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Characterization of bioactive components and antioxidant activity of *opuntia ficus indica* peels.

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ABSTRACT

Opuntia ficus-indica peels contain a number of active substances. Bioactive secondary metabolites have effective role in the maintenance of health and the treatment of some common disorders. The chemical composition showed moisture (6.78±0.065), total protein (4.78±0.030), fats (0.9±0.010), total ash (10.49±0.233), total carbohydrates (50.12±0.045), and crude fiber (26.93±0.025) on the dry weight. Total phenolics in the methanolic and ethanolic extracts of *Opuntia ficus indica* peels were (292.9±0.0198) and (165.6±0.0553) mg GAE/100 g, while total flavonoids were (20.39 ±0.0031) and (11.39±0.0346) mg QE/100g, respectively. Antioxidant activity in the methanolic extract was (79.100±0.005), while in the ethanolic extract it was (35.09±0.003). Using HPLC analysis, identification of thirteen phenolic compounds were: Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Pyro catechol, Coumaric acid, Ferulic acid, Naringenin, Daidzein and Querectin. While the highest concenterations were Pyro catechol and Catechin which have concentrations 60.36 µg/ml and 58.47µg/ml respectively. LC/MS/MS analysis identified the main phenolic compounds (5peaks) in *opuntia* peel ethanolic extract as piscidic, eucomic, malic, iso-rhamnetin and behenic acid.

KEYWORDS: *Opuntia ficus indica*, HPLC analysis, LC/MS/MS analysis and Antioxidant

1. INTRODUCTION

Antioxidants are substances that, presented in low concentrations compared to easy oxididated compounds that can be slow down and/or stop its oxidation. Li et al., (2006). Regular cell reinforcements, which are bountiful in numerous food sources and restorative plants, have an exceptional free revolutionary searching potential that might be helpful in the treatment of oxidative pressure. El Tanbouly et al., (2017). The development of new therapeutic agents is anticipated to be aided by the consumption of vegetables and fruits which identified as rich sources of bioactive phenolic compounds that must be ingested over the course of a lifetime in a balanced diet. Mata et al., (2016). Opuntia

ficus-indica which known as cactus pear and prickly pear. Barba et al., (2017). In family Cactaceae. El-Hawary et al.,(2020). One of the first fruit crops to adapt to a variety of soil conditions, it is also regarded as one of important cactus specie in agricultural field. In different conditions (dry and semi-arid conditions). opuntia can be cultivated and grown. Daniloski et al., (2022). Many food products, including jam, drinks, nectars, and candies, are made from processed opuntia fruit. Its peels, which made up roughly 30% of the weight of the fruit and were recognized as an agricultural industrial waste, Melgar et al., (2017). Around 45% to 50% of the weight of this fruit is made up of its skin. According to previous results, annually, millions of kilograms of fruit peels are thrown away, which are disposal and causing environmental problems. Elkady et al., (2020 (. In Egypt, prickly pear peel production amounts to about 58,344 tonnes annually Ali et al., (2022). Opuntia peels can be consumed or utilised as functional food material to increase their health value for people and animals. Oniszczuk et al .,(2020).

In several countries folk remedy is among the uses of, opuntia ficus indica, to treat wound edoema, burns, and dyspepsia. Its alcoholic extracts contain antibacterial, anti-inflammatory, and hypoglycemic activities, according to data from numerous investigations. Also, historically, diabetics have been treated with the stem of the prickly pear cactus.(Tilahun and Welegerima, 2018).

The prickly pear peel contains nutrients with high nutritional value, including flavonoids and phenolics that are frequently used in pharmaceutical and food industries.. Prickly pear peels, which are regarded as vegetable waste, can be used to extract a significant quantity of bioactive components like vitamin C, and dietary fibre (Anaberta et al., (2011) (Jiménez-Aguilar et al., 2015) and (Shimaa et al., (2022). It is considered healthy compounds, lowering the risk of illnesses including diabetes, cancer, and inflammation, among others (Dillard and German, 2000). Nevertheless, the agronomic techniques cultivar factors, environmental and climatic circumstances may have an impact on the distribution of bioactive molecules such as the characteristics of chemicals of food items like fruit and peels of Opuntia pear. (Moussa-Ayoub et al., 2014). Therefore, this study investigated the phenolic compound profile by HPLC and LC/MS/MS analysis in the ethanolic extract of O.ficus indica peels and antioxidant activity present in both ethanolic and methanolic extracts as well as their chemical composition.

