International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Ziziphus oxyphylla fruit as a source of secondary metabolites: Extraction and isolation techniques

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Abstract

Ziziphusoxyphylla is a member of the Rhamnaceae family. This plant is highly significant in medicine and is used to cure various illnesses. It has a variety of biological properties, including antinociceptive, antibacterial, antioxidant, and antipyretic ones. The goal of this investigation was to separate bioactive substances from *ziziphus oxyphylla* fruit. The ethanol and chloroform aqueous component chromatographic filtration. Several connotations were formed by berries comprising flavonoids and phenolic acid. While the both of caffeine and ferulic acid are acids generated by the chloroform fraction ethyl acetate fraction generated quercetin and catechin. Components one to four were identified found due to the use of mass spectrometry and contrasted with information presented in the scientific literature.

Keywords: petroleum ether (PE), Ziziphusoxyphylla (ZO), methanol (MeOH), n-butanol (n-BuOH)

Full length article **Corresponding Author*, e-mail*shreya.sharma@vgu.ac.in*

1. Introduction

Ziziphus jujubais according to this investigation they looked at metabolism and transcriptase properties obtained from developed leaves including leaf bud follicles of two varieties associated with ziziphus exhibiting purple foliage. Additionally, it was discovered how associated proteins expressed themselves, along with the amount of light impacted the formation produced by phytonutrients [1].The vast majority of the biologically active products identified in jujubes leaves belong into one of six categories alkaloid substances, fatty acids flavonoids polyphones, saponins that while triterpenic acids. The jujube plants used in earlier investigations were obtained at an identical phase of growth. An accurate approach believed to can describe the intricate chemical constituents of Ziziphus is made possible by metabolism science. The complex Investigation of metabolic improvement has enabled a specific strategy for characterizing the chemical components of ziziphus [2].Improving the quality of jujube through genetics requires a comprehension of the processes of nutrient accumulation and composition throughout fruit growth. However for most the jujube varieties this is still unidentified. Partially due to its great quantity of nutrients, tasty flavor, and therapeutic benefits. Fruits species ziziphus jujuba have been traditionally used in conventional food and medicinal products spanning over 4,000 years [3]. Particular studies have been conducted on jujubes despite their decades of use in medicine and nourishment. In this study we looked at eight different jujube communities from Tehran as well as one imported jujube to determine their physicochemical features nutritional values and antioxidant capacities. The intention through such an environment was to explore the phytochemistry and evaluate both the antioxidant and hemorrhagic potential of two indigenous plants, Ziziphus jujube mill, and Rhamnus alaternus. Both of having unique qualities that make further research into them worthwhile. They are two plants that belong to an identical family that grows on each other with several uses in traditional Algerian medicines [4].The emphasis therefore is on two native plants the Ziziphus jujube Mill and Rhamnus alaternus were because varied qualities make them plants worth investigating further their photochemical composition and the antioxidant and hemolytic activities they exhibit. They are both members of the same plant family therefore each of them grow wild and are widely used in traditional Algerian medicines. Consequently, in the current investigation, they concentrated on determining if *ziziphus oxyphylla* and its isolates had any inhibitions on the enzymatic processes of Glucose and this enzyme. There

produced plant-fruit extracts of n-butanol (n-BuOH) petroleum ether (PE) and methanol (MeOH) for this intention [5]. Ziziphus the purpose of has been used to treat a wide variety of medical conditions, including diabetes, diarrhea, skin infections, liver complaints, urinary disorders, obesity, fever, pharyngitis, bronchitis, anemia insomnia cancer and more producers for therapeutic substances and innovative chemicals in the last several decades. The goal of this investigation was to separate bioactive substances from *Ziziphus oxyphylla* fruit. Four chemicals, including quercetin and phenolic acids, were obtained after chromatographic purification of the *Ziziphus oxyphylla* fruit's methanolic extract's the ethyl acetate component plus the portion accessible in formaldehyde.

