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Phytochemical Investigation Of Some Phenolic Compounds In Carthamus Tinctorius That Grown Naturally In Iraq

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ABSTRACT

The study provides the first comprehensive research done in Iraq to study the phytochemicals and the methods of extraction and separation of some phenolic compounds from carthamus tinctorius grown naturally in Iraq. Preliminary phytochemical screening of Carthamus tinctorious crude extract was revealed the presence of flavonoids, phenols, terpenoids, cardiac glycoside and tannin. Leaves parts of plant was extracted by hot extraction methods using 90% ethanol and fractionation was done to separate the active constituents according to difference in polarities using pet. Ether, chloroform, ethyl acetate and n-butanol. The n-butanol, fraction was used for detection and separation of flavonoids and phenolic acids by TLC, and HPLC. Flavonoid (apigenin), and the Phenolic acid (gallic acid) were isolated and purified by preparative thin layer chromatography. The isolated compounds were subjected to various chemical, chromatographic and spectral analytical techniques for their identification such as TLC, HPLC, and FTIR.

Keywords: Carthamus plant, Phenolic acid, Flavonoids

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INTRODUCTION

Professor H. L. Chakravarty mention in his book PLANT WEALTH OF IRAQ that there are more than three thousands species of plant in Iraq among them the plant *Carthamus tinctorius.* This plant related to ***Asteraceae*** or ***Compositae*** **family**) which is a very large and widespread family of flowering plants (Angiospermae)[(1)(2)]. The family includes over 32,000 currently accepted species, in over 1,900 genera in 13 subfamilies[(3)]. In Iraq carthamus tinctorius found in Baghdad, Zafraniya Expt. Station; Ramadi, cult. Haji Omran, in cult. Land and near streams; North –east of Rania, mountain-slope[(4)]**.** Historically, the plant has been restricted to the Middle East, part of Asia and Africa, but over time it has also been adapted to the semi-arid climatic areas [(5)(6)]. The florets of this plant have been used as a remedy for stroke, gynecological disease, coronary heart disease, angina pectoris, and hypertension in Chinese folk medicine [(7)]. In Korea, the safflower seed extracts have traditionally been used for the treatment of blood stasis, the promotion of bone formation and the prevention of osteoporosis[(8)(9)].

Plant material

Carthamus tinctorius the plant parts were obtained from garden of college of pharmacy Baghdad University where it was cultivated in in garden for scientific research and studies. *Carthamus tinctorius* was identified and authenticated by Prof. Dr. Khansaa Mohammed Rasheed /Department of Biology /College of Sciences/ University of Baghdad. All parts that's were obtained were washed thoroughly, dried in the shade followed by grinding with an electrical grinder to a fine powder.

Experimental work

The experimental work is divided into:

- Extraction and fractionation of different active constituents.
- Preliminary phytochemical screening of various secondary Metabolites like alkaloids, flavonoids, steroids, tannins, saponins, anthraquinioin, terpenoids and cardiac glycosides) in the parts of *Carthamus tinctorius* plant.
- Isolation and purification of different active constituents.
- Identification and characterization of the isolated compounds.

EXTRACTION METHOD

(Hot method)

250 grams of shade-dried pulverized plant materials were packed in the thimble of soxhlet apparatus and extracted with 250 mL of 90% Ethanol for 24 hours. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract. The residue was suspended in 60 ml water and partitioned successively with petroleum ether (B.p. 30-60), chloroform, ethyl acetate, and n-butanol (3x30ml) for each fraction. The first three fractions were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. Each fraction was weighted and assigned for further analysis[(10)(11)].

