RESEARCH ARTICLE

Extraction, Isolation and Identification of Caffeic Acid and p-Coumaric acid from N-butanol Fraction of Iraqi *Osteospermum ecklonis* (F. Asteraceae)

Hind M. Jewely*, Thukaa Z. Abdul-Jalil

Department of Pharmacognosy and Medicinal Plants. College of Pharmacy, University of Baghdad, Baghdad, Iraq

Received: 14th February, 2022; Revised: 10th April, 2022; Accepted: 04th May, 2022; Available Online: 25th June, 2022

ABSTRACT

Osteospermum ecklonis, known as the African daisy, is a member of the Asteraceae family that includes more than 600 genera of various daisies. Traditionally *O. ecklonis* was used for its whitening effect to fight skin hyperpigmentation, implemented recently in several anti-scar topical formulations and as a promising remedy with anti-parasitic activity in India to treat malaria. The available literature survey revealed no studies about Iraqi *O. ecklonis* phenolic acids, especially caffeic acid, and p-coumaric acid. Therefore, the objective of this study was to extract and isolate these powerful phenolic acids for their promising pharmacological activities. This goal was achieved using 85% methanol followed by fractionation with different solvents (petroleum ether, chloroform, ethyl acetate, and n-butanol) that differ in their polarities. Two phenolic acids were isolated from aerial parts n- butanol fraction by preparative thin-layer chromatography (PLC), then identified by measuring melting point (m.p.), thin-layer chromatography (TLC), Fourier transforms infrared (FTIR) spectra, and by analytical high-performance liquid chromatography (HPLC). This study implements a consensus about the Iraqi *O. ecklonis* plant as a promising plant for its different secondary metabolites, especially the phenolic acids, which detected novelty in this study.

Keywords: Caffeic acid, Fourier transforms infrared (FT-IR) spectra, High-performance liquid chromatography (HPLC), p-Coumaric acid, *Osteospermum ecklonis.*

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.2.31

How to cite this article: Jewely HM, Abdul-Jalil TZ. Extraction, Isolation and Identification of Caffeic Acid and p-Coumaric acid from N-butanol Fraction of Iraqi *Osteospermum ecklonis* (F. Asteraceae). International Journal of Drug Delivery Technology. 2022;12(2):648-653.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Osteospermum ecklonis F. Asteraceae is also known as African Daisy, cape daisy, cape marigold or Suncape daisy. Its native origin is south Africa, Arabian Peninsula and lately Southern Europe. *O. ecklonis* F. is a subshrub usually characterized by annual growth with green leaves that are partly or totally toothed and florets that are symmetrical with different colors like white, yellow or purple.[1,2]

It has been used over decades as a remedy for certain health issues, as cardiovascular,[3] antimicrobial,[4] anti-parasitic,[5] whitening, antitumor,[4] and the flowers used as a relaxing aid not surprisingly as it was reported to have several phytochemicals of pharmacological importance such as flavonoids and phenols, terpenoids, essential oils, saponins, polysaccharides, coumarins[6] carotenoids[7] as well as phytosterols. Triterpenes and sesquiterpenes isolated using ultrasonication technique,[8] are proved to have anti-parasitic effect with a suggestive promising future alternative for quinolines antimalarial.[9,10] The main target of the present study is to investigate the two phenolic acids in Iraqi origin *O. ecklonis* aerial parts by preparative layer chromatography using silica gel plates. Qualitative identification was conducted by determination of melting point, TLC, IR and analytical HPLC.

MATERIALS AND METHODS

Apparatus and Instruments

Rotatory evaporator (Buchi rotatory evaporator attached to vacuum pump), oven (Memmert 854), and Electrical sensitive balance (Sartorius, Germany), melting point was determined by electro-thermal melting point Stuart, UK, Fourier transforms infrared spectra (FT-IR) were scanned on-FTIR Schimadzu-IR Affinity-1 spectrometer (Schimadzu, Japan) and specac quest ATR-diamond type (UK) at University of Baghdad, Collage of Pharmacy. and HPLC analysis was carried out using the Schimadzu Technology of Japan at Iraqi National Centre for Drug Control and Research.