The goal of [6] was to the outcome of the present investigation unequivocally demonstrate that ziziphus jujube is a valuable resource for medicinal research. Extract of n-BuOH and water extract, when used as a healthy control, proved a more significant inhibitory impact of the sugar. The objective of [7] was too according to the present investigations, the efficacy of fruit extracts, polyphone antioxidants, and quantity of essential protein in the Ziziphus species are significantly influenced by atmospheric variables, including soil properties. Research [8] provides the opportunity to demonstrate a unique technique for the long-term preservation of *Ziziphus oxyphylla* fruits, particularly those of mahdia provenance. It was suggested that we eat Ziziphus fruit as an element of our food. The study [9] was the initial strategy to examine variance in the secondary metabolome of three of the most frequently observed Ziziphus leaf species presented in this study comparison. The goal of [10] was to show that unripened fruit in both species demonstrated much greater phenolic, flavonoid compounds, anti-oxidants, and antibacterial activity than ripe fruits.Research[11] to increase the economic value and competition in the marketplace of dried jujube; these studies offered guidance for the assessment of fruit quality in dried-out jujube and the development of graded requirements. The goal of [12] was too most importantly, the discoveries provide an empirical basis for comprehending precisely the coloring of jujube fruit varies as it ripens, enabling a better understanding of the essential components involved in maturing fruity color. Research [13] utilizing a high-resolution mass spectrometer and mass spectrum libraries, a quick and easy approach was devised in the current work to identify the primary chemical elements in both closely related species of the plant Ziziphus. The study [14] was that these fruits' strong antioxidant capabilities, alongside their low metal concentrations, make them a suitable environmental preventive for preserving food. Research [15] subsequently discovered revealed an ethanol extract of the Ziziphus fruit was high in polyphones with antioxidant activity. Ziziphus fruit has been endorsed to be a possible renoprotective chemical due to its reactive and antigen-toxic qualities, and this consumption needs to become more widely recognized worldwide.

2. Materials

2.1 Botanical Sample Gathering

Manipal university of Jaipur department of bioscience collected the ziziphus species oxyphylla edge in Jaipur in which we placed the plant specimen with an assigned voucher number, recognized and

These organisms have emerged as significant confirmed the plant. The rewards of the plant were removed out of the complete vegetation and cleaned using G_2P dried.The fruits were processed into finely ground dust.

2.2 Extraction of Plant Material

Three days of RT were spent extracting the fruit powder three times with alcohol. The methanolic extract was concentrated before filtration at $40^{\circ}C$ advertising vacuum. To produce enough methanolic sections, the procedure was carried out repeatedly. The methanol extract was mixed using water that had been distilled, and then fractionated using n-hexane in stages of chlorine dioxide polycarbonate of ethanol, both n-butanols. The percentage of ethyl acetate is determined. The mixture was divided into four portions using gradients n-hexane-ethyl-acetate elution on a silicon dioxide gel column. Subsequently, column chromatography fraction three was eliminated n-Hexane-Chloroform is used to create the remaining fractions. The columns enacted through part the gradient of n-hexane and chlorine dioxide. The chemical with n-hexane chloroform angles was produced by the component. After employing n-hexane chloroform gradation and elements the chloroform fraction was separated via silicone gel-based chromatographic stages. Eight distinct divisions F1 through F8 have been determined to be treated with n-hexane chloroform gradient column Ceramic gel electrophoresis with hexane-chloroform to produce the substance. The chemical was retrieved from fraction seven after it had been subjected to column chromatography employing an n-hexane-chloroform gradient on a gel composed of silica.

3. Results and Discussions

After evaporation using a circular evaporator the *ziziphus oxyphylla* fruit methanol extract after it has been purged numerous polarity based substances that include the solvent n- ethyl acetate chlorine dioxide and n-butanol after being dissolved in alcohol. Substances were isolated using the procedures described in the material and technique section after performing an array of column chromatographic separations of the ethyl-acetate and chloroform soluble fractions. . The isolates were characterized using a variety of spectroscopic techniques and the outcomes subsequently contrasted with earlier studies**.**

***3.1 Quercetin*:** According the form of a powder yellow mp=315-316.2°C, H-NMR: δ: 6.10, 6.31, 6.83, 7.44, and 7.57. CNMR: δ 176.5, 165.1, 162.1, 157.7, 147.1, 145.7, 144.0, 136.1, 124.1, 120.1, 118.1, 116.1, 103.1, 99.1, 95.0. EI/MS m/z (%): 303.3 $M+H^+$, $M-C_6H_5O_2$= 193.2 $M-CH_9H_5O_5$ = 109.1 by comparing it to spectroscopic data published in the literature, quercetin was identified as the substance.