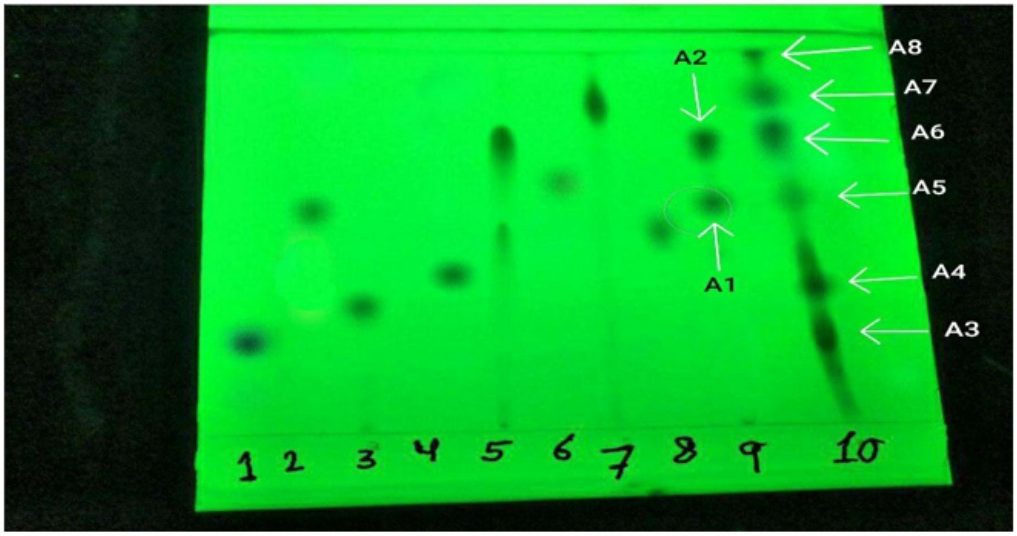
RESULTS AND DISCUSSION

Phytochemical Screening of *Carthamus tinctorious* crude extract: show the presence of Flavonoids, Steroids, Tannins, Terpenoids also Cardiac glycoside and absence Saponins and Alkaloids.

Thin layer chromatography (TLC) examination of ethyl acetate fraction and n-butanol fraction:

Preliminary TLC analysis using Ethyl acetate: Formic acid: Acetic acid: Water (84: 4: 4: 10) [(12)]

Solvent system demonstrate the presence of flavonoids and phenolic compounds in ethyl acetate and n-butanol fraction.



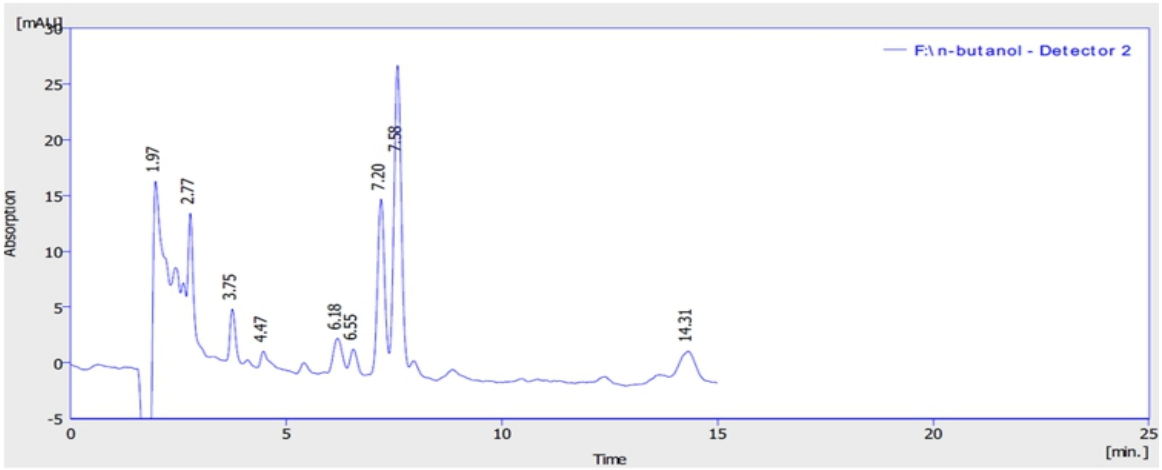
Thin layer chromatography for standards and analyzed fractions

(1: Rutin, 2: apigenin, 3: quercetin, 4: kaempferol, 5: gallic acid, 6: myricetin, 7: ellagic acid, 8: catechin, 9: ethyl acetate fraction, 10: n-butanol fraction. Developed in the S8 solvent system, at 254 nm).

