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### Chromatographic screening of the Ethanolic Extracts of *Zizyphus xylopyrus* (Retz.) Willd.

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#### Abstract

To evaluate the presence of phytoconstitute of ethanolic fractions of *Zizyphus xylopyrus* on anti ulcer activity. Root powder of the plant was extracted successively with ethanol; Extract had pungent odour. showed the presence of desired phytochemicals i.e. Flavonoids, Tannin, Phenol. On the basis of all the qualitative tests performed in each extracts; ethanolic extract was subjected for the further chromatographic screening to isolate the flavonoids; this was subjected for the further pharmacological studies because only the ethanolic extracts and Saponins

**Key-Words:** *Zizyphus xylopyrus*, Anti- Ulcer, Isolate Fraction

#### Introduction

The drug consists of the whole dried herb of *Zizyphus xylopyrus* (Retz.) Willd. (Family: Rhamnaceae) *Zizyphus* is a genus of about 40 species of spiny shrubs and small trees in the buckthorn family *Rhamnaceae*. The leaves are alternate, entire, with three prominent basal veins, and 2-7 cm long; some species are deciduous, others evergreen<sup>1</sup>. The common name of this plant in Hindi - Kat-ber, Gote, Kakor, Ghont. A large, straggling shrub or a small tree, armed with spines, up to 4 m. in height<sup>2</sup>. The major chemical composition of *Z. xylopyrus* are rich in flavonoids in particular quercetin, quercitrin, Kempferol-4'-methylether and Kaempferol, Tannins (7.2%), d-7, 3', 4'-trihydroxyflavan-3, 4-diol and oleanolic acid<sup>3-4</sup>. It also contains Cyclopeptide alkaloids namely Amphibine H, Nummularine- K, Xylopyrine - A and Xylopyrine - B<sup>5</sup>. The bark was also found to contain Betulinic acid (1%), Betulin<sup>6</sup>. Fruit contains Catechol-type of tannins (8-12%). Fruits were also reported to have Oleanolic acid, 1-leucocyanidin, 3, 3', 4-tri-O-methyl-ellagic acid<sup>7</sup>. Seeds unsaponifiable matter (0.8%) consists of a Sterol, insoluble mixed fatty acid found to contain Myristic, Linoleic and Oleic acid<sup>8</sup>. this plant is widely used in Turkish folk medicines as a potent Sedative<sup>9</sup>. The root bark of this plant is reported to have Antinociceptive, Anti-convulsant and Anti-inflammatory activity. Apart from that the leaf of this plant has been reported to have antidepressant and antioxidant activities.<sup>10</sup>

Thin layer Chromatography (TLC) is a method of analysis in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate; and the mobile phase, a liquid is allowed to migrate across the surface of the plate. Although separation efficiencies equivalent to those obtained with gas or high-pressure liquid chromatography cannot be obtained by this method, it has the advantages of speed, versatility and simplicity<sup>11</sup>. the present study was undertaken to evaluate the antiulcer activity of ethanolic extract of *Zizyphus xylopyrus* and prove tribal claim scientifically.

#### Material and Methods

##### Plant

Whole plant of *Zizyphus xylopyrus* (Retz.) Willd. was collected near from the college campus, Bhopal (M.P.) India in the months of March to April 2010. The leaf of this plant was shade dried and finely powdered with the help of mixer. A small amount of powdered drug was spread on a white tile and physically examined for general appearance i.e. color, nature, texture etc.

##### Preparation of ethanolic extracts

About 100 gm. leaves of *Z. xylopyrus* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The marc obtained after petroleum ether was subjected to ethanol extraction in soxhlet apparatus. The extraction was continued for a period of 6-7 days to achieve complete extraction. The extract was concentrated and dried to a constant

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weight. The dried extracts of the drugs were evaluated for physical parameters such as consistency, color, odor and taste. the presence of desired phytochemicals i.e. Flavonoids, Tannin, Saponins .

#### **Preliminary phytochemical investigations**<sup>12-13</sup>

The plant after shade drying, was powdered and it was subject for the organoleptic characterization. The colour of the powdered drug was found to be light green with aromatic an odour and coarse texture. After this 100 gm of powdered drug was weighed and used direct ethanolic extraction through Soxhlet apparatus. The extractive value was calculated and was found to be 10.2 %. The extract was further examined physically like colour, odour, consistency, etc. The colour of the extract was brown, with a semi-solid consistency. Extract had pungent odour. Qualitative chemical tests were carried out for the ethanolic extract of *Z. xylopyrus*. The results of the tests showed the presence of carbohydrates, reducing sugars, saponins, phenolics, tannins, and flavonoids.

