Isolation of Beta-Sitosterol, Campesterol and Stigmasterol from Alcea Kurdica Roots Petroleum Extract Using Preparative HPLC[1]

***Dilbreen H. Abdulqader, *Sami R. AL-Zubaydi, **Enas Jawad Kadhim**

**College of Pharmacy, University of Duhok, Duhok, Iraq.*

***College of Pharmacy, University of Baghdad, Duhok, Iraq.*

phcdilbreen@gmail.com

Date of Receiving: 11 Feb 2023, Date of Acceptance: 18 March 2023, Date of Publication: 25 March 2023

ABSTRACT

Isolation of plants components represents as a main bottleneck step for the discovery of drugs and/or the identification of a template for the discovery of new drugs. Some of these components undergo destruction during separation and isolation techniques, therefore, the selection of appropriate techniques for the separation of these components are very important to avoid the loss of some of the herally derived components. The aim of the present study is to isolate beta-sitosterol, campesterol and stigmasterol from Alcea kurdica roots petroleum extract using Preparative HPLC. Methods: the extraction of the components of the roots of Alcea kurdica was performed using petroleum extraction technique followed by separation of beta-sitosterol, campesterol and stigmasterol using HPLC techniques were used. Results: Peaks of chromatograms of the standards of beta-sitosterol, campesterol and stigmasterol were compared the separated sample for confirmation. Conclusion: the extract contain enough and sufficient outcome of these components (beta-sitosterol, campesterol, and stigmasterol) using HPLC as a separation techniques.

Keywords: *sterol; Alcea Kurdica; beta-sitosterol; campesterol; stigmasterol*

INTRODUCTION

In plant kingdom, sterols are important plant byproduct involving in maintenance of plasma membrane fluidity and cellular permeability during cellular exposure to stress conditions or any other abnormal metabolic derangements, being a precursors for many steroidal-derived compounds, such as, testosterone, estrogen, glucocorticoids, and mineral corticoids in mammals[1]. The nutritional plants consumed by human represents the main sterol components with a majority of the form of β- sitosterol 65%, campesterol 30%, and stigmasterol3% of the overall nutritional composition[2]. Researchers has focused on Alcea species because of their antimicrobial potential besides antioxidant, anti-inflammatory and cytotoxic activities, particularly due to flavonoids and other phenolic constituents[3].

[1] *How to cite the article:* Abdulqader D.H., Al-Zubaydi S.R., Kadhim E.J.; Jan-Mar 2023, Isolation of Beta-Sitosterol, Campesterol and Stigmasterol from Alcea Kurdica Roots Petroleum Extract Using Preparative HPLC, *International Journal of Pharmacy and Pharmaceutical Studies*, Vol 7, Issue 1, 6-12

The first official record of Alcea (Althaea) Kurdica in Iraq was done in August 1841 , the specimen collected from Gara mountain, and it preserved till now in the herbarium of Royal botanic Gardens, Kew[4]. Alcea kurdica were utilized for medical reasons by the extinct Neandarthal people, who are believed to have existed in Iraq around 60000 years ago. Herbs remain in use presently[5]. Alcea kurdica leaf and root decoction traditionally used for treating kidney ache, kidney stone and asthma[6]. Although Alcea kurdica is native to Kurdistan region and has a historical importance and cultural symbol, there is no previous phytochemical screening nor isolation attempts had been done. However, other Alcea species contain a variety of active substances e.g. starch, mucilage, pectin, sucrose, phenolics etc.[7]. As a result of the low concentrations of alkaloids, secondary metabolites isolated from Alcea species were found not to exhibit such toxic effects as psychotropic and mind-fogging[8]. Few reports mentioned the presence of sterols; β-sitosterol found in fruits, leaves and seeds of Althaea rosea[9]. More sterols isolated from other Malvaceae members, β- sitosterol, campesterol and stigmasterol were determined in Malva sylvestris and Althaea officinalis[10].

MATERIALS AND METHODS

Plants source and collection: Parts of *Alcea kurdica* were collected in May 2021 from natural fields in Dohuk. Voucher specimen kept at herbarium of pharmacognosy department in pharmacy college - Duhok University. the plant roots were cleaned, washed, sliced and then shade dried at room temperature in well ventilated room for two weeks. The dried roots slices were milled by using mortar and pestle, the resultant powders placed into a well closed container to be ready for extraction process.