*Author for Correspondence: hmchmc7jan@gmail.com

Chemicals and Reagents

p-coumaric acid standard and caffeic acid standard were purchased from Biopurfy, China, Silica gel GF 254_{nm} with thickness 0.25 mm from Silicycle (Canada) while 1mm from Sanpont (China), absolute methanol, ethanol, and HPLC grade methanol, ethyl acetate, petroleum ether, chloroform, were purchased from Tomas Backer (India), Toluene was purchased from CDH, India while formic acid and glacial acetic acid were from GCC, U.K.

Collection and Authentication of Plant Materials

Plant material was harvested from a herbarium in Baghdad, Greyaat district, during April, 2020, then the specimen was authenticated by Dr. S. A. Aleiwy, Assistant Professor at the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Preparation of Plant Extract

The collected plant aerial parts were first rinsed with water to remove any contaminants and then air dried in a shady room at room temperature for one month. Later on grounded by using mortar and pestle, then further grounded by an electrical grinder to obtain fine homogenous powdered plant material, assembled in an amber-colored jar for further investigation.

A total of 100 grams of aerial parts were assigned for extraction by maceration with 85% methanol for 15 days with frequent stirring, daily for few hours using magnetic stirrer; the macerated material was filtered every 5 days using Whitman's filter paper, the filtrate was collected and gathered, while the marc was being re-macerated with the fresh, same, solvent mixture. The collected filtrate was assembled, dried and concentrated by a rotatory evaporator, weighted and kept aside in closed containers for further processing.[11]

Fractionation of Plant Extract

The plant extract was fractionated by suspending it with distilled water then the mixture was partitioned with petroleum ether, chloroform, ethyl acetate and n-butanol, respectively, repeated three times for each solvent (100 mL*3). The collected fractions (except the n-butanol fraction) were dried over anhydrous sodium sulfate, filtered, and then all of them were brought to dryness by a rotatory evaporator under reduced pressure, weighted and assigned for further analysis.[12,13]

Preliminary Phytochemical Investigation

The preliminary phytochemical screening for various compounds in the plant extracts was done via a series of chemical tests, each of which was designed for a certain chemical class and was performed according to the Trease and Evans (1989) Harborne (1998) guidelines.[14,15]

Identification of Bioactive Constituents by Thin-layer Chromatography (TLC)

TLC aluminum plates coated with silica gel GF_{245} 0.25 mm from Silicycle (Canada) in size 20x20 were utilized to run the analytical chromatography, an aliquot of standard solutions of caffeic acid and p-coumaric acid were introduced to the baseline, that is 2 cm away from the lower edge of the plate in 1.5 cm away from each other spot.

Aliquots of sample from the n-butanol fraction was introduced to the baseline as well, and the plate is set aside to dry then was introduced to the TLC jar, that was previously saturated with vapors of the mobile phase, then, covered tightly and left to develop at room temperature. Each of the mentioned mobile phases below was examined separately, one at a time. When development was finished, the plate was removed from the jar, set aside to dry, before visualization under UV light, then, the Rf values of standards were compared to that of eluted samples, to determine the best separation.[16]

A volume of 50 mL from each of the following mobile phases with the denoted proportions was prepared and filtered with Whitman's filter paper before each TLC analysis:

S1: Chloroform: methanol (9:1)[17]
S2: Chloroform: acetone: formic acid (75:16:8) (13)
S3: Toluene: Ethyl acetate: methanol: formic acid (32:14:12:5) [13]
S4: Methanol: water: formic acid (40:57:3) [13]
S5: Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26) [17]
S6: Ethyl acetate: acetic acid: water (7:1.5:1.7) [13]
S7: Toluene: ethyl acetate: formic acid: acetic acid (20:10:10:7.5) [13]

Isolation and purification of bioactive constituents (H1, H2)

