Detection and Isolation of P-Coumaric Acid from Chloroform Extract of Iraqi, *Cicer arietinum* as well as Characterization Using Various Techniques (HPTLC, TLC, Preparative TLC, UV, IR)

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ABSTRACT

In all stages of the drug discovery and development process, analysis of pharmaceutical and natural chemicals, as well as newer pharmaceuticals, is routinely used. The entire potential of thin layer chromatography is exploited by high performance thin layer chromatography, a complex instrumental technique. It is a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples and food items. These features include automation, scanning, full optimization, selective detection principle, minimal sample preparation, hyphenation and other substances.

Key words: Thin layer chromatography, Instrumental technique, Pharmaceuticals, Hyphenation, Scanning

INTRODUCTION

Chickpea (*Cicer arietinum*) is the world's fifth most important legume in terms of total grain production and Spain's first common food legume (Figure 1).
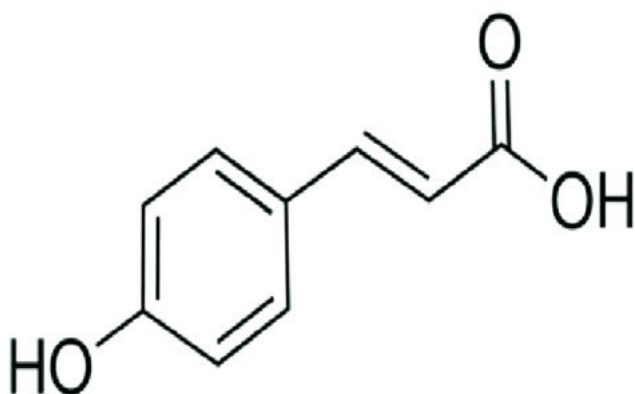

Figure 1: p-Coumaric acid structure.

Among legumes, chickpea is generally recognized as a valuable source of dietary proteins and an important part of a daily diet in many countries.

There are several naturally occurring bioactive molecules termed phytochemicals that can provide considerable long term health advantages, according to epidemiologic and nutritional studies. Polyphenols have been identified as the most abundant source of antioxidants in our diet among these substances [1,2]

A phenolic acid, p-coumaric acid (4-hydroxycinnamic acid) is a hydroxyl derivative of cinnamic acid. It suppresses cellular melanogenesis, reduces LDL peroxidation, has anti-mutagenesis, anti-genotoxicity and anti-microbial properties and plays a function in immunological regulation in humans [3].

The shikimate process, which uses phenylalanine and tyrosine as precursors, produces P-Coumaric Acid (p-CA), a phenolic acid belonging to the hydroxycinnamic acid family. Tyrosine ammonia lyase transforms tyrosine to p-CA in plants and mushrooms. Because it can regulate secondary metabolism, p-CA plays a crucial role. Caffeic acid, for example, can be converted to phenolic acids, flavonoids, lignin, ferulic acid, chlorogenic acid and sinapic acid secondary metabolites and precursors [4,5]. According to the benefit of p-comuaric acid in plants for

humans, we focused on it and used different methods to detect it in (*Cicer arietinum*) extract, isolated it and approved it by different technique [6].

MATERIALS AND METHODS

Experimental

The collection of the plant, preparation of the crude extract, determination of the extractive value and determination of various phytochemicals [7]. The plants have been collected from Kirkuk city, located in the north of Iraq (Figure 2).

Figure 2: Chickpea (Cicer arietinum) pods and seeds.

General procedure

All parts of the plant (seeds, leaves, root) have been chopped and ground to produce powder, then defatting in hexane overnight, later the extract filtered and kept for evaporation under reduced pressure on vacuum rotary evaporated the extract, then after dried put the plant in soxhlet apparatus with methanol for 16 hours then evaporation on vacuum rotary evaporated under reduced pressure, then dissolved with 150 ml water then fractionation with various solvents (chloroform, ethylacetate, n-butanol), phytochemical analysis using thin layer chromatography and HPTLC and preparative TLC, UV, IR (Figure 3) [8-10].

Figure 3: Cicer arietionum extracted by soxhlet apparatus.