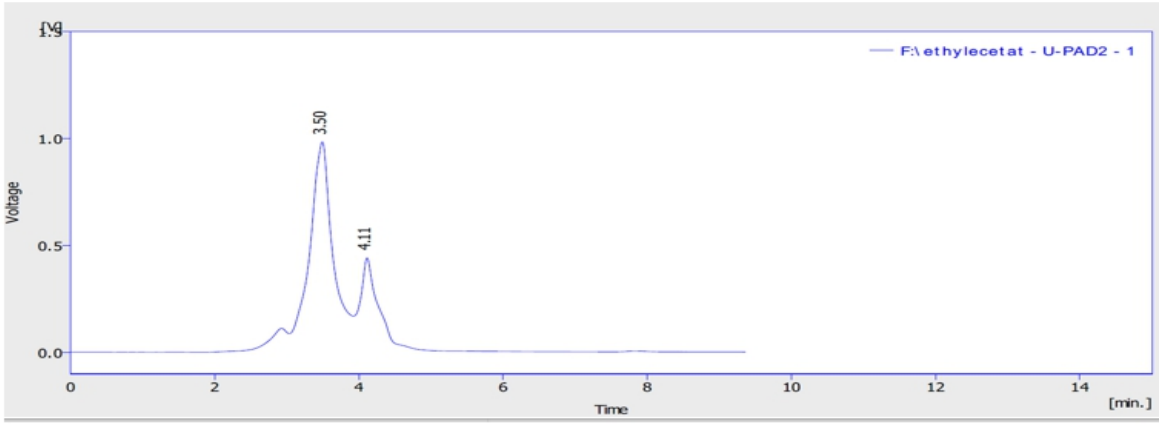
Figure 1: Thin layer chromatography for standard and analyzed fractions

Identification by HPLC

The HPLC results of the ethyl acetate and n-butanol fractions given in the figure 2.



HPLC chromatogram of n-butanol Fraction.



HPLC chromatogram of ethyl acetate Fraction.

HPLC chromatogram of n-butanol Fraction and ethyl acetate Fraction.

Figure 2: HPLC chromatogram of n-butanol Fraction and ethyl acetate fraction

Isolation and purification of flavonoids by preparative TLC

For isolation and purification of compounds present in n-butanol fraction, preparative TLC was done utilize n-butanol fraction, one gram of n-butanol developed in Ethyl acetate: Formic acid: Acetic acid: Water (84: 4: 4: 10) solvent system for isolation of rutin, quercetin, apigenin and Gallic acid.

Identification of the isolated compounds

a. **TLC**: Precise measurement of R_f values in thin layer chromatography for the isolated compound is essential for identification, it must give a single spot similar to standard material, having the same quenching of fluorescence at 254 and 366 nm, and give the same color after reacting with various spraying reagents. The measured R_f values for standard **Apigenin** and the isolated flavonoid **A1** and standard **Gallic acid** and the isolated phenolic acid **A2** should be equal.

