International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319-6718, ISSN (Print): 2319- 670X
www.ijpsi.org Volume 12 Issue 2 ǁ Mar. –Apr. 2023 ǁ PP. 01-05

Chromatographic evaluation of aqueous and ethanolic stem extracts of ZiZiphus *xylopyrus* (Retz) Willd.

Dr. Upendra B. Gandagule [1].
Professor, Shri Gulabrao Deokar college of pharmacy. Jalgaon M.S.

Kavita Yogesh Pawar [2].
Asst. prof. Institute of management and research Jalgaon. M.S.

Dr. Yogesh Pawar [1].
Principal, Shri Gulanrao Deokar college of pharmacy. Jalgaon M.S.

Rohit Sawant. [1].
Asst. prof. Shri Gulanrao Deokar college of pharmacy. Jalgaon M.S.

Abstract*- The stem of ZiZiphus xylopyrus, (Retz) Willd. Was collected dried, powdered and subjected for extraction. Preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, steroids and sterol, glycosides, saponins, Flavonoids, phenolic compounds, triterpenoid and showed the absence of amino acids, proteins and acidic compounds. in ethanolic and aqueous extracts of stem of ZiZiphus xylopyrus, (Retz) Willd. Fractions were prepared according to polarity. TLC was carried out.*
Keywords: *ZiZiphus xylopyrus, aqueous and ethanolic stem extract, Chromatographic evaluation.*

Date of Submission: 08-04-2023 Date of acceptance: 22-04-2023

I. Introduction

Ziziphus xylopyrus family Rhamnaceae is commonly known as Jujab in English.[1] It is a large, straggling shrub or a small tree, armed with spines, up to 4 m in height. Fruits are globose, 3-rarely 2 or 4-celled, with usually a seed in each cell, very hard and woody. It is found in Pakistan and China, North-Western India, Uttar Pradesh, Bihar, Central and South India.[2] The fruit decoction of this plant is used in birth control in some parts of Rajasthan, India.[3] This plant is widely used in Turkish medicine as a potent sedative.[4] Bark and leaf powder paste are applied externally for chest pain arising from cough. In stomach ache and indigestion, fruit powder (3-4 g) is administered with a pinch of ginger powder thrice in a day. The leaves contain quercetin and quecitrin. The bark contains tannins (7.2%) 7, 3, 4-trihydroxy flavan-3, 4-diol and oleanic acid.[5] *Z. xylopyrus* is used in Pyorrhoea and to check oogenesis.[6] The bark is used for its astringent activity and as dental sticks for teeth cleaning. In different parts of India this plant is also used in the treatment of diarrhoea.[7] Leaf paste is applied on pimples and it is ground along with latex of *Ipomea carnea* and applied on boils.[8] One to two inches of the fresh stem bark of this species are chewed with 1-2 peppers (*Piper nigrum*) and the sap swallowed once a day for 5 days in the treatment of cough.[9] Xylopyrine-A and Xylopyrine-B, the two new13-membered cyclopeptide alkaloids are also present in *Ziziphus* species i.e. *Z. xylopyra*.[10] There are no literatures supporting chromatographic studies on aqueous and ethanolic stem extract of *ZiZiphus xylopyrus* Therefore, the present study was carried out.

II. Materials and methods

2.1 Plant material

The Plant *Z. xylopyrus was* collected in the month of July from Tirupati district, Andhra Pradesh, India. The Plant was authenticated by the botanist, Dr. K. Madhavachetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India. The plant material was subjected for processing which included initially, removal of other than the plant parts (foreign matters) such as grass, sand and mud to ensure the plant quality [11].

2.2 Extraction of selected plant material

The dried parts were ground into coarse powder with the help of a grinder and passed through sieve size mesh 60. The coarse powdered materials of the plant parts were subjected to the cold maceration. 500 gm. of stem of *Z. xylopyrus* parts was extracted by cold maceration process in 3 liter round bottom flasks. For extraction, solvents were used, ethanol and distilled water. The nature and yield of the extract was noted. Preliminary phytochemical analysis was carried out for both extract [12, 13, 14,]. plants were found to contain different phytoconstituents such as alkaloids, glycosides, steroids and sterols, saponins, Flavonoids, carbohydrates, and tri- terpenoids and showed the absence of amino acids, proteins and acidic compounds, Results were mentioned in table 1.TLC for fractions were carried out according to their polarity [15]. Results were mentioned in table 2 and 3.

2.2.1 Fractionation of aqueous and ethanolic stem extracts of *ZiZiphus xylopyrus*

The column chromatographic technique is for separation, isolation and purification of the natural products. The principle involved in this is the adsorption towards the adsorbent packed in the column. By changing the polarity of the mobile phase, the separation can be achieved in the column chromatography technique. Here fractions were prepared using solvent extraction techniques. Selection of the solvent was carried out based on their polarity.