Detection by thin layer chromatography

First of all, I activated the thin layer chromatography, which is pre coated silica gel 20 cm × 20 cm (0.25 mm thick) TLC plates from Whatman, in an oven at 60°C for 10 min [11]. Then I used three different mobile phases of toluene: Ethyle acetate: Formic acid (36:12:6), which is ideal, toluene: Acetone: Formic acid,

38:10:5, petroleum ether: Ethyl acetate: Formic acid, 30:15:5 and leave ajar for 20 minutes to saturated [12]. This time, I applied my sample (chloroform extract of *Cicer aretienum*) and p-coumaric standard as spot by capillary on a TLC plate, then put it in a jar and waited for it to develop. After I took it from the jar and waited for it to dry, after drying, visualization was performed in short UV light (254 nm)[12]. We can notice that the spot of extract and spot of p-coumaric standard are matched (Figure 4).
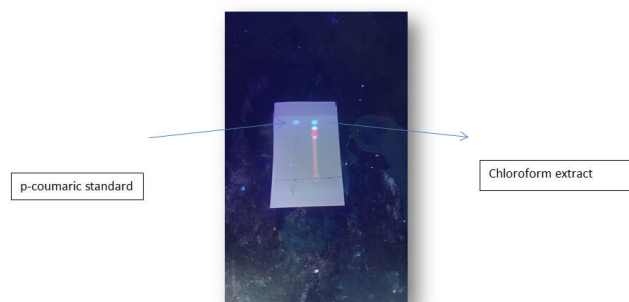

Figure 4: Thin layer chromatography of chloroform extract and p-coumaric acid standard.

RESULTS AND DISCUSSION

Detection by High Performance Thin Layer Chromato graphy (HPTLC)

HPTLC was used to determine the phenolic profile for the specified fractions utilizing Eike Reich/CAMAG–laborator (Figure 5).

Using a Hamilton syringe and camag linomat 5 instruments, 10 cm x 20 cm silica gel TLC plates were loaded with standards and samples (2 μl of each) [13]. The TLC plate was developed in an automatic developing chamber (ADC, CAMAG), then pre conditioned with 10 ml of a mobile phase containing toluene, ethyl acetate and formic acid (36:12:6) [14]. The plate was developed in the mobile phase up to 75 mm and the solvent was evaporated off the formed plate using hot air [15]. The plate was stored in a photo documentation chamber and photos were taken under white light and UV light (254 nm and 366 nm) (Tables 1 and 2, Figures 6-8) [16].
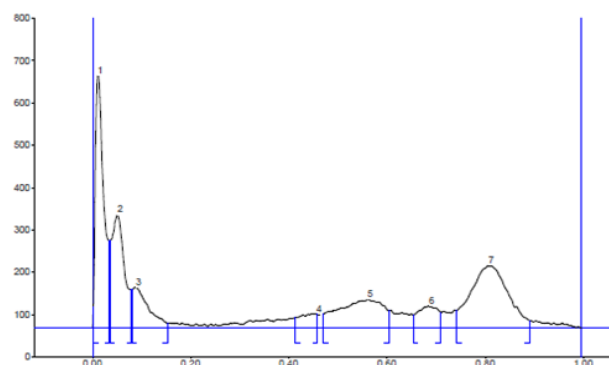

Figure 5: Average Rf values of chloroform fraction.

Table 1: Average Rf values of chloroform fraction.

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%
1	0.00	29.9	0.01	535.9	30.29	0.04	183.9	8157.1	15.64
2	0.04	184.8	0.05	263.3	14.88	0.07	158.7	4507.6	8.64
3	0.07	161.6	0.09	259.1	14.64	0.13	40.0	6071.4	11.64
4	0.13	40.7	0.14	52.0	2.94	0.19	22.5	1745.3	3.35
5	0.23	11.0	0.27	34.8	1.97	0.29	24.0	1080.0	2.07
6	0.39	27.1	0.42	51.0	2.88	0.43	47.3	1455.0	2.79
7	0.49	41.5	0.56	181.8	10.28	0.61	60.3	8769.2	16.81
8	0.64	71.4	0.68	281.8	15.92	0.74	64.8	11569.4	22.18
9	0.76	63.9	0.82	109.9	6.21	0.94	20.7	8808.8	16.89
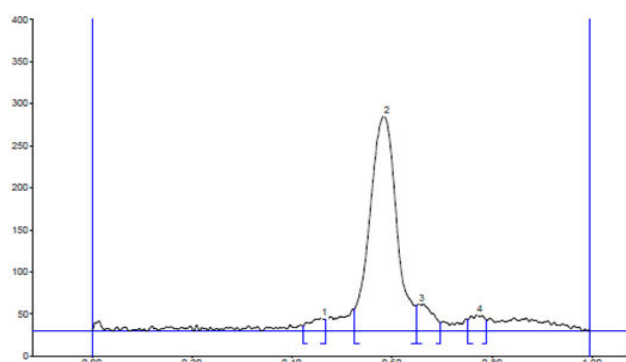
Figure 6: Average Rf value of p-coumaric standard.