For isolation and purification of **H1,H2** compounds, readily prepared glass TLC plates covered with 1 mm of silica gel GF_{254} in size 20x20 from Sanpont (China) were utilized, a streak of the sample was introduced into the baseline, set to dry then introduced to the TLC jar that was saturated with vapors of solvent that gave the best separation, which was (S7). The jar is tightly covered and let to develop at room temperature for a while, and the plate was removed, set aside to dry and visualized under UV to locate the targeted bands using a wooden skewer. The bands then were scraped with a spatula, and the scrapped silica-containing eluted component was assembled in a closely stoppered amber color container. A sufficient quantity of absolute methanol was added, and the flasks were shaken on a warm water bath, cooled and kept for crystallization for 4–5 hours in a refrigerator. The crystalline constituent was filtered through double filter paper and dried to get pure constituents. Two bands were separated from the n-butanol fraction of *O. ecklonis* aerial parts, isolated and named as H1 and H2.[18]

Identification and Characterization of the Isolated Constituents (H1, H2)

Isolated substances H1and H2 were then identified in different identification ways, including:

- Measuring the melting point and comparing it to that of the standard.
- Development in different mobile systems to check the Rf values of the standard and the isolated substance

- Analyzing the IR spectra to reveal the active chemical groupings
- Analytical HPLC that revealed the peak area at the same retention time as the standard

RESULTS AND DISCUSSION

Extraction and Fractionation of *O. ecklonis* Aerial Parts

Extraction is the crucial first step in analyzing medicinal plants because it is necessary to extract the desired chemical components from the plant materials for further separation and identification.[19,20] Since most of the proposed active constituents found in *O. ecklonis* may be polar to non-polar and thermally labile depending on the literature survey, so the selection of the solvent system as alcohol (85% methanol) to extract the proposed constituents was made, and the suitability of the methods of extraction as a cold method has been considered. For full phytochemical profile screening for a given plant, fractionation of crude extract is advisable, so the main classes of plant constituents will be isolated from each other according to the difference in polarity and solubility before chromatographic analysis is performed since crude extract contains diverse classes of chemical constituents with various polarities. Fractionation of crude extracts of *O. ecklonis* aerial parts was done with a series of solvents towards increasing polarities (petroleum ether, chloroform, ethyl acetate and n-butanol).[21]

Preliminary Phytochemical Investigation by Chemical Tests

After obtaining the crude extract and active fraction from *O. ecklonis* aerial parts, phytochemical screening assays were performed with the appropriate tests to get an idea regarding the type of phytochemicals existing in the extract fractions as they are simple, quick, and inexpensive procedures.

The results of phytochemical screening assay for *O. ecklonis* fractions indicate the presence of terpenoids, steroids and cardiac glycosides in both petroleum ether and chloroform fractions. At the same time, phenolic compounds (phenolic acid and flavonoids) and cardiac glycosides present in both ethyl acetate and n-butanol fractions of Iraqi *O. ecklonis* also the results indicate the absence of alkaloids in all Iraqi *O. ecklonis* fractions**.**

Identification of Bioactive Constituents by Thin Layer Chromatography (TLC)

The TLC may be performed on the analytical scale to monitor the progress of a reaction, giving the researcher a quick answer as to how many components are in a mixture.[22] The goal of TLC is to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound, to obtain well-defined and well-separated spots.[23] Thin layer chromatography of n-butanol fraction obtained from Iraqi *O. ecklonis* aerial parts confirms the presence of two different phenolic acids designated as H1 and H2 by using different developing solvent systems (S1-S7) (Figure 1). H1 and H2 appeared as a single compact spot having the same color and R_f value as that of caffeic acid standard (for H1) and p-coumaric acid standard (for H2) on TLC plates in S2 and S7 (best developing solvent systems), detected by using UV-light at 254 nm and 365 nm.