2. MATERIALS AND METHODS

2.1. Materials

O.ficus indica fruits were obtained from a supermarket in Benha city. Peels were separated, washed, cut into smaller pieces and dried at room temperature and ground into fine powder for extraction.

2.2. Preparation of extracts

Each fine powder of peels (100 g) was mixed with different solvent ethanol –methanol 80%, 1:10 ratio (w/v) at room ambient for 3 weeks and mixed gently every day by steering. (Abou-Elella and Ali. 2014).

2.3. Analytical methods

(A.O.A.C., (2019) used for determination of ash, total lipids, crude protein, and moisture.

Determination of total carbohydrates

The concentration of total carbohydrates was determined according to DuBoiset al., (1956).

Determination of total phenolic.

Total phenol analysis was carried out according to the method of Muntana and Prasong, (2010). Using spectrometry method

Determination of Total flavonoid.

Total flavonoid analysis was achieved by the method of Tandoro et al., (2020).

Determination of antioxidant activity by DPPH:

Antixidant activity in opuntia ficus indica peels ethanolic extract was measured by Tandoro et al., (2020). Percentage of antioxidant activity of free radical DPPH was calculated as follows:

Antioxidant activity (inhibition) % = [($A_{control}$ - A_{sample}) / $A_{control}$] × 100

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the plant extract.

HPLC analysis

Separation of phenols and flavonoids in ethanolic extract of O.ficus indica peel, was carried out by Verma et al. (2011).

LC/MS/MS analysis

we used (4000 QTRAPLC–MS/MS) method for detection of phenolic compounds in *O.ficus indica* peels ethanolic extraction. The LC/MS optimization was performed by direct infusion with the Pump HARVARD APPARATUS11PLUSat. The flow-rate of was 0.1 mL/min. with mobile phase contains of Water: Methanol: formic acid (50:50:0.1 v/v/v).

3. RESULT AND DISCUSSION

3.1. Chemical composition of *O. ficus indica* peels

The chemical composition of O. ficus indica peels were determined and reported in Table (1). The peels contain moisture 6.78 g/100g, protein (4.78 g/100g), fats (0.9 g/100g), ash (10.49 g/100g), carbohydrates (50.12 g/100g) and Crude fiber(26.93 g/100g) dry bases. Current results are agreement with (Kossori et al., (1998) and (Mahfouz and Abd-Elnoor., (2020). The chemical composition in O.ficus indica peels were observed Based on their dried weight by El-Said et al., (2011). (18.50%) moisture, (4.5%) crude protein, (8%) total ash, (4.9%) crude fibre, and (81.5%) total solid,respectively.

Table 1. Chemical composition of *opuntia* peels (mean ±SD)

Components	Contents(g/100g)
Moisture	6.78±0.065
Crude Protein	4.78±0.030
Total Lipids	0.9±0.010
Ash	10.49±0.233
Crude fiber	26.93±0.025
Total Carbohydrates	50.12±0.045

3.2. Total Phenolic, Total flavonoids compounds and Antioxidant activity of *O.ficus indica* peels extracts.

Table (2) showed that ethanolic extracts of opuntia peels was 21.35%, while methanolic extract yield was 25.815% while, contents of phenolics opuntia peel methanol extract were 292.9 and ethanolic extract were 165.6 (mgGAE/100g(.While, Total flavonoids in methanolic extract were 20.39 and ethanolic extract were 11.39 (mgQE/100g(. Antioxidant activity in the methanolic extract was 79.100 % while,The ethanolic extract was 35.09%, .our results are in agreement with those reported by Abou-Elella and Ali (2014), Aparicio-Fernández et al., (2018) and Nabil et al., (2020) extract yields of Fruit pulp ranged from 0.64 to 40.76%, whereas extract yields of peel ranged from 0.80 to 37.60%. The highest TPC was detected through dry/freeze peel methanol extracts, at 17.59 and 16.51 mg GAE/g, respectively. (Aruwa et al., (2019) Peels of O. ficus-indica exhibit substantial phenolic content variation. The type of phenolic component that extracts include, as well as variations in the processing techniques and solvents employed, may all be related to variations in TPC. (Chavez-Santoscoy et al., 2009).