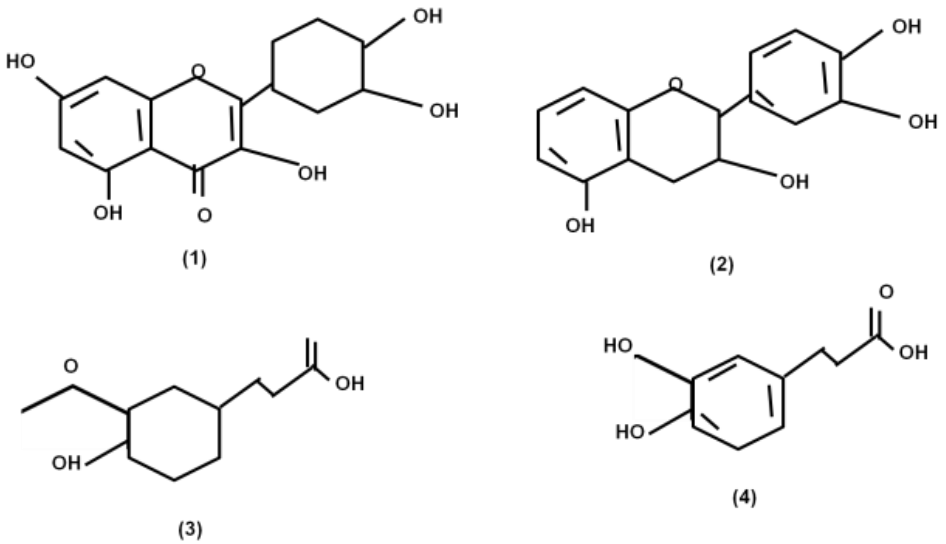

Figure 1. Chemical Structure For Quercetin, Catechin, Ferulic Acid, And Caffeic Acid

Table 1. Secondary Metabolites

	Secondary Metabolites
Alkaloids	0.022
Flavonoids	0.258
Saponins	0.9265
Tannins	0.3245

