

Isolation of secondary metabolites from *Ziziphus oxyphylla* fruit

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Abstract

Ziziphus oxyphylla belongs to family Rhamnaceae. This plant is medicinally very important and is used in treatment of various ailments. It possesses various biological activities as antinociceptive, antimicrobial, antioxidant, antipyretic etc. This study was designed to isolate bioactive compounds from fruits of plant *Ziziphus oxyphylla*. Chromatographic purification of ethyl acetate and chloroform soluble fraction of the methanolic extract of the *Ziziphus oxyphylla* Fruit yielded four compounds which included flavonoids and phenolic acids. Quercetin (1) and Catechin (2) were isolated from ethyl acetate fraction and chloroform fraction yielded ferulic acid (3) and caffeic acid (4). The compounds 1-4 were characterized by using ¹H, ¹³C NMR and mass spectrometry (EI-MS) and in comparison with the reported data in the literature.

Key words: Quercetin, Isolation, Fruit, Medicine and *Ziziphus oxyphylla*

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1. Introduction

Plants are major source of conventional medicines used to cure various diseases. It has been estimated that more than 80% of the developing countries in the world relies on herbal medicines [1]. *Ziziphus oxyphylla* is small or medium sized tree habitated mostly in the northern areas of Pakistan where it is used by tradition as a fruit [2]. *Ziziphus oxyphylla* belong to the family Rhamnaceae having more than 100 species distributed all over the warm-temperate and subtropical regions of the world among which only six species are native of Pakistan [3]. Locally this plant is called as Mamyau or Elanai. Different parts of this plant as fruits and roots are used for treatment of gas trouble and jaundice, hypertension. The extract of roots obtained after boiling in water is used against scabies, pustules and diabetes. Fruits are edible and leaves are browsed by goats [4]. A variety of *Ziziphus* species have been used as medicine in ayurvedic and folk type system to treat skin infection, allergy, urinary problems and fever etc. [5]. *Ziziphus oxyphylla* possess various types of pharmaceutical importance such as antioxidant [6], antinociceptive [7] and antipyretic [8], anti-infective [9] and antipyretic [10]. As part of our efforts to explore the flora of Pakistan for nutritional and medicinal importance for chemical and biological activities [11-27]. In the current study four different secondary metabolites have been isolated from fruit of *Ziziphus oxyphylla* plant.

2. Materials and Methods

2.1. Collection of Plant Material

The plant *Ziziphus oxyphylla* Edgew was collected from Ziarat, Baluchistan and Dr. Rasool Bakhsh Tareen from Department of Botany, University of Balochistan, Quetta Pakistan identified and recognized the plant, where we have deposited the plant specimen with allotted Voucher number ZO-RBT-09. The plant fruits were separated from the whole plant, washed with H₂O and shade dried. The fruits were grinded to fine powdered form.

2.2. Extraction of Plant Material

The fruit powder (1kg) was extracted thrice at rt for 3 days with methanol (3L). The filtered methanolic extract was concentrated at 40°C in vacuo. The process was repeated thrice to obtain a sufficient quantity of methanolic extract (50g). The methanol extract was suspended in distilled water and fractioned successively with n-hexane (10g), chloroform (8g), ethyl acetate (8g) and n-butanol (10g). The ethyl acetate fraction (8g) was subjected to column chromatography over silica gel eluting with gradient n-hexane-ethyl-acetate to obtain 4 fractions. Fraction 3 (3g) was subjected to column chromatography eluted with n-hexane-chloroform at different ratios to further three fractions A, B, C. Fraction A was passed through column

with n-hexane-chloroform gradient (100:1 to 100 chloroform) give compound (1) (20 mg). Fraction C yielded the compound (2) (10 mg) with n-hexane-chloroform gradient (100:1 to 100 chloroform).

Then the chloroform fraction was subjected to column chromatography on silica gel using n-hexane-chloroform gradient as eluents. Eight different fractions were obtained F1-F8. F2 fraction (2g) was subjected to column chromatography on silica gel using n-hexane-chloroform gradient (hexane-chloroform 50:1 to chloroform) yielded the compound (3) (10mg). Then fraction 7 was subjected to column chromatography on silica gel using n-hexane-chloroform gradient and it yielded the compound 4 (5mg).

3. Results and Discussion

The methanolic extract of dried plant fruit (1kg) of *Z. oxyphylla* was evaporated with rotary evaporator then it was suspended in water and partitioned with different polarity based solvents as n-hexane, chloroform, ethyl-acetate and n-butanol. After doing a series of column chromatography of ethyl-acetate and chloroform soluble fractions, compounds 1-4 were isolated as methods describes in material and method section. Isolated compounds were characterized by using different spectroscopic techniques as ^1H NMR, ^{13}C NMR and EI-MS and results were compared with already reported literature as well.