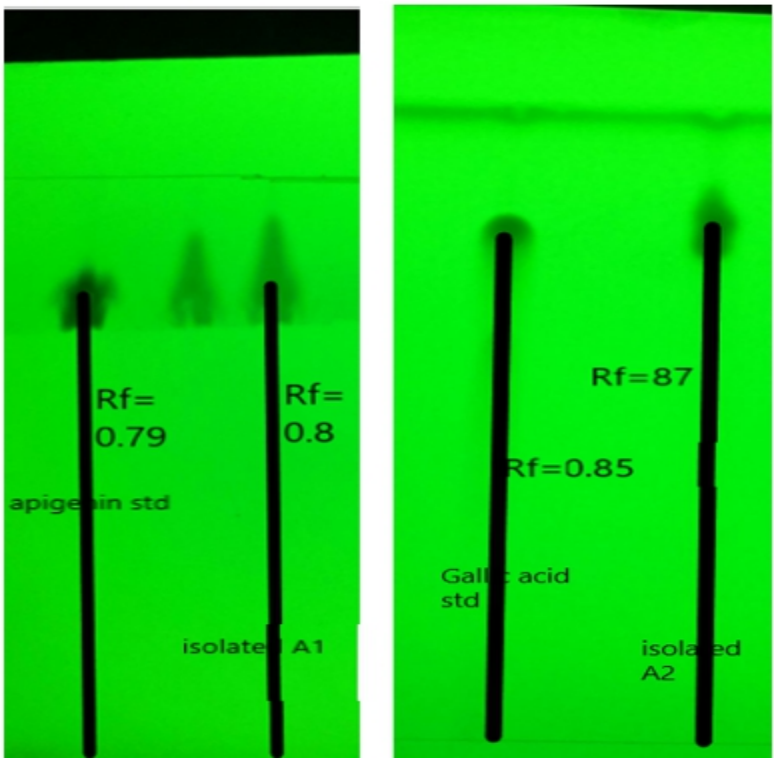
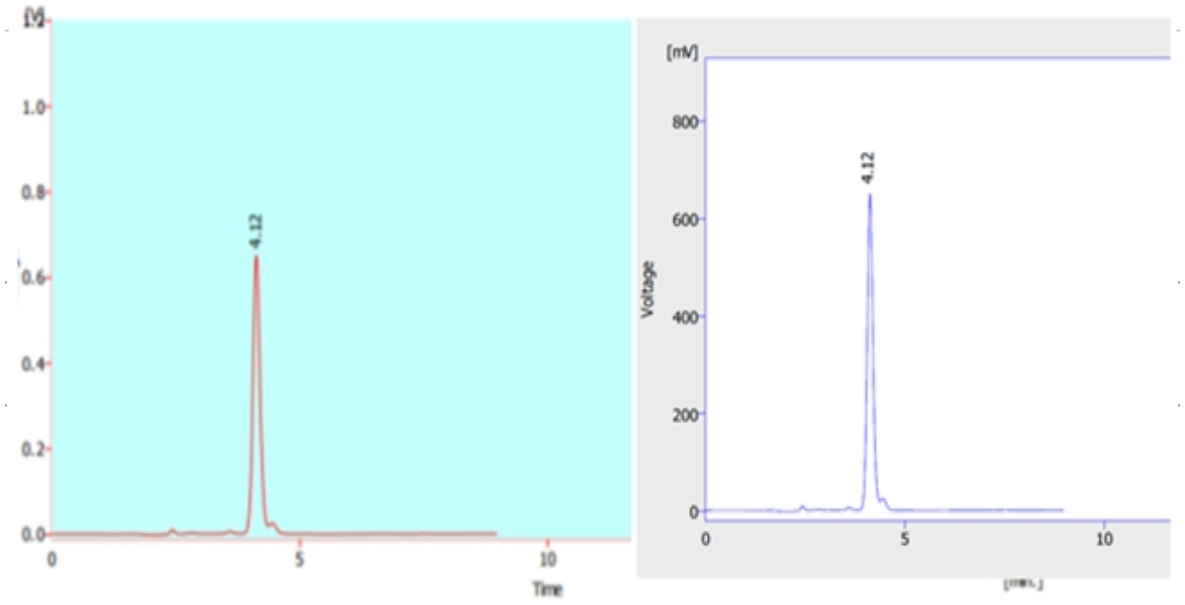


Figure 3: Thin layer chromatogram of Apigenin standard **and isolated A1 and Gallic acid** standard **isolated A2**