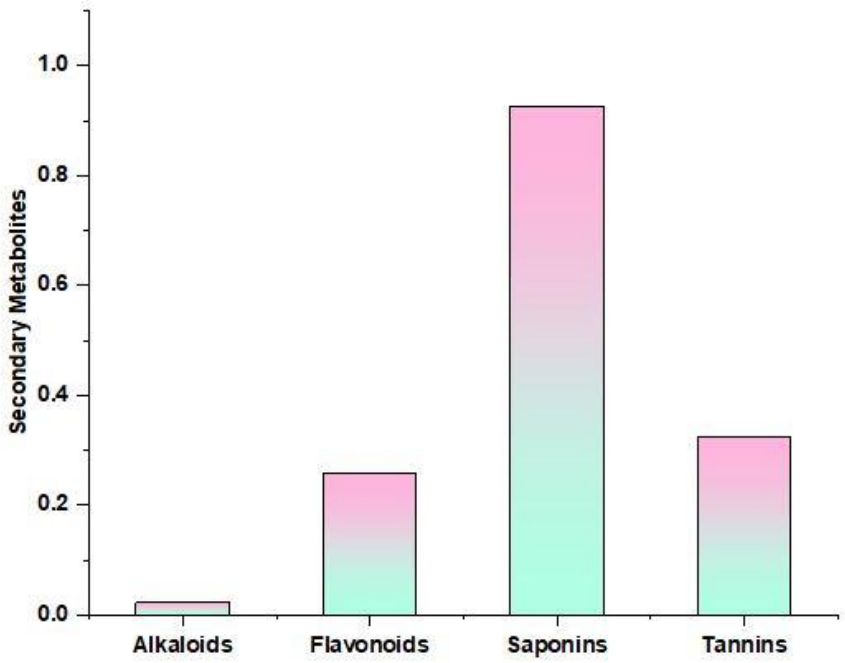

Figure 2. Ferulic Acid

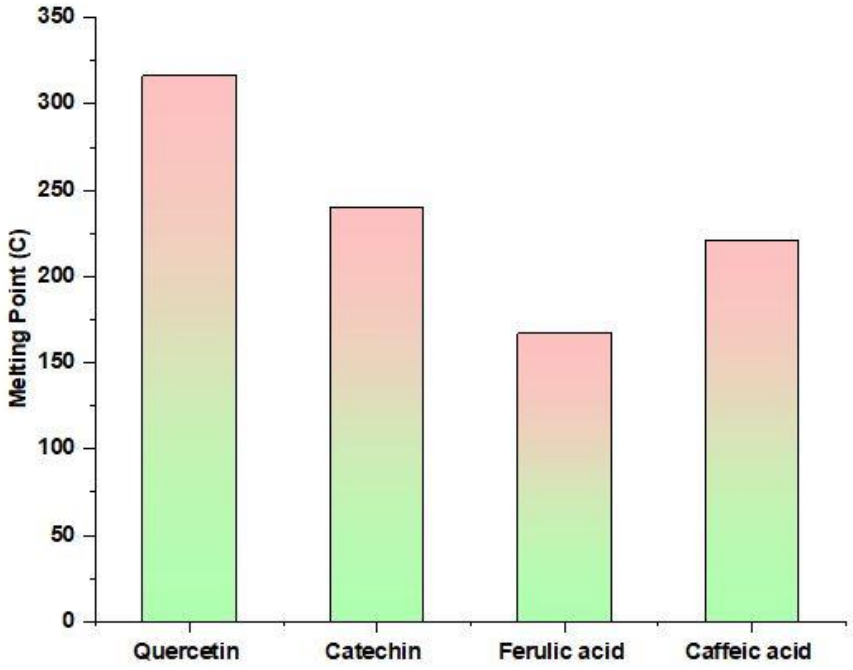

Figure 3. Melting point

Table 2. Melting Point (C)

	Melting Point (C)
Quercetin	316.2
Catechin	240
Ferulic acid	167
Caffeic acid	221

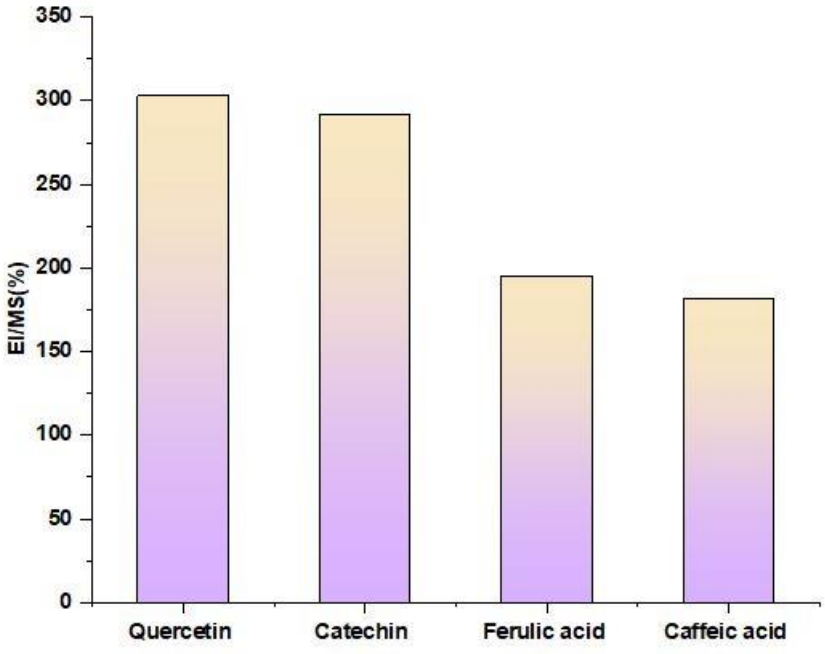

Figure 4. EI/MS(%)