Quercetin (1): obtained as yellow powder, mp=315-316.2°C, ^1H -NMR (400 MHz, CDCl_3): δ : 6.10 (1H, d, J=2.5 Hz), 6.31 (1H, d, J=2.8 Hz), 6.83 (1H, d, J=7.5 Hz), 7.44 (1H, dd, J=1.3, 8.0 Hz), 7.57 (1H, d, J=4.4 Hz). ^{13}C -NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{H}]^+$, $[\text{M}-\text{C}_6\text{H}_5\text{O}_2]=193.2$, $[\text{M}-\text{CH}_9\text{H}_5\text{O}_5]=109.1$. It was determined as quercetin by comparison with the spectroscopic data reported in the literature

Catechin (2): obtained as yellowish crystal like compound, mp=240-242°C, ^1H -NMR (400 MHz, CDCl_3): δ : 2.67 (m, 2H), 4.55 (d, J=7.2 Hz, 1H), 4.81 (m=1H), 6.80 (d, J=8.07 Hz, 1H), 6.72 (d, J=7.5 Hz, 1H), 6.60 (d, J=3.07 Hz, 1H), 5.90 (m, 2H). ^{13}C -NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{H}]^+$, $[\text{M}-\text{CH}_6\text{H}_5\text{O}_2]=181.4$, $[\text{M}-\text{C}_9\text{H}_9\text{O}_4]=109.2$. It was determined as Catechin by comparison with the spectroscopic data reported in the literature

Ferulic Acid (3): obtained as yellowish solid like compound, mp 167-168°C, ^1H -NMR (400 MHz, CDCl_3): δ : 12.2 (s, 1H), 7.55 (1H, d, J=10 Hz), 7.15 (d, J=7.3 Hz, 1H),

7.01 (1H, d, J=2.5 Hz, 1H), 6.98 ((d, J=7.0 Hz, 1H), 6.76 (m, 1H), 3.53 (s, 3H). ^{13}C -NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{H}]^+$, $[\text{M}-\text{C}_3\text{H}_3\text{O}_2]=123.4$, $[\text{M}-\text{C}_7\text{H}_7\text{O}_2]=71.1$. It was determined as ferulic acid by comparison with the spectroscopic data reported in the literature

Caffeic acid (4): obtained as yellow solid like compound, mp=224 to 225.5°C, ^1H -NMR (400 MHz, CDCl_3): δ : 12.1 (s, 1H), 7.53 (1H, d, J = 8.6 Hz), 7.12 (d, J=7.1 Hz, 1H), 7.06 (1H, d, J=2.0 Hz, 1H), 6.88 (d, J=6.5 Hz, 1H), 6.56 (d J=5.5 Hz, 1H). ^{13}C -NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{H}]^+$, $[\text{M}-\text{OH}] = 164.1$. It was determined as caffeic acid by comparison with the spectroscopic data reported in the literature

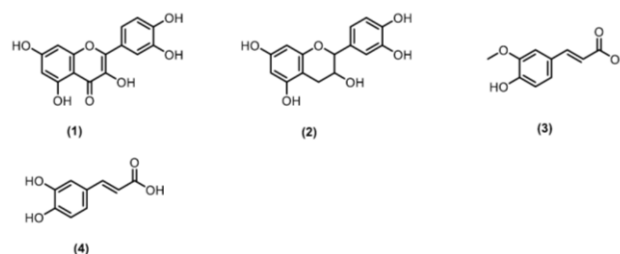


Figure: 1 Structures of the compounds isolated from Fruit of *Zizyphus oxyphylla*, Quercetin (1); Catechin (2); Ferulic acid (3); Caffeic acid (4)

In our previous investigation, the crude methanolic extract and its different fractions n-hexane, chloroform, ethyl-acetate, n-butanol and oils (essential and fixed oils) of fruit of *Zizyphus oxyphylla* Edgew were analyzed for antimicrobial, antioxidant and hemolytic activities with mineral analysis of fruit. The fruit extracts showed potent antimicrobial and antioxidant activities. The isolated compounds in this study are excellent antioxidant and antimicrobial agents. Further investigations are suggested on this plant for benefits of human beings [20]. These compounds are reported for the first time from *Zizyphus oxyphylla* fruit. Further study is ongoing on different fraction of this plant.

4. Conclusion

In this study secondary metabolites have been isolated from fruit of *Zizyphus oxyphylla* plant. Two flavonoids Quercetin (1) and Catechin (2) were isolated from ethyl-acetate soluble fraction and two phenolic acids ferulic acid (3) and caffeic acid (4) were obtained from chloroform fraction of fruit. Metabolites were characterized by using ^1H , ^{13}C NMR and mass spectrometry (EI-MS) and in comparison with the reported data in the literature. These compounds are reported for the first time from *Zizyphus oxyphylla* fruit. The current results may provide more

information about flavonoids and phenolic acid profiles of this plant.

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