Isolation and Purification of Bioactive Cconstituents (H1, H2)

The bioactive constituents H1 and H2 were isolated by PTLC technique in which these two phenolic acids were isolated from n-butanol fraction of *O. ecklonis* aerial parts. The separated bands of H1 and H2 were observed under UV light according

Figure 1: S7 TLC chromatogram of n-butanol fraction of *Osteospermum ecklonis* aerial parts, A= n-butanol fraction, B: = caffeic acid standard, C =p-coumaric acid spot

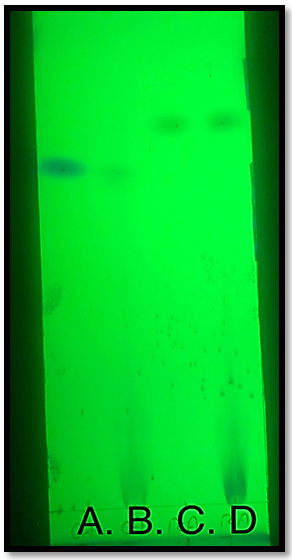

Figure 2: TLC S7 fot identification of H1 and H2, the applied spots are as follows: A= caffeic acid standard, B= H1, C=P-coumaric acid standard, D=H2

to the reference standards (caffeic acid and P-coumaric acid standards, respectively) (Figure 2).

Isolation and Purification of Bioactive Constituents (H1, H2)

The faint yellow crystals (H1) and colorless needles (H2) that yield from PTLC were subjected to different identification analysis techniques to determine the purity of the isolated constituents and several techniques were carried out to obtain their structure elucidation of them (Figure 3).

Identification of Isolated Compounds:

Melting Point

This physical character helps to estimate the isolated compound by measuring it and comparing it to that of a standard. In this study, the compound H1 melting point was 222–225°C which is comparable to that of caffeic acid while compound H2 melting point was 211–213°C which was comparable to that of p-coumaric acid.

TLC

From the results of the TLC test in different variations of mobile phase polarity, H1 and H2 still provide a single spot that had the same R_f value and color as that of a caffeic acid standard (Figure 4 spots A and B) and p-coumaric acid standard (Figure4 spots C and D), respectively.

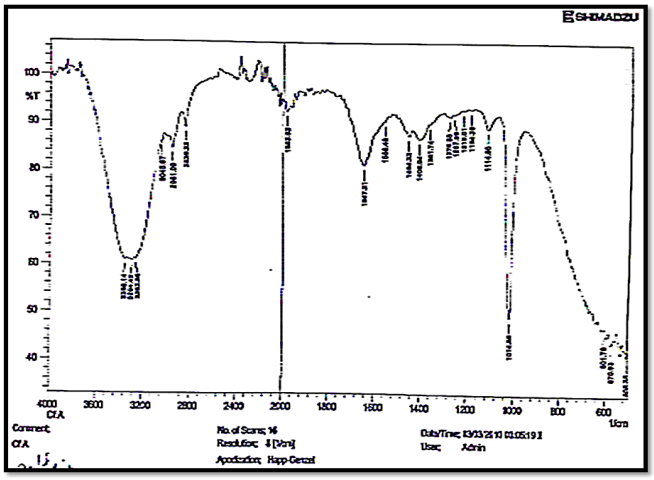

Figure 3: FTIR IR spectra for compound H1

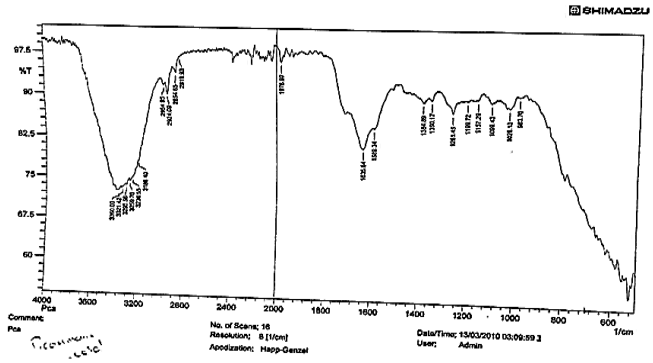

Figure 4: FTIR Spectra of compound H2

The R_f values of H1 and H2 with their corresponding standards (caffeic acid and p-coumaric acid, respectively) in the best-developing solvent systems were observed and revealed that spots of isolates (B and D) are very close to the Rf values of the standards spots (A and C) using the developing systemS7 (toluene: ethyl acetate: formic acid: acetic acid) (20:10:10:5).