b. **HPLC**

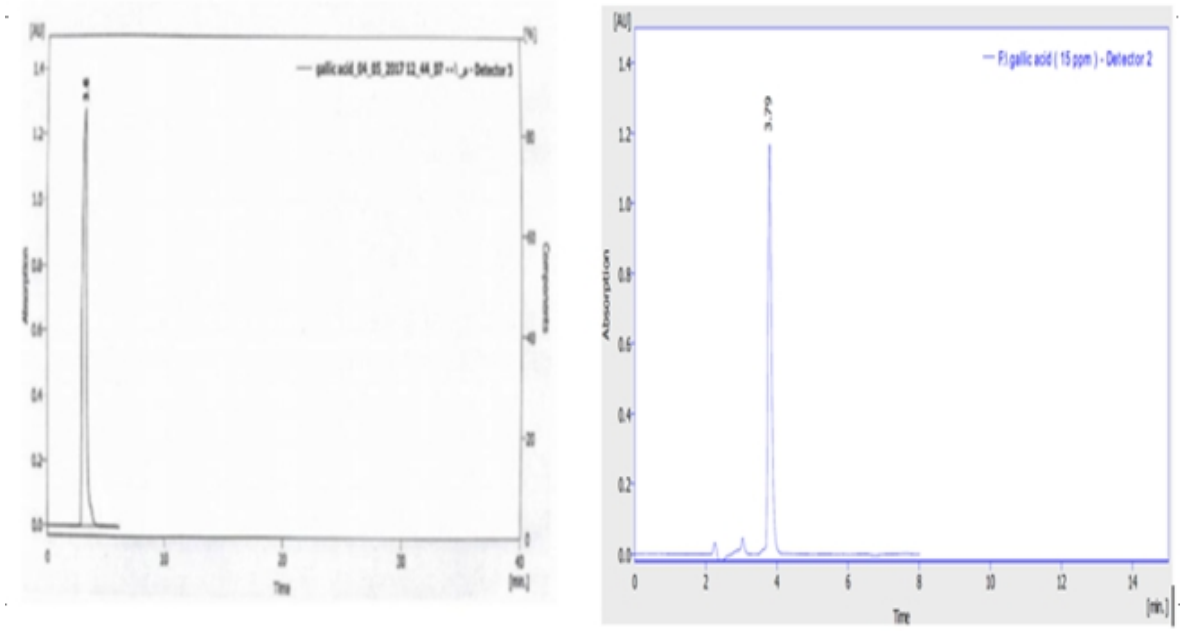
Retention times in minutes for standard **Apigenin** and **isolated A1** is 4.124 and 4.124 respectively.

Retention times in minutes for **gallic acid** standard and **isolated A2** is 3.8 and 3.792 respectively.



HPL C chromatogram of Apigenin standard Retention Time (RT =4.12).

HPLC chromatogram of isolated Apigenin Retention Time (RT=4.12)



HPLC chromatogram of standard Gallic acid Retention Time (RT=3.8).

HPLC chromatogram of isolated compoundA2 Retention Time (RT=3.8).

Figure 4: HPLC chromatogram of Apigenin standard **and isolated A1 and Gallic acid** standard **isolated A2**

c. Fourier transform infrared spectroscopy (**FTIR):** is a technique for material analysis it offers quantitative and qualitative analysis of the sample. FTIR identified chemical bands in molecules. IR spectra for a compound was recorded in KBr disk, the range of scanning 4000-400 cm-1.

