 2009; 1: 12- 17.
- [8.] Singhal U, Senthilkumar K, Antidiarrhoeal Activity of Fruit extract of *Ziziphus Xylopyrus* (Retz.) Wild in Rats, International Journal of Pharmaceutical Erudition, 2011; 1(2): 57- 61.
- [9.] Khan S, Naqvi M, Kalia K, Evaluation of in- vitro Antidiabetic Activity of *Ziziphus Xylopyrus*, World Journal of Pharmaceutical Research, 2022; 11(6): 983- 990.
- [10.] Sharma V et al, A Study of Antioxidant and Antiulcer Activity of *Ziziphus Xylopyru*, 2013; 3(4): 167- 174.
- [11.] Modi A, Kumar V, Phytochemical Analysis, Antioxidant Activity and Hepatoprotective Effect of *Ziziphus Xylopyrus* (Retz.) Wild Leaves Extracts Against Carbon Tetrachloride Induced Hepatotoxicity in In-vitro and In- vivo Models, International Journal of Green Pharmacy, 2017; 11(1): 42- 56.
- [12.] Shah UK, Ratha B, Anticonvulsant and Antinociceptive Effect of *Ziziphus Xylopyrus* Wild (Rhamnaceae) Stem Bark Ethanolic Extract, Journal of Pharmacognosy and Phytochemistry, 2018; SP1: 3228- 3231.
- [13.] Sameera NS, and Manadakini BP, Investigations into the Antibacterial Activity of *Ziziphus Maceritiana* Lam. And *Ziziphus Xylopyrus* (Retz.) Wild, International Food Research Journal, 2015; 22(2): 849- 853.
- [14.] Alsayari A, Wahab S, Genus *Ziziphus* for the Treatment of Chronic Inflammatory Disease, Saudi Journal of Biological Sciences, 2021; 28: 6897- 6914.
- [15.] Jena BK et al, Wound Healing Potential of *Ziziphus Xylopyrus* Wild. (Rhamnaceae) Stem Bark Ethanol Extracts Using In- vitro and In- vivo Model, Journal of Drug Delivery Therapeutics, 2012; 2(6): 41- 46.
- [16.] Raghvendra H et al, Phytochemical Analysis, Antifungal and Antioxidant Activity of *Ziziphus Xylopyrus* (Retz.) Wild (Rhamnaceae), Star Technology Arts Research Journal, 2015; 4(4): 83- 88.
- [17.] Khandelwal KR, Practical Pharmacognosy, Techniques and Experiments, 9<sup>th</sup> Edition, Nirali Prakashan, Pune, 2008, 175.