Table 2. Total phenolics, total flavonoids and antioxidant activities of *opuntia ficus indica* peels extract

Parameters	Methanol extract	Ethanol extract
Yields (w/w)%	25.815±0.100	21.35±0.157
Total polyphenols (mg GAE /100g)	292.9±0.0198	165.6±0.0553
Total Flavonoids (mg QE /100g)	20.39 ±0.0031	11.39±0.0346
Antioxidant activity(%) by DPPH	79.10٠±0.005	35.09±0.003

Value are mean of three replicates ± standard deviation (n = 3).

3.3. Identification of some phenolic and flavonoid components of *opuntia ficus indica* peel Ethanolic extract by HPLC:

Phenols are one of the most significant plant chemicals because of their capacity to scavenge free radicals and because they contain hydroxyl groups Heim et al., (2002) and Abou-Zaid et al., (2022).(Table 3, table 4)and figer1 shows the separation and identification of thirteen phenolic compounds were Chlorogenic acid (3.32µg/ml), Catechin (58.47µg/ml), Methyl gallate (2.83 µg/ml), Coffeic acid (1.29 µg/ml), Pyro catechol (60.36 µg/ml), Coumaric acid (13.48 µg/ml), Ferulic acid (52.78 µg/ml), Naringenin (2.27 µg/ml), Daidzein(0.17 µg/ml), Querectin (6,83 µg/ml), Cinnamic acid (0.32 µg/ml), Apigenin (0..77 µg/ml) and Hesperetin (0.34 µg/ml).by Abou-Zaid et al ., (2022), who found that Chlorogenic acid (ranging from 56.44 to 60.91 µg/ml), Catechin (ranging from 79.12 to 97.04) µg/ml , Methyl gallate (ranging from 2.50to2.80 µg/ml), Coffeic acid (ranging from 4.10 to 3.72) µg/ml , Pyro catechol (22.05-22.50) µg/ml, Coumaric acid (ranging from 2.75 to 3.01) µg/ml, Ferulic acid(21.01to23.99) µg/ml, Naringenin (ranging from 15.80 to 20.55) µg/ml, Daidzein (ranging from 3.05to3.35 µg/ml), Querectin (ranging from 0.80 to 0.10) µg/ml and Cinnamic acid (ranging from 0.22 to 0.05) µg/ml)