#### **Chromatographic investigation**<sup>14</sup>

The solvent system was poured to a depth of 0.5 cm in a rectangular chromatographic glass chamber. The chamber was lined with a piece of filter paper to ensure proper saturation. The spots of extract were applied on a silica gel-G plate with the help of capillary tube. The distance between two spots was kept approximately 2.0 cm. The applied spots were dried at room temperature and the plate was gently placed inside the glass chamber. The angle of the plate with the vertical was kept approximately 15°. The chromatogram was developed till the solvent front migrated to about 10.0 cm. The plate was taken out and the solvent front was marked. The plate was dried at room temperature and inspected either under UV light or sprayed with the specific detecting reagent. The colored spots were marked and the  $R_f$  value of each separated component was calculated and best resolution was obtained in, Methanol:Hcl (9:1) Benzene:Ethyl acetate(1:0.5). The TLC plate examined under sun light showed the presence of 8 spots. The same plates were derivatized with anisaldehyde-sulphuric acid reagent and ferric chloride sol<sup>n</sup>., but after using these spraying reagents non of them showed good resolution of the spots so only sun light detection was choosed for the further investigations as it showed the best resolution. Many other solvent systems were investigated before developing the best solvent system but none of those gave the satisfactory resolution or the good separation. Ethyl acetate: nButanol: water: Formic acid (10:10:4:2) gave the same separation as by ethyl acetate: nButanol: water (10:10:4) but the resolution of the spots were not as clear the best solvent chosen.

#### **Results and Conclusion**

The plant after shade drying, was powdered and it was subject for the organoleptic characterization. The colour of the powdered drug was found to be light green with aromatic an odour and coarse texture. After this 100 gm of powdered drug was weighed and used direct ethanolic extraction through Soxhlet apparatus. The extractive value was calculated and was found to be 10.2 %. The extract was further examined physically like colour, odour, consistency, etc. On the basis of all the qualitative chemical tests performed in extract; ethanolic extract was subjected for further phytochemical and pharmacological studies as the ethanolic extract showed the presence of variety of desired phytochemicals .Ethanolic extract of the drug was chromatographed on TLC plates and best resolution was obtained in, Methanol:Hcl(9:1) Benzene:Ethyl acetate(1:0.5). The TLC plate examined under sun light showed the presence of 8 spots. The same plates were derivatized with anisaldehyde-sulphuric acid reagent and ferric chloride sol<sup>n</sup>., but after using these spraying reagents non of them showed good resolution of the spots so only sun light detection was choosed for the further investigations as it showed the best resolution. Many other solvent systems were investigated before developing the best solvent system but none of those gave the satisfactory resolution or the good separation. Ethyl acetate: nButanol: water: Formic acid (10:10:4:2) gave the same separation as by ethyl acetate: nButanol: water (10:10:4) but the resolution of the spots were not as clear the best solvent chosen.

This work was conducted to explore the hidden potential of this unexplored herb. Initially due to the less data available in this plant we felt tough to decide the basis of our work ,so the strong basis was opted to work on that plant was the other species of the same genera and phytochemical reported on this plant mainly flavonoids .In recent findings done on these plants as well as flavonoids also proved tha way for the selection of this plant for peptic ulcer diseases.It was figured out that the other species of this genus are being used as in ulcer so it was thought worthwhile to investigate and to provide the scientific data on its use as an antiulcer agent. ethanolic extract of this plant contains major phytochemicals viz. Phenolics, flavonoids, tannins, saponins, and traces of alkaloids. It was thought worthy to select this extract for the further studies.TLC of ethanolic extract was done in order to separate out as many as compounds possible.After trying to many solvents and solvents system Benzene:Ethyl acetate (1:0.5) came out to be the best solvent system as it gave 8 spots in glass plate coated with silica gel G.After that we conducted the Co-TLC of ethanolic

extract with all the available standards. Co-TLC results revealed that this plant contains quercetin and rutin in it. Rutin is a flavonol glycoside which was yet not reported in this plant so it was thought to be valuable to isolate this compound after the pharmacological screenings.

