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Research article

Isolation and characterization of the ethanolic extract of *Zizyphus xylopyrus* (Raz) wild

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ABSTRACT

To isolate and characterization of the phytoconstitute of ethanolic fractions of *Zizyphus xylopyrus* .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; ethanolic extract was subjected for the further perform spectroscopic Characterization to isolated flavonoids compound.

Keywords: *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. The common name of this plant in Hindi - Kat-ber, Gote, Kakor, Ghont. A large, straggling shrub or a small three, armed with spines, up to 4 m. in height. 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 (3-4). It also contains Cyclopeptide alkaloids namely Amphibine H, Nummularine- K, Xylopyrine - A and Xylopyrine – B. The bark was also found to contain Betulinic acid (1%), Betulin). Fruit contains Catechol-type of tannins (8-12%). Fruits were also reported to have Oleanolic acid, l- leucocyanidin, 3, 3', 4-tri-O-methyl-ellagic acid. Seeds unsaponifiable matter (0.8%) consists of a Sterol, insoluble mixed fatty acid found to contain Myristic, Linoleic and Oleic acid. this plant is widely used in Turkish folk medicines as a potent Sedative. 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. Herbal product contains multiple constituents that might be responsible for its therapeutic effects. It is thus necessary to define as many of the constituents as possible in order to understand and explain the bioactivity. The concept of phytoequivalence has been introduced in Germany to ensure consistency of phytotherapeuticals. According to this concept, a chemical profile for a herbal product is constructed and compared with the profile of a clinically proven reference product. Since many of these preparations contain flavonoids, it is essential to have adequate analytical techniques at hand for this class of natural product. Knowledge of the flavonoid content of plant-based foods is paramount to understanding their role in plant physiology and human health. flavonoids are indispensable markers for chemotaxonomic purposes. Both the isolated fractions were first off all characterized by their morphological characteristics i.e. colour, texture, etc. then various analytical parameters were employed so as to characterize the isolated compounds, these parameters are Melting points, Solubility profiles, Qualitative tests, UV-Vis Spectroscopy and some other spectral analysis methods. [1].

MATERIAL AND METHOD

Chemical and Plant material

Plant Zizyphus xylopyrus leaves were collected from botany department of Rani Durgavati University Jabalpur M.P. India. The entire chemical was analytical grade used.

Sample Preparation

The plant, Zizyphus xylopyrus leaves were washed properly with tap water followed by rinsing with double distilled water and shade drying for seven days. The fine powder was obtained from dried plant by using kitchen mixer grinder. The plant powder was stored under desiccators for further studies.

Extraction of flavonoids

Solvent extraction of dried leaves powder (50g) of Zizyphus xylopyrus was done using 2L of 99% ethanol in a soxhlet extractor for 24h.The extract was concentrated by evaporator. The characterization of two fractions isolated from the aqueous ppt. fraction of Z. .

UV/Visible Spectroscopy

The application of standardized UV (or UV– Vis) spectroscopy has for years been used in analyses of flavonoids. One feature that is of immense benefit for flavonoid analysis is the presence of the phenyl ring. This excellent chromophore is, of course, UV active and provides the reason why flavonoids are so easy to detect. Their UV spectra are particularly informative, providing Considerable structural information that can distinguish the type of phenol and the oxidation pattern.

Wavelength determination of isolated fraction

As stated by Markham, flavonoids appear as dark spots when observed in UV light (249 nm) on plates containing a UV-fluorescent indicator (such as silica gel F254). In 365 nm UV light, depending on the structural type, flavonoids show dark yellow, green, or blue fluorescence. These polyphenolic compounds reveal two characteristic UV absorption bands with maxima in the 240 to 285 and 300 to 550 nm ranges. Individual flavonoids are dissolved in methanol (concentration such that the maximum absorbance is between 0.05 and 1.00 AU), and the basic spectrum is measured. Most flavonoids show a band in the 210- to 290- nm region (band II) and a second band at 320-380 nm (band I). Compilations of spectral data are available for comparison, for anthocyanins, the latter band is in the visible region [490-540 nm] .

Preparation of samples and UV / Vis determination

1 mg of fraction I was dissolved in 1ml of methanol similarly 1 mg of fraction II was dissolved in 1 ml of dist. water, blanks solution was kept as methanol for I and dist. Water for II. Both samples were scanned in UV / Vis. Spectroscopy; scanned reports are reported.

Infra Red Spectroscopy

In recent years, IR and Raman spectroscopic techniques have been applied for the characterization of flavonoids-containing systems with rather complex composition. A rapid analytical method involving attenuated total reflection (ATR) mid-IR spectroscopy and UV–Vis spectroscopy, combined with multivariate data analysis, has been applied for the discrimination of Austrian red wines.

The broad and intense IR absorption observed in the range 3400 to 1900 cm_1, assigned to the hydrogen-bonded OH-group stretching vibration, exhibited the characteristic ABC structure of strong hydrogen-bonded complexes in good agreement with previous x-ray data showing that cis-formic acid was strongly hydrogen bonded to 2',6'-dimethoxyflavone. IR spectrum of Isolated fraction of flavonoids compound is shown in figure no.1. And Assigned peaks in IR spectra and interpretations of isolated flavonoids compound.

Nuclear MagneticResonance Spectroscopy

NMR spectroscopy is an extremely powerful analytical technique for the determination of flavonoid structures, Recent developments have, however, made NMR arguably the most important tool for complete structure elucidation of flavonoids. Today, it is possible to make complete assignments of all proton and carbon signals in NMR spectra of most flavonoids isolated in the low milligram range. These assignments are based on chemical shifts (d) and coupling constants (J) observed in 1D 1H and 13C NMR spectra combined with correlations observed as cross peaks in homo- and heteronuclear 2D NMR experiments.The purpose of a standard 1H NMR experiment is to record chemical shifts, spin–spin couplings, and integration data, thus providing information about the relative number of hydrogen atoms. 13C-NMR spectra of isolated flavonoid compound shown in Figure no.2, Data interpretation of 13C-NMR of isolated flavonoids compound shown in Table no.3, 1H-NMR spectra of isolated flavonoids compound shown in Figure no. 3, Data interpretation of 1H-NMR of isolated flavonoids compound [8].

RESULT AND DISCUSSION

The ethnolic extract of *Zizyphus xylopyrus*

It was fractionated to three different portions i.e. ethyl acetate, water and precipitate fractions. Then just after the fractionation of the drug all the fractions were underwent through the qualitative chemical tests for flavonoids, phenolics, tannins and saponins and both the fractions except water part found to contain flavonoids which was the desired for the purpose of isolation. Saponins were present in all three fractions. The test for tannins was found to be positive in all three fractions, Phenolics were absent in water portion but it was present in other two. The results obtained from qualitative tests, ethyl acetate. (Fraction 3) found to have maximum colour intensity in Magnesium ribbon test. For the pharmacological screening both the fractions as well as ethanolic extract were opted. The results obtained from the various screening models which were reported for antiulcer [9].

These approch of phytochemical work was undertaken so as to investigate the active moiety present in this plant, which

was found to be rutin. It also paved the way for the upcoming researchers to further investigate the different constituent of this plant as they are still not found. It also guides one fact that this plant is medicinally potent and still there is a lot of work to be done in it. This study was conducted so as to prove the medicinal potential of this plant which still not being proved at a greater extent [10].

CONCLUSION

It may be mentioned that flavonoids have been isolated successfully from the *Zizyphus xylopyrus* leaves under the present study. Identified on the basis of spectral data, the compounds were identified as rutin.

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