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR has proven to be a valuable tool for the characterization and structural elucidation of constituents or functional groups present in unknown compounds of plant extracts. The FTIR spectra of pure constituents are usually unique, resembling molecular fingerprints.[20] Thus, the identification of H1 and H2 were further confirmed by using FTIR spectroscopy. The FTIR spectra of H1 and H2 agree with those of caffeic acid and p-coumaric acid standards, respectively, as shown in Figures 5 and 6.

The characteristic FTIR absorption bands of the isolated compounds H1 and H2 been compared to that of the corresponding standards (caffeic acid and p-coumaric acid). indicated that H1 is suspected to be caffeic acid while H2 is suspected to be p-Coumaric acid.[24,25]

High-Performance Liquid Chromatography (HPLC)

The HPLC is a simple, sensitive, reliable technique for qualitative and It utilizes an integrated system of essential components that are operated via a software application that controls the analysis procedure, which aims to get the time of spiking of a sample compared to that of a standard.[26-28]

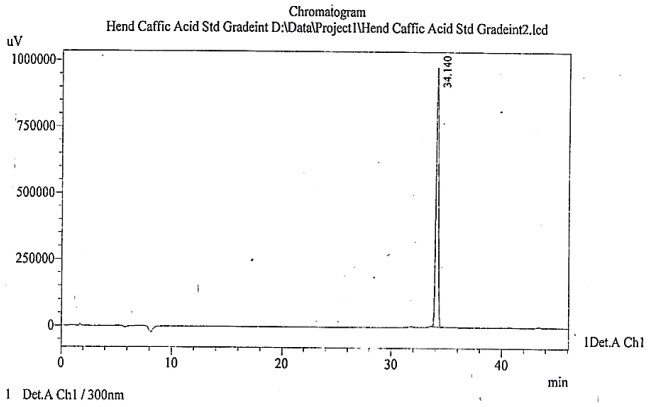

Figure 5: HPLC diagram for standard Caffeic acid

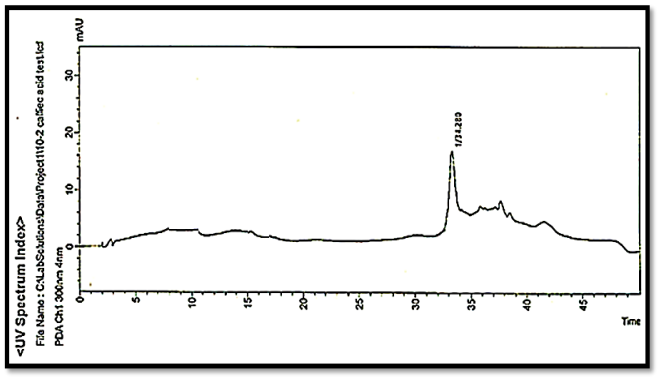

Figure 6: HPLC diagram for isolated H1

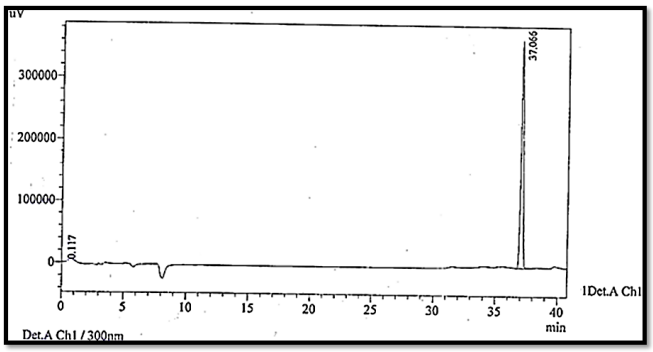

Figure 7: HPLC diagram for standard p-coumaric acid

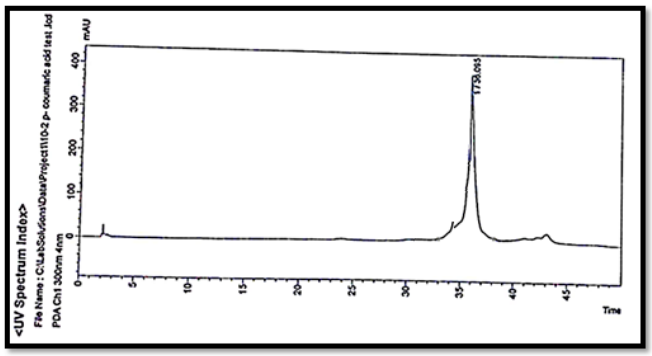

Figure 8: HPLC diagram for isolated H2

In this study, the qualitative analysis was performed using a mobile phase consisting of 1% aqueous acetic acid solution (A) and methanol (B). The following gradient elution used to elute the samples, 90% A from 0 to 27 min, from 90 to 60% A in 28 min, for a 60% A for 5 minutes was introduced, while from 60 to 57% A in 2 