Table 3. EI/MS(%)

	EI/MS(%)
Quercetin	303
Catechin	291.3
Ferulic acid	195.3
Caffeic acid	181.3

***3. 2Catechin*:** Produced into a substance that resembles yellow diamonds, $mp = 240 - 242°C$, H-NMR: δ: 2.67, 4.55, 4.81, 6.80, 6.72, 6.60, 5.90, C-NMR: δ 158.1, 156.1, 155.1, 146.3, 144.8, 132.1, 122.3, 117.1, 116.4, 98.3, 94.5, 86.1, 66.1, 29.0. EI/MS m/z (%): 291.3 $M + H^+$, $M - C_6H_5O_2$=181.4, $M - CH_9H_5O_5$ = 109.2 by comparing it to the spectroscopic data provided in the literature, it was found to be catechin.

3.3 Ferulic Acid: produced as a compound that resembles a yellow solid, mp= $167 - 168°C$, H-NMR: δ: 12.2, 7.55, 7.15, 7.01, 6.76, 3.53, C-NMR: δ 172.1, 148.1, 146.1, 145.1, 128.1, 123.4, 117.1, 116.7, 112.1, 57.1. EI/MS m/z (%): 195.3 $M + H^+$, $M - C_6H_5O_2 = 123.4$, $M - CH_9H_5O_5$= 71.1 Comparing it to spectroscopic data published in the literature allowed researchers to identify it as ferulic acid.

***3.4 Caffeic acid*:** According to a yellow solid-like substance is produced, mp=224 to $225.5°C$, H-NMR: δ: 12.1, 7.53, 7.12, and 7.06, 6.88, 6.56, C-NMR: δ 171.1, 147.2, 146.2, 144.1, 127.1, 124.4, 117.1, 115.7, 111.1. EI/MS m/z (%): 181.3 $M + H^+$, $M + OH$=164.1 by comparing it to the compound caffeine was found to be a compound based on spectroscopic information found in the literature. Zizyphus oxyphylla edgew's crude methanolic extract and its different fractions, including a solvent called chlorine dioxide, ethyl-acetate, and n-butanol, were analyzed for their antibacterial, antioxidant, and hemolytic characteristics, were examined along with the fruit's mineral composition. Fruit extracts had strong antibacterial and antioxidant effects. The isolated chemicals used in this investigation are potent antibacterial and antioxidants. For the benefit of humans, further research on this plant is advised. For the first time, these substances have been found in *Ziziphus oxyphylla* fruit. More research is being done on various plant parts.Quercetin, Catechin, Ferulic Acid, and Caffeic Acid, four chemicals isolated from the fruit of Ziziphus oxyphylla, show their structures in figure 1. Table 1and figure 2 indicates the secondary metabolites of alkaloids, Flavonoids, Saponins and Tannins. Figure 3 table 2 depicts the catechin and melting point (c). Figure 4 table 3 depicts the ferulic acid and EI/MS (%).

4. Conclusions

Throughout these investigations, additional metabolites from *Ziziphus oxyphylla* plant fruit have been discovered and established. This formaldehyde portion of the berry produced two separate phenolic acids the ferulic acid and caffeine acid. At the same time, two flavonoids, quercetin, and catechin and were extracted from the ethyl-acetate soluble fraction. Zizyphusoxyphyllaedgew's crude methanolic extract and its different fractions, including a solvent called chlorine dioxide, ethyl-acetate, and n-butanol, were analyzed for their antibacterial, antioxidant, and hemolytic characteristics, were examined along with the fruit's mineral composition. By using mass spectrometry and comparison with the information published in the literature, metabolites were identified. For the first time, these substances have been found in *ziziphus oxyphylla* fruit. The present findings might reveal further details concerning the plant's compositions containing polyphenols and phenol acids.

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