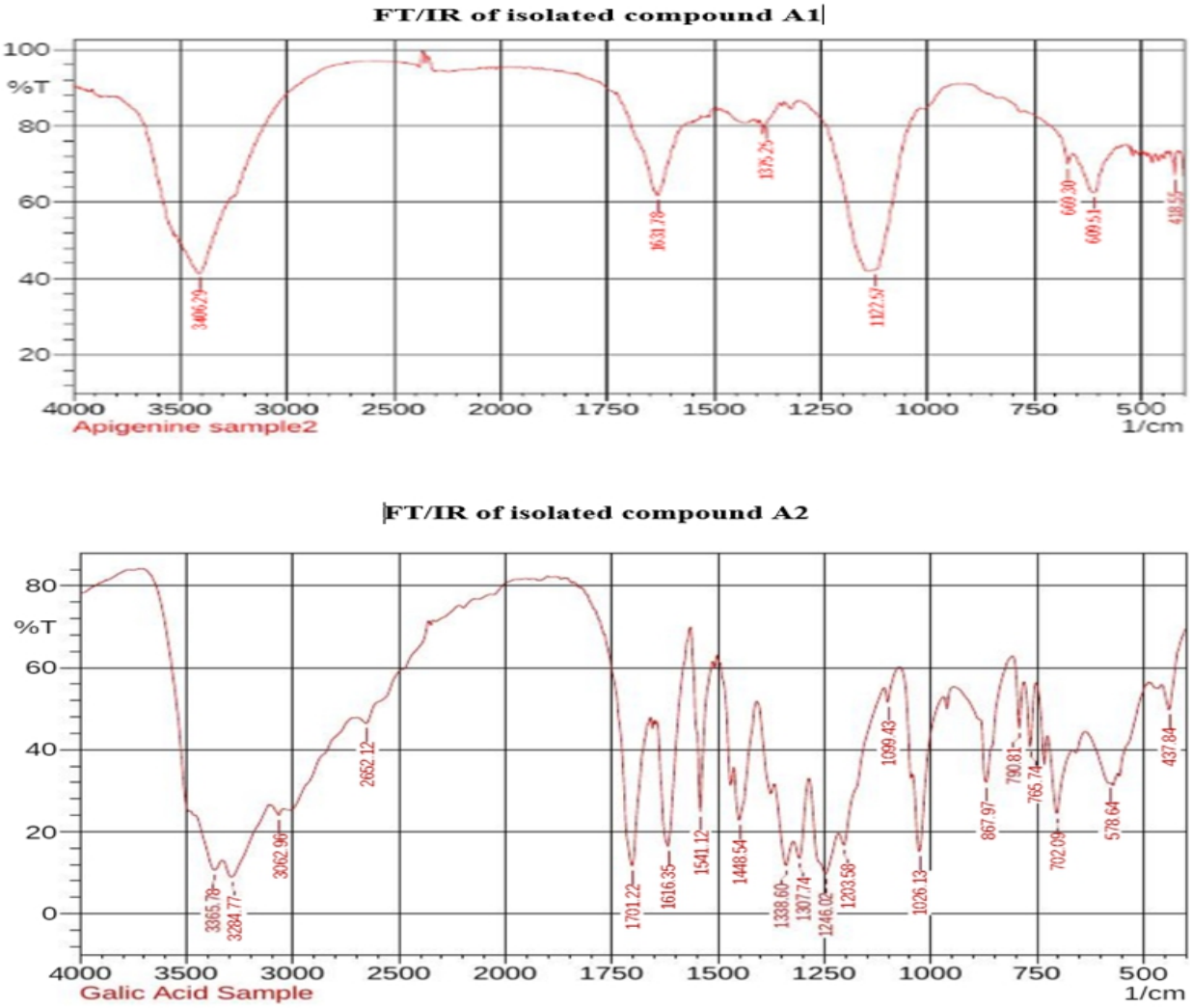


Figure 5: FTIR of Isolated **A1 and** isolated **A2 compounds**

FT/IR of isolated compound A1

IR band v cm^{-1}	*Interpretation*
3406	**Phenolic OH stretching**
1632	**Cyclic ketone carbonyl group stretching**
1122	**(C-O-C) asymmetrical stretching vibration of cyclic aliphatic ether overlapped with C-O stretching vibration of C-O-H**

FT/IR of isolated compound A2

IR band(v cm^{-1})	Interpretation
3500-2652	Broad stretching of carboxylic acid OH
3366	Phenolic OH stretching
1701	Carboxylic acid carbonyl group stretching
1616 and 1541	Ar. (C=C) stretching
1448	(C-O-H) bending vibration of phenyl-O-H
1026	C-O stretching vibration of C-O-H

Figure 6: FTIR interpretations of Isolated **A1 and** isolated **A2 compounds**

CONCLUSIONS

1- Phytochemical investigation of *Carthamus tinctorius* revealed the presence of flavonoid (apigenin) and phenolic acid (Gallic acid) that considered as important natural products with valuable medicinal uses.
2- Chromatographic analyses were carried out for isolation and purification of the isolated compounds: preparative TLC, and analytical HPLC for flavonoids (Apigenin) and phenolic acid (Gallic acid).

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