minutes, 56% A in 8 minutes, 56 to 90% A in 1 min and 90% A in 4 minutes used to re-establish the initial conditions. A flow rate of 1 mL/min was used in an injection volume of 5 µL when the column temperature was at 20°C. three different wave lengths was utilized to determine the reading (254,278,300).[29] The retention time for each sample was determined and compared to that of a standard at the same conditions, in which both of isolated H1 and H2 with caffeic and p-coumaric acid standards gave identical retention time.

The spiking for Caffeic acid standard is 34.140, while the isolated H1 was 34.280.

The Figures 7 and 8 shows the spiking time for p-Coumaric acid standard is 37.066 and 37.005.

From the above finding, all results favor that H1 is caffeic acid and H2 is p-Coumaric acid.

CONCLUSION

Traditionally, *O. ecklonis* was used as a remedy for many health issues and now its increasing use in many modern pharmaceutical preparations is due to the presence of valuable phytochemicals in it. The presence of phenolics in the n-butanol fraction was detected first by chemical test, followed by chromatographic techniques, isolation, and further identification by measuring the melting point, IR spectra, and the Rf value in three different solvents systems, and the most powerful and sensitive technique of HPLC. All the above methods gave data coincided with the suggested standards (caffeic acid and p-coumaric acid. This study implements a consensus about the Iraqi *O. ecklonis* plant as a promising plant for its different secondary metabolites, especially the phenolic acids, having many pharmacological actions that were detected as a novelty in it.

ACKNOWLEDGMENTS

The researcher is very grateful to all the helpful staff of the college of Pharmacy \Baghdad University that assisted in the conduction of this research