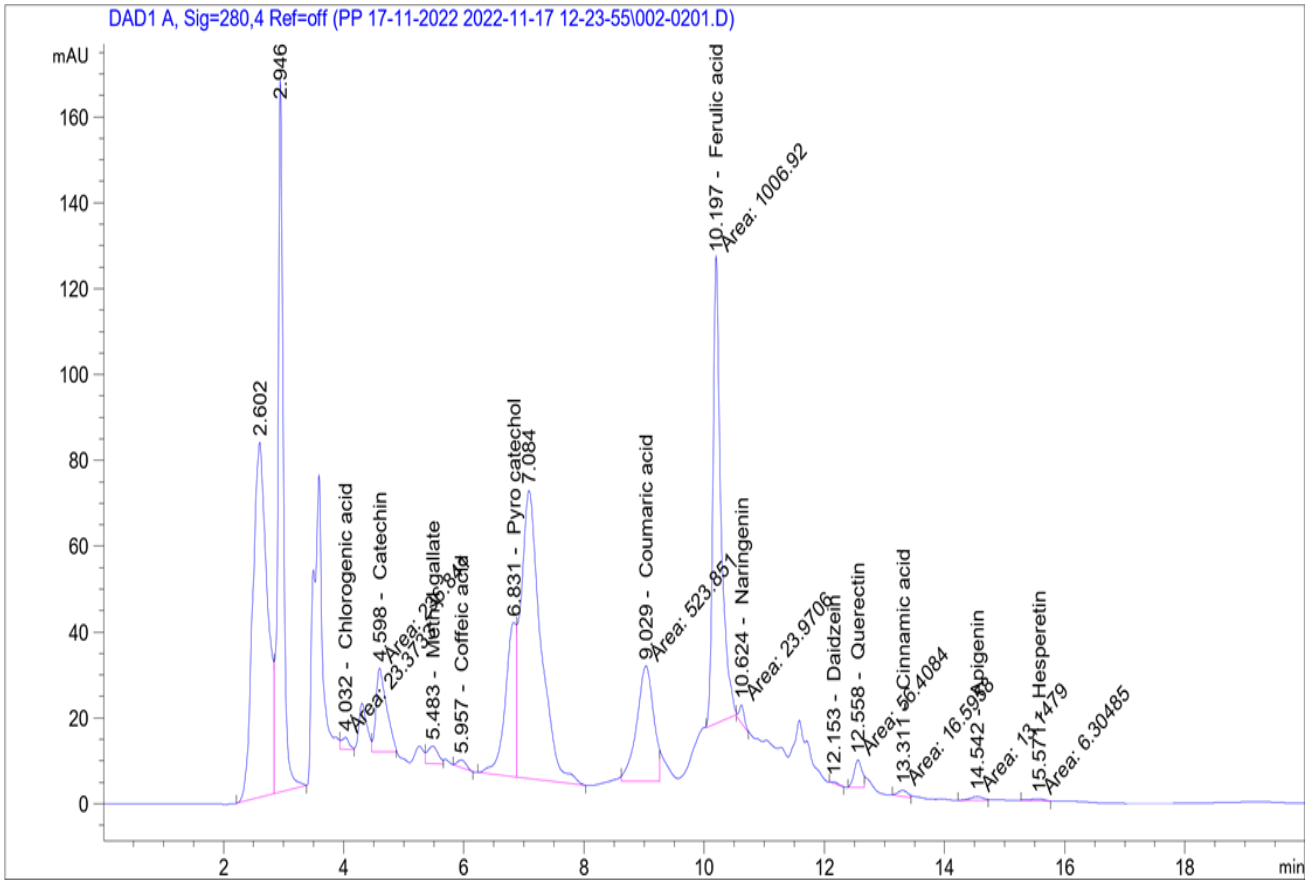


Fig 1. HPLC analysis profile of Opuntia ficus-indica peels of ethanolic extract.

Table 3. Phenolic compounds of *O.ficus indica* peels ethanolic extract analyzed by HPLC.