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**Table I: Organoleptic characters of powder of crude drugs**

Drug	Nature	Color	Odour	Texture
<i>Zizyphus xylopyrus</i> (Retz.) Willd.	Coarse	Dark green	Aromatic	Rough

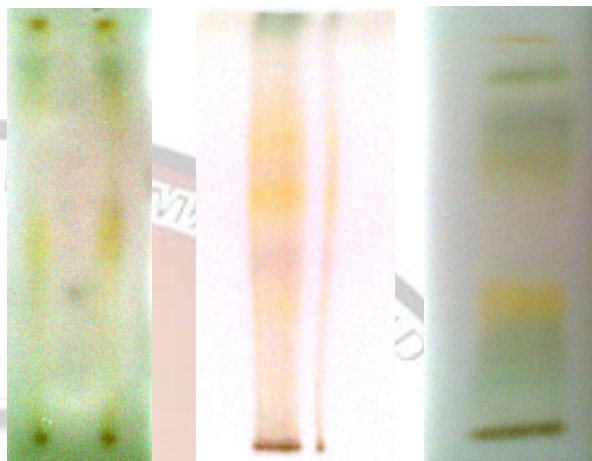
**Table II: Physical characteristics of extracts of *Zizyphus xylopyrus***

Name of Extract	Consistency	Color	Odor	Extractive value (%w/w)
Petroleum ether extract	Semi-Solid	Dark green	Characteristic	7.7%
Ethanollic extract (Direct)	Semi-Solid	Brown	Pungent	10.2%

**Table III : Qualitative chemical tests performed in the ethanollic extract of *Zizyphus xylopyrus***

Phytoconstitute	Ethanollic extract
Alkaloids	-
Glycosides	+
Phenols/Tannins	+
Flavonoids	++
Saponins	+
Gums & Mucilage	-
Carbohydrates	+
Amino acids	-
Steroids	+

+ = Present      - = Absent



**Ethanollic Extract in sunlight**

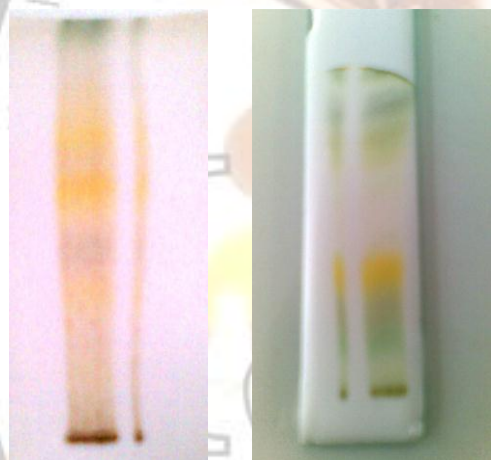
**Table IV: Solvent system used in Chromatographic procedure**

Solvent System	No. of Spots	Conclusion
Benzene-Ethyl acetate(1:0.5)	8	Very good
Methanol-Hcl (9:1)	7	Very good
Ethyl Acetate-n-Butanol-Water (10:10:2)	4	Good
Choloroform:Hcll (2:1)	4	Good
Ethyl Acetate-n-Butanol-Water (10:10:4)	4	Good
Ethyl Acetate-n-Butanol-Water-Formic acid (10:10:4:2)	4	Good
Benzene-n-Butenol-Ethyl Acetate (1:0.5:0.5)	3	Poor

Detection: Under Sun light

**Table V: TLC Profile of ethanolic extract of *Zizyphus xylopyrus* (Retz.) Willd. In Methenol-Hcl (9:1), Benzene: Ethyl Acetate (1: 0.5)**

S.No.	R <sub>f</sub>	Spot Colour In Sunlight
1	0.09	Dark yellow
2	0.17	Not visible
3	0.23	Not visible
4	0.28	Not visible
5	0.39	Light yellow
6	0.40	Not visible
7	0.9	Yellow



**Figure IV Co-TLC Ethanolic Extract & standard rutin**

**Figure V Co-TLC EA Extract & standard rutin**

**Table 2.6: Comparative co-TLC of ethanolic ext. with standard Rutin**

S. No.	Samples	R <sub>f</sub>	Colour of the Spot in Sunlight
1	Ethanolic extract (Direct)	0.50	Dark yellow
		0.91	Yellow
2	Standard Rutin	0.50	Dark yellow

Solvent system used: Methenol-Hcl(9:1), ethyl acetate-n-Butanol-Water (10:10:4)

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