1. Petroleum ether
2. Petroleum ether: Chloroform (1:1)
3. Chloroform
4. Chloroform: Ethyl Acetate (1:1)
5. Ethyl acetate
6. Ethyl acetate: Methanol (1:1)
7. Methanol
8. Water

The aqueous and ethanolic stem extract of *ZiZiphus xylopyrus*, were subjected for fractionation using above mentioned solvents to separate the phytoconstituents. 8 fractions were prepared. The extracts were triturated with activated silica gel using pestle and mortar. After that the mixture was dissolved in the solvent of low polarity, stirred, heated moderately (Luke warm), and filtered. The filtrated solution was collected in a labeled conical flask, and the marc was air dried, which was used for the next solvent system of high polarity. The process was followed according to polarity of solvent system as mentioned above.

2.3 Chromatographic evaluation

2.3.1 Thin layer chromatography (TLC) [15]

TLC is a very effective technique for the separation of chemical constituents of an extract and for their identification. TLC profile developed for an extract and its fractions using a defined solvent system (mobile phase) and other parameters could be used as fingerprints in the qualitative evaluation of herbal drugs.

Separation of components

The extract was dissolved in respective solvents separately and spotted using a capillary tube on a prepared TLC plate 1cm above from the bottom of the plate. The spot was equally sized and had a diameter ranging from 2-3 mm.

Selection of mobile phase:

The selection of solvent or mobile phase depends upon various factors such as
Nature of substance to be separated,
Nature of stationary phase (polar / non polar),
Mode of chromatography (normal / reverse phase),
Extent of separation to be achieved (analytical / preparative).

Thin layer chromatographic observations for successive fractions of aqueous and ethanolic stem extract of *ZiZiphus xylopyrus.*

The aqueous and ethanolic extracts were subjected for TLC using different solvent systems, but the only solvents showed good separation with maximum number of spots were finalized and mentioned below in the table along with the number of spots. Abbreviations - Petroleum ether – (Pet.ether); Chloroform – ($CHCl_3$); Ethyl acetate – (Et. acetate); Methanol – (MeOH); Glacial acetic acid – (G.A.A.); Acetic acid- (A.A); Water- (H_2O); and fraction- fr. The result of TLC was mentioned in table 2 and 3.

III. Results and Discussion.

3.1 Phytochemical studies

The aqueous and ethanolic extract of stem of *ZiZiphus xylopyrus* were subjected to preliminary phytochemical screening for the detection of phytoconstituents. The results obtained were given below

Abbreviations used for different prepared extract and are as follows

ZISA- *ZiZiphus xylopyrus* stem aqueous extract.
ZISE- *ZiZiphus xylopyrus* stem ethanolic extract

Table 1 Preliminary phytochemical screening of the prepared extract of *ZiZiphus xylopyrus.* aqueous and ethanolic stem.

Sr. No.	Phytochemical tests	Stem extract	
		ZISE	ZISA
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Steroids and sterols	+	-
4	Glycosides	-	-
5	Saponins	+	+
6	Protein and amino acids	-	-
7	Flavonoids	+	-
8	Phenolic	+	+
9	Acidic	-	-
10	Fixed oils	+	-
11	Triterpenoids	-	-

3.2 TLC studies

The aqueous and ethanolic extract of stem of ZiZiphus *xylopyrus* were subjected for TLC studies. The results obtained were given below in table 2 and 3

TLC for fractions of aqueous and ethanolic extract of stem of ZiZiphus *xylopyrus.* Abbreviations - Petroleum ether – (Pet.ether); Chloroform – (CHCl3); Ethyl acetate – (Et.acetate); Methanol – (MeOH); Glacial acetic acid – (G.A.A.); Acetic acid- (A.A); Water- (H2O); and fraction- fr.

Table 2 TLC for the different fractions of aqueous extract of *Z. xylopyrus* stem

S. No	Fraction	Solvent System	Ratio	No. of spots
1.	Pet. ether	No residue, colourless	-	Nil
2.	Pet.ether: CHCl3 (1:1)	No residue, colourless	-	Nil
3.	Chloroform	No residue, colourless	-	Nil
4.	CHCl3: Et. acetate (1:1)	No residue, colourless	-	Nil
5.	Ethyl acetate	No residue, colourless	-	Nil
6.	Et. acetate: MeOH (1:1)	Ethyl acetate: MeOH	4: 1	1
7.	Methanol	Ethyl acetate: MeOH	4: 1	1
8.	Aqueous	Ethyl acetate: MeOH	3: 2	2