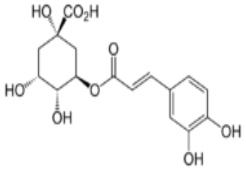
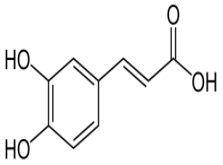
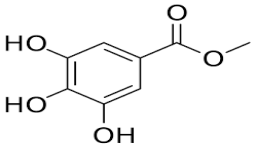
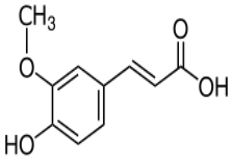
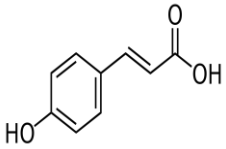
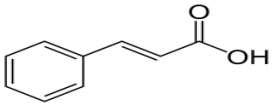
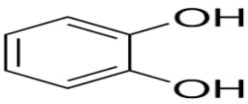
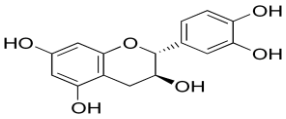
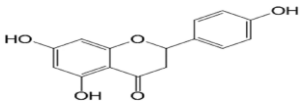
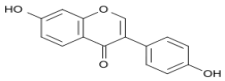
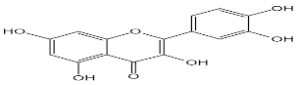
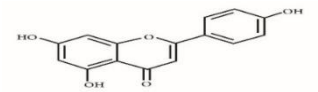
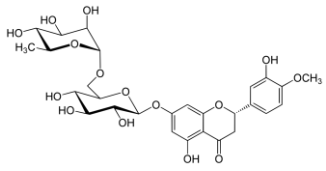
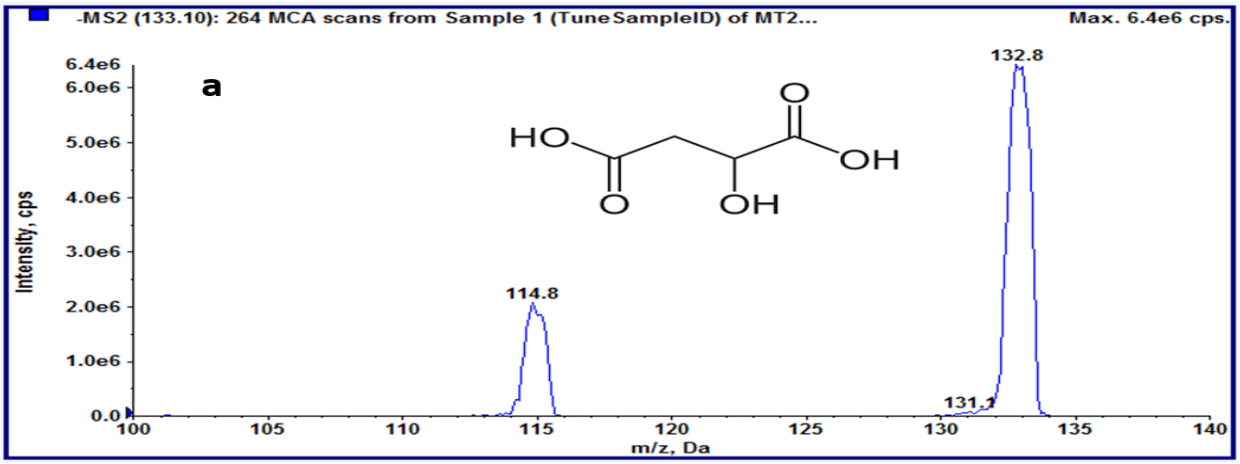
Phenolic compounds	structure	Concentraton (µg/ml)	References
Chlorogenic acid		3.32	Abou-Zaid et al .,2022
Coffeic acid		1.29	El-Mostafa et al.,(2014)and Belhadj Slimen et al .,(2020)
Methyl gallate		2.83	Abou-Zaid et al .,2022
Ferulic acid		52.78	El-Mostafa et al.,(2014)and Belhadj Slimen et al .,(2020)
Coumaric acid		13.48	Aruwa et al .,(2019)and Belhadj Slimen et al .,(2020)
Pyro catechol		60.36	Abou-Zaid et al .,2022
Cinnamic acid		0.32	Belhadj Slimen et al .,(2020)

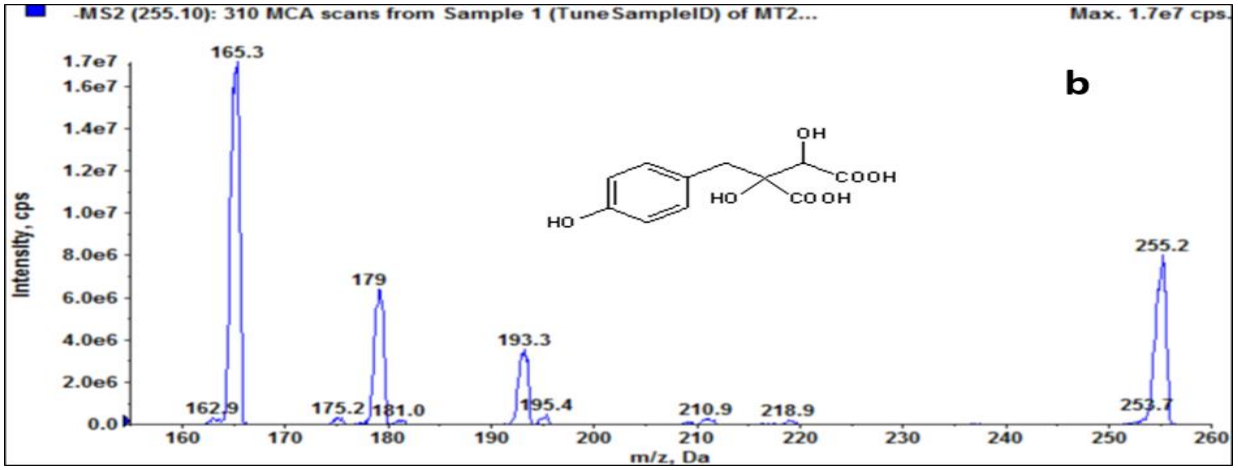
Table 4. **flavonids compounds of *O.ficus indica* peels ethanolic extract analyzed by HPLC.**