Table 2: Average Rf value of p-coumaric standard.

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%
1	0.41	15.0	0.43	27.6	3.60	0.44	23.4	396.9	1.22
2	0.44	23.4	0.47	46.4	6.05	0.48	42.6	1148.3	3.53
3	0.52	50.8	0.58	590.7	77.02	0,64	52.7	27805.8	85.41
4	0.65	53.3	0.67	69.7	9.09	0.72	25.4	2640.5	8.11
5	0.72	26.4	0.72	32.5	4.24	0.75	23.4	563.4	1.73
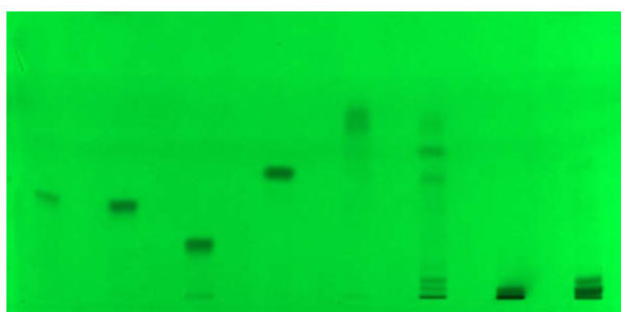
Figure 7: HPTLC chromatogram at 254 nm.
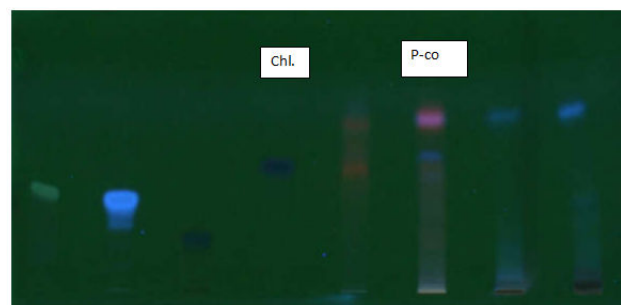
Figure 8: HPTLC chromatogram at 366 nm.

Based on the similarity of the max Rf and the hue of the bands, the following phenolic compounds were identified: We determined that *Cicer aritenum* in chloroform fraction has p-coumaric acid by HPTLC because the maximum Rf value of the chloroform fraction is 0.56 and the maximum Rf value of the p-coumaric acid standard is 0.58 and the band color is navy blue.

Isolation by preparative thin layer chromatography

I prepared the mobile phase with toluene, ethyle acetate and formic acid (36:12:6) after activating the preparative TLC in the oven for 30 minutes [17]. I put the p-coumaric standard in the first quarter of preparative TLC, then put it in the gar and waited until it reached the front line [18]. I removed it from the gar and allowed it to dry before identifying the p-coumaric band with UV light, scratching the silca on the p-coumaric band and washing it all in methanol for one night before filtering [19]. This filter liquid is p-coumaric, which I confirmed using Thin Layer Chromatography (TLC) in the same mobile phases, the outcome is identical to that of a p-coumaric stander with the same RF value (Figures 9 and 10) [20].
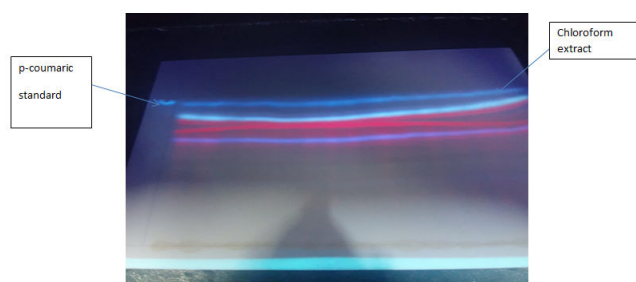