REFERENCES

1. Bussmann, R. W. et al. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. J Ethnobiol Ethnomed 2 22. 2006;2(22).
2. Suzuki A, Metzger JD. Vernalization in a greenhouse promotes and synchronizes flowering of Osteospermum ecklonis Norl. HortScience. 2001;36(4):658–60.
3. Oyedemi SO, Bradley G, Afolayan a J. Ethnobotanical survey of medicinal plants used for the management of cardiovascular diseases in the Nkonkobe municipality of South Africa. J Med Plants Res. 2011;5(17):4256–60.
4. Gouda YG, Abdallah QMA, Elbadawy MF, Basha AA, Alorabi AK, Altowerqe AS, et al. Cytotoxic and Antimicrobial Activities of Some Compositae Plants Growing in Taif Area, Saudi Arabia. 2014;3(5):43–8.
5. Panda SK, Luyten W. Antiparasitic activity in Asteraceae with special attention to ethnobotanical use by the tribes of Odisha, India. Parasite. 2018;25.
6. Sülsen VP, Lizarraga E, Mamadalieva NZ, Lago JHG. Potential of Terpenoids and Flavonoids from Asteraceae as Anti-Inflammatory, Antitumor, and Anti-parasitic Agents. Evidence-based Complement Altern Med. 2017;2017:6–8.
7. Kishimoto S, Ohmiya A. Review studies on carotenoids in the petals of compositae plants. J Japanese Soc Hortic Sci. 2009;78(3):263–72.
8. Bohlmann F, Wallmeyer M, Jakupovic J, Ziesche J. Diterpenes and sesquiterpenes from Osteospermum species. Phytochemistry. 1983;22(7):1645–51.
9. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, et al. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J Ethnopharmacol. 2004;92(2–3):177–91.
10. Pillay P, Maharaj VJ, Smith PJ. Investigating South African plants as a source of new antimalarial drugs. J Ethnopharmacol. 2008;119(3):438–54.
11. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Med (United Kingdom) [Internet]. 2018;13(1):1–26. Available from: https://doi.org/10.1186/s13020-018-0177-x
12. Abdul-Jalil Th. Z.: Screening of rutin seedsand leaves extractof dill,coriander and fennel cultivatedin Iraq. PharmacyGlobalIJCP. 2013;3(2):1–6.
13. Hamad MN. Detection and isolation of flavonoids from Calendula officinalis (F. Asteraceae) cultivated in Iraq. 2016;25(2):1–6.
14. Bart HJ. Extraction of Natural Products from Plants - An Introduction. Ind Scale Nat Prod Extr. 2011;1–25.
15. Sticher O. Natural product isolation. Nat Prod Rep. 2008;25(3):517–54.

16. Stahl E. Thin-layer chromatography. A laboratory handbook. Vol. 21, Journal of Colloid and Interface Science. 1966. 124–125 p.
17. Wagner, H.Baldt S. PLANT DRUG ANALYSIS. The Cambridge Companion to Wittgenstein, Second Edition. 2017. xiii–xiv.
18. Harborne JB. Phytochemical Methods A Guide To Modern Tecniques Of Plant Analysis, Third Edition. Chapman Hall. 1998;58.
19. Mohammad Kamil Hussain MSMFK. Natural bio-active compounds: Chemistry, pharmacology and health care practices. Nat Bio-active Compd Chem Pharmacol Heal Care Pract. 2019;2(November):1–492.
20. S. Sasidharan1,**, Y. Chen1, D. Saravanan2, K.M. Sundram3 LYL, 1Institute. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1–10.
21. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017;6(4).
22. Braz R, Wolf LG, Lopes GC, de Mello JCP. Quality control and TLC profile data on selected plant species commonly found in the Brazilian market. Rev Bras Farmacogn. 2012;22(5):1111–8.
23. Mohammad A, Bhawani S, Sharma S. Analysis of Herbal Products by Thin-Layer Chromatography: A Review. undefined. 2010;
24. Al-shammaa DA, Saour KY, Abdul-khalik ZM. Phytochemical Investigation for the Main Active Constituents in Arctium lappa L. Cultivated in Iraq. Iraqi J Pharm Sci. 2013;22(1):18–24.
25. Xing Y, Peng HY, Zhang MX, Li X, Zeng WW, Yang XE. Caffeic acid product from the highly copper-tolerant plant Elsholtzia splendens post-phytoremediation: Its extraction, purification, and identification. J Zhejiang Univ Sci B. 2012 Jun;13(6):487–93.
26. Sirard T. Fundamentals of HPLC. Waters [Internet]. 2012;1–103. Available from: http://books.google.com/books?id=yBEeEqzGCnsC&pgis=1
27. Patel DB. Journal of Global Pharma Technology Available Online at www.jgpt.co.in. System. 2009;(June):85–90.
28. Hashim HO. chromatography and HPLC principles Chromatography and HPLC principles By : Dr Hayder Obayes Hashim Chromatography : 2018;(January).
29. Nour V, Trandafir I, Cosmulescu S. HPLC determination of phenolic acids, flavonoids and juglone in walnut leaves. J Chromatogr Sci. 2013;51(9):883–90.