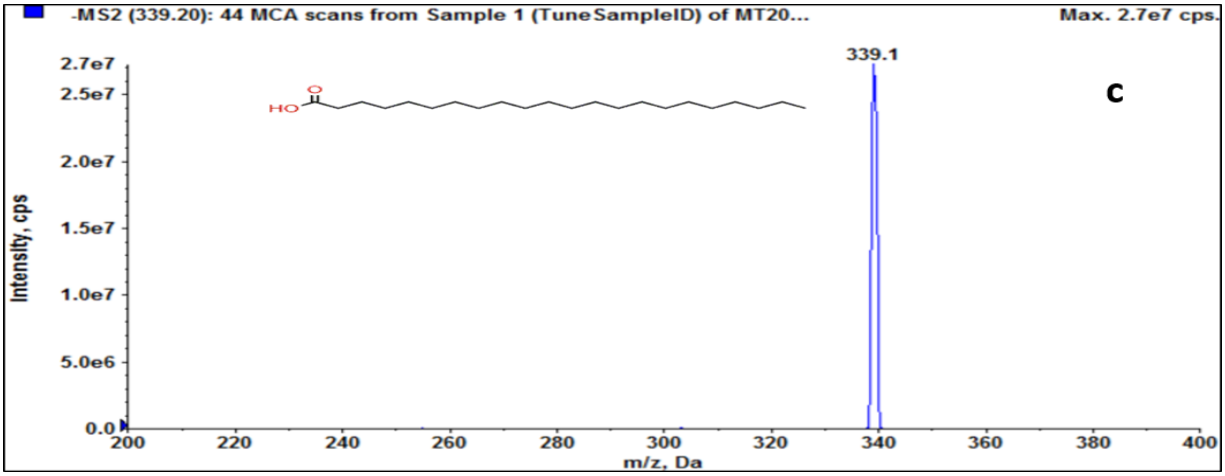
Flavonid compounds	structure	Concentraton (µg/ml)	References
Catechin		58.47	Abou-Zaid et al .,2022
Naringenin		2.27	Belhadj Slimen et al., (2017) and Belhadj Slimen et al .,(2020)
Daidzein		0.17	Abou-Zaid et al .,2022
Querectin		6.83	El-Mostafa et al.,(2014)
Apigenin		0.77	Abou-Zaid et al .,2022
Hesperetin		0.34	Abou-Zaid et al .,2022