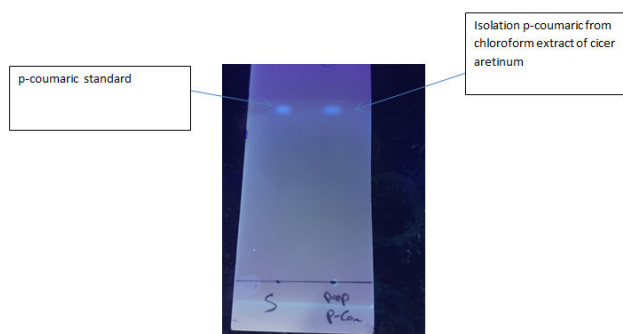**Figure 9: Preparative thin layer chromatography of chloroform extract with p-coumaric acid standard.**

Figure 10: Thin layer chromatography for isolated p-coumaric acid by preparative TLC.

Detection by Ultraviolet spectroscopy (UV)

The UV spectra were taken between 200 and 800 nm and the findings demonstrated that the maximum absorbance of the isolated chemical was the same as p-coumaric measured at the same wavelength (Figure 11) [21,22].
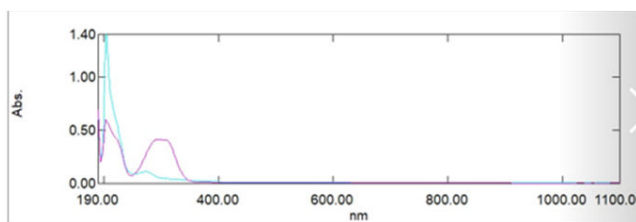

Figure 11: UV spectrum of the p-coumaric acid std. and the isolated compound from the chloroform fraction.

Detection by Fourier Transforms Infrared (FT-IR) spectroscopy

FT-IR spectroscopy is commonly used as a fingerprinting device in phytochemical research to compare a natural with a synthetic reference standard [23]. Chemical IR spectra and different IR absorption bands were distinguished as a result (Figures 12 and 13) [24,25].
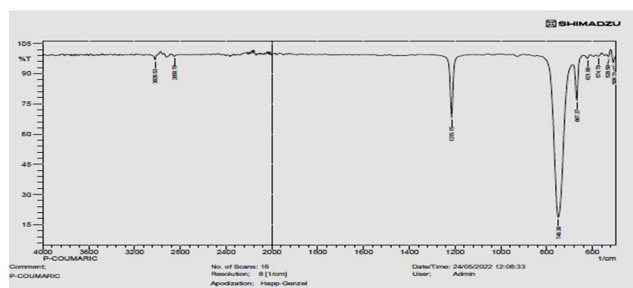

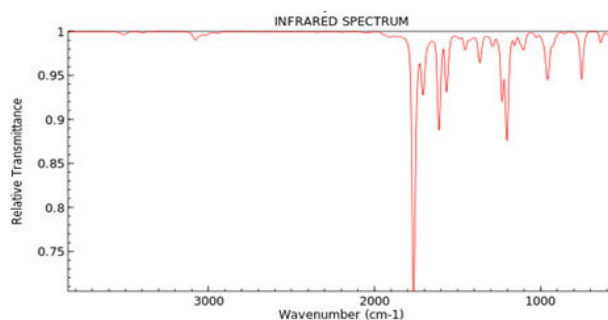**Figure 12: The UV spectra recorded for isolated p-coumaric acid.**

Figure 13: The UV spectra recorded for p-coumaric acid standard.

CONCLUSION

Due to its applications in phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis and HPTLC future to combinatorial approach, HPTLC-FTIR, HPTLC has grown to be a potent analytical tool in the field of analysis. In the future, more samples important to the examination of drug formulations, bulk medications, natural goods, clinical samples, food and the environment are likely to be investigated using instrumental HPTLC.

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