Table 3 TLC for the different fractions of ethanolic extract of *Z. xylopyrus* stem

Sr. No	Fraction	Solvent system	Ratio	No. of spots
1.	Petroleum ether	Pet.ether: CHCl3: MeOH	3.9: 1.1:0.1	7
2.	Pet.ether: CHCl3 (1:1)	CHCl3: Et. acetate: A. A	5: 0.5: 0.1	4
3.	Chloroform	CHCl3: Et. acetate	4.5: 0.5	3
4.	CHCl3: Et. acetate (1:1)	CHCl3: Et. acetate	4.5: 0.5	1
5.	Ethyl acetate	CHCl3: Et. acetate	3.9: 1.1	1
6.	Et. acetate: MeOH (1:1)	CHCl3: Et. acetate	5: 0.6	1
7.	Methanol	CHCl3: Et. acetate: MeOH	3.8: 1.9: 0.1	1
8.	Aqueous	Et. acetate: MeOH: H2O	3: 0.5: 1.5	1

Summary- Aqueous and ethanolic extract were prepared from the stem of *ZiZiphus xylopyrus* Preliminary phytochemical analysis was carried out. Extract was found to contain different phytoconstituents such as alkaloids, glycosides, steroids and sterols, saponins, flavonoids, carbohydrates, and tri- terpenoids and showed the absence of amino acids, proteins and acidic compounds. Extract was subjected to TLC. Result was noted.

Conclusion- These parameters which reported for the first time, could be useful in isolation and identification of new lead molecule from *ZiZiphus xylopyrus* plant. The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigation and application.

Acknowledgements

Authors thanks to J. S. S. University Mysore, (India) for providing facilities to conduct this research work. Authors also acknowledge Dr. K. Madhavachetty, Assistant Professor, Department of Botany, Sri

Venkateshwara University, Tirupati, Andhra Pradesh (India) in collection and authentication of the plant material.

References.

[1]. Department of AYUSH. *Ayurvedic Pharmacopoeia of India.* Part-1, Vol. 5. Delhi: Govt. of India, Department of ISM and H, Published by the Controller of Publications; 2006. 47.
[2]. Anonymous. *The Wealth of India*: Raw Materials Series. Vol. 11 (X-Z). New Delhi: NISCAIR, CSIR; 1976. 123-4.
[3]. Jain A, Katewa SS, Chaudhary BL, Galav P. Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India. *J Ethnopharmacol* 2004; 90:171-7.
[4]. Jagtap SD, Deokule SS, Bhosle SV. Some unique ethnomedicinal uses of plants used by the Korku tribe of Amravati district of Maharashtra, India. *J Ethnopharmacol* 2006; 107:463-9.
[5]. Yadav M, Meena AK, Rao MM, Kapil P, Panda P, Chahal J, *et al*. Review on *Ziziphus xylopyrus*: A potential traditional drug. *J Pharm Res* 2011; 4:922-3.
[6]. Meena AK, Rao MM. Folk herbal medicines used by the Meena community in Rajasthan. *Asian J Tradit Med* 2010; 5:19-31.
[7]. Dash SK, Padhy S. Review on ethnomedicines for diarrhoea diseases from Orissa pre valence versus culture. J Hum Ecol 2006; 20:59-64.
[8]. Naidu KA, Khasim SM. Contribution to the floristic diversity and ethnobotany of Eastern Ghats in Andhra Pradesh, India. *Ethnobotanical Leaflets* 2010; 14:920-41.
[9]. Reddy KN, Reddy CS, Trimurthulu G. Ethno botanical survey on respiratory disorders in Eastern Ghats of Andhra Pradesh, India. *Ethnobotanical Leaflets* 2006; 10:139-48.
[10]. Singh AK, Pandey MB, Singh VP, Pandey VB. Xylopyrine-A and Xylopyrine-B, two new peptide alkaloids from *Zizyphus xylopyra*. *Nat Prod Res* 2007; 21:1114-20.
[11]. Chaudhary RD. *Herbal Drug Industry.* 1st ed. New Delhi: Eastern Publication; 1996, 373-375,473.
[12]. Kokate CK, Purohit AP, Gokhale SB. *The Text Book of Pharmacognosy.* 3rd ed. New Delhi: Vallabh Prakashan; 1991, 606-611.
[13]. Khandelwal KR. *Practical Pharmacognosy.* Pune: Nirali Prakashan; 1998, 149-156.
[14]. Raaman N. Qualitative phytochemical screening in: Phytochemical techniques. New Delhi: New India publishing agency 2006.19-24.
[15]. Chatwal GR, Anand S.K. Instrumental method of chemical analysis. New Delhi: Himalayas publishing house 2002. 2599-2600.