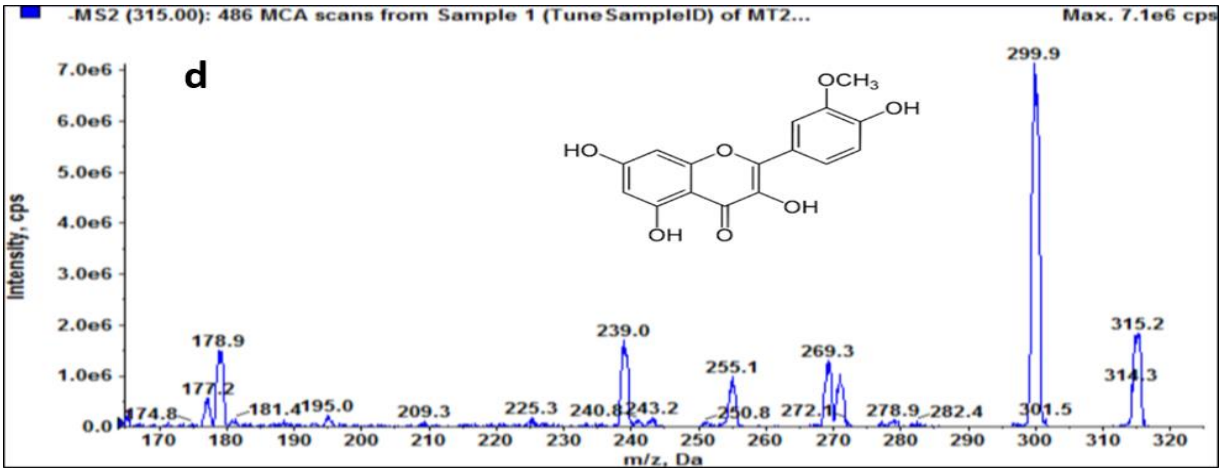
3.4.LC/MS/MS Analysis

Because of its higher sensitivity, durability, and increased sample molecular weight range made possible by the creation of multiple charged ions, commonly used LC-MS ionisation method. Seemann et al ., (2015). The results in Figure 3 confirmed that O.ficus indica peels extracts have a significant number of phenolic compounds. The LC/MS/MS detected phenolic (5 peaks) in O.ficus indica peels extracts as piscidic , Eucomic , Iso Rhamnetin, Behenic and malic acid The compounds' precursor–product ion pairs (quantification transitions) were discovered to be m/z (179.0,177.0,149.1) –(193.3,179.1,165.3)-114.8 and(178.9, 239.0, 255.1 ,269.3), respectively. These fragments were also observed by Aruwa et al., (2018), García-Cayuela et al., 2019 and El-Hawary et al., (2022), found that opuntia peels extract have Eucomic, piscidic, malic and Iso Rhamnetin. Piscidic acid, along with eucomic acid, was the predominant phenolic found in the prickly pear pulp and peel of opuntia ficus indica, and it was the most prevalent among phenolic acids found in the peels. Da Silveira Agostini-Costa et al.(2022). Recently, piscidic acid derivatives have shown in vitro neuroprotective properties. Petruk et al., (2018). Cladodes and peels have a sour flavour because of malic acid. (Wiberg et al., (1996).


-MS2 (133.10): 264 MCA scans from Sample 1 (TuneSampleID) of MT2...
Max. 6.4e6 cps.
a
132.8
114.8
131.1
Intensity, cps
m/z, Da


-MS2 (255.10): 310 MCA scans from Sample 1 (TuneSampleID) of MT2...
Max. 1.7e7 cps.
b
165.3
179
193.3
255.2
162.9
175.2
181.0
195.4
210.9
218.9
253.7
Intensity, cps
m/z, Da


-MS2 (339.20): 44 MCA scans from Sample 1 (TuneSampleID) of MT20...
Max. 2.7e7 cps.
c
339.1
Intensity, cps
m/z, Da


-MS2 (315.00): 486 MCA scans from Sample 1 (TuneSampleID) of MT2...
Max. 7.1e6 cps
d
299.9
239.0
315.2
269.3
255.1
178.9
177.2
Intensity, cps
m/z, Da

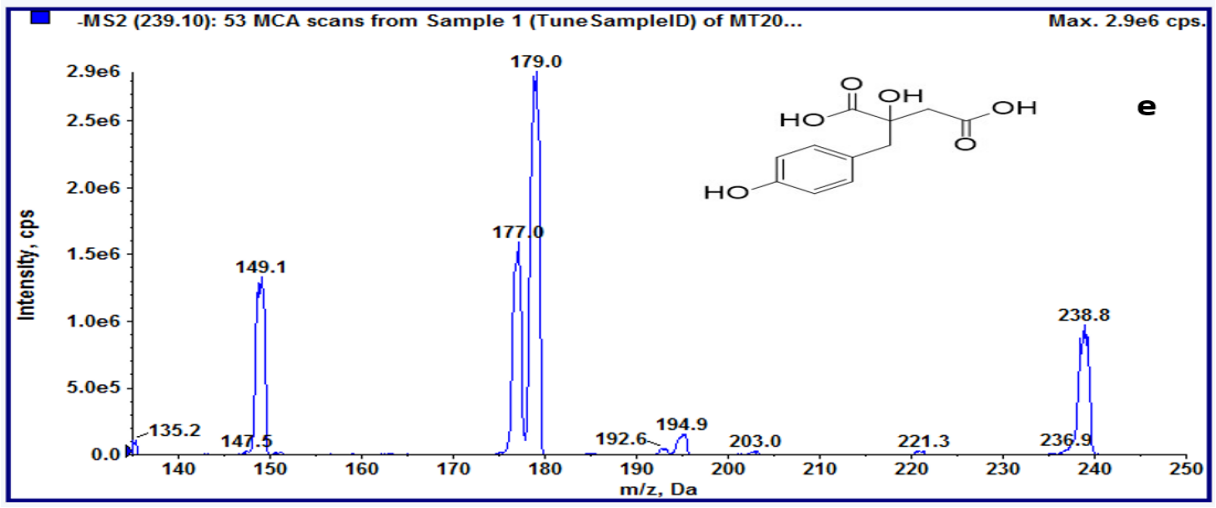


Fig 2. Product ion spectra and the main fragmentation pathway of [M + H]- for malic acid (A), pisidic acid (B), behenic acid (C), Iso Rhamnetin((D), and eucomic acid (E)

4. CONCLUSION.

The ethanolic extract of opuntia ficus indica peels is valuable source of bioactive compounds, including piscidic , eucomic, malic, iso-rhamnetin and behenic acid were investigated by LC/MS/MS analysis In addition to, some phenolic and flavonoid compounds were identified by HPLC analysis, were Chlorogenic acid (3.32 µg/ml), Catechin (58.47µg/ml), Methyl gallate (2.83 µg/ml), Coffeic acid (1.29 µg/ml), Pyro catechol(60.36 µg/ml), Coumaric acid (13.48 µg/ml), Ferulic acid (52.78 µg/ml), Naringenin (2.27 µg/ml), Daidzein (0.17 µg/ml), Querectin (6,83 µg/ml), Cinnamic acid (0.32 µg/ml) , Apigenin (0..77 µg/ml) and Hesperetin (0.34 µg/ml) and It also contains a high of crude fiber (26.93), which indicates the significance of *opuntia ficus indica* peels .

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الملخص العربي

توصيف المكونات الفعاله ومضادات الأكسده في قشور التين الشوكى

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قشور التين الشوكى غنيه بمحتواها من الالياف ومضادات الاكسده الهامه والتى لها العديد من الفوائد الصحيه والغذائيه تم تقدير التركيب الكميائي لهذه القشور على أساس الوزن الجاف فكان محتواها من الرطوبه ٦,٧٨ والبروتين ٤,٧٨ والكربوهيدرات ٥١,١٢ والالياف ٢٦,٩٣ والزيت ٠,٩ والرماد ١٠,٤٩ كما تم تقدير الفينولات الكليه للمستخلص الميثانولى كانت حوالى ٢٩٢,٩ والايثانولى ١٦٥,٦ بينما الفلافونيدات للمستخلص الميثانولى كانت حوالى ٢٠,٣٩ والايثانولي ١١,٣٩ وكانت نسبه التثبيط للمستخلص الميثانولى ٧٩,١٠٠% بينما للمستخلص الايثانولى ٣٥,٠٩% كما تم التعرف على بعض من مضادات الاكسده الهامه بواسطه التحليل الكروماتوجرافي السائل للمستخلص الايثانولى ومن هذه المركبات الهامه الكورستين والكلوروجنك وميثيل جالات وسينامايك والكويومارك والابجنينين والبيروكاتيكول والكافيك والكاتشين والديدازين والهسبارتين بالاضافه الى التعرف على مركبات فعاله اخرى بواسطه التحليل الكروماتوجرافي السائل المزود بمطياف الكتله والذي يعتبر اكثر حساسيه ودقه من التحليل الكروماتوجرافي السائل ومن ضمن المركبات التى تم التعرف عليها من خلاله حمض البهينك والمالك والايزوريهمنتين والايكومك وال بيزدك مما ياكد إن قشور التين الشوكى غنيه بمحتواها من المركبات الفعاله والتى لها العديد من الفوائد في المجالات الصناعيه والطبيه .

الكلمات المفتاحية: التين الشوكى , والتحليل الكروماتوجرافي السائل, مضادات الاكسده, التحليل الكروماتوجرافي السائل المزود بمطياف الكتله