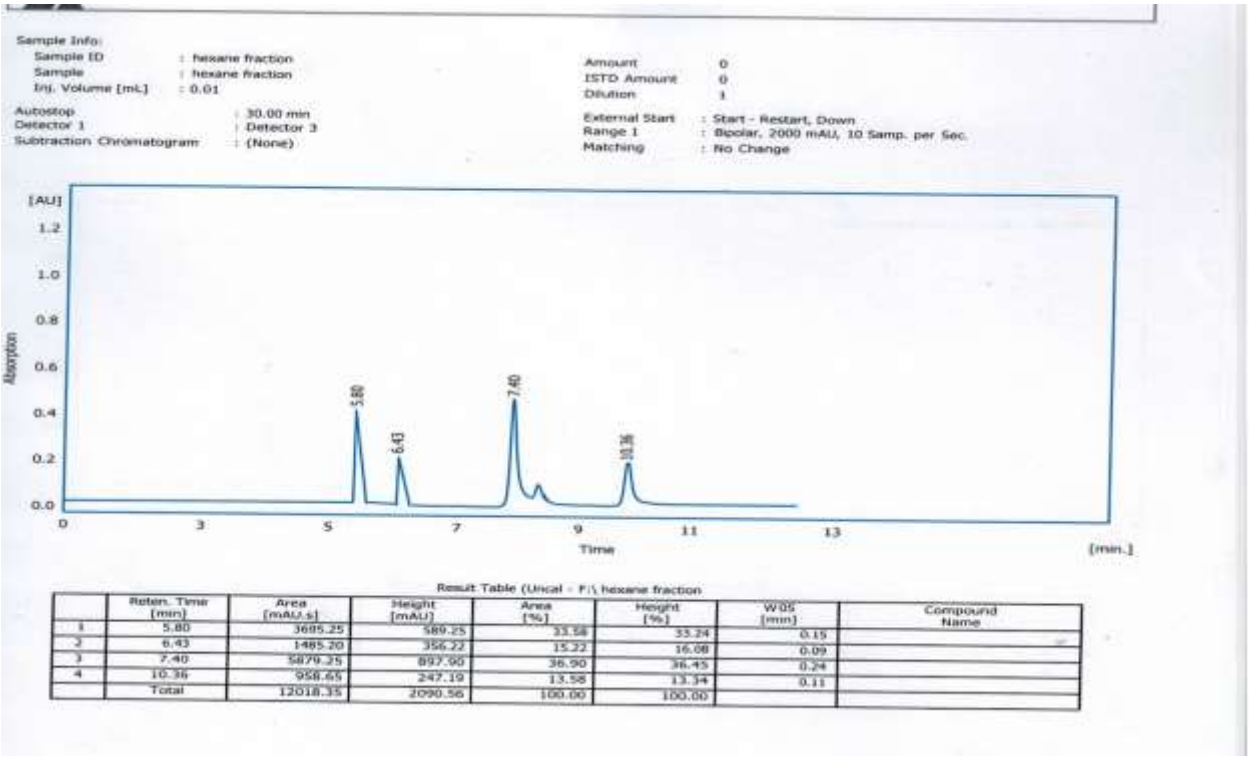
Preparation of extracts: The phytochemical extraction was performed by Maceration extraction method. This extraction was done by taking 100 gm of dried plant's roots powder and was placed into a well stoppered storage glass bottle then extracted with 500 ml of petroleum ether (40-60 ^{o}C). The extraction processes carry on under continuous electro-magnetic stirring for 7 days. the extract collected and the volume was condensed by using rotary evaporator under vacuum at 40 ^{o}C then transferred to trays to be totally dried. Obtained extracts were filtered through a cellulose filter (fine pore, 0.45 µm) and reconstituted filtrates were properly diluted with the solvent to the required concentrations.

Isolation and purification of phytostrols by preparative HPLC: A 0.5gm of sample (plant root hexane fraction obtained by maceration) was dissolved in a minimum quantity of chloroform and injected in to preparative HPLC using :-

acetonitrile: absolute methanol: water (60:30: 10) as a mobile phase (experimental work)
Column: mediterranea C18, 5 µm 15 X 2.12 cm.
Flow rate: 5 ml / min.
Injection volume: 1 ml.
Detection: UV. Detector at λ 280 nm.(experimental work)

RESULTS:

Characteristic preparative HPLC traces of phytosterols from A. kurdica roots extracts and are shown in Figure1.



	Retén. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [mm]	Compound Name
1	5.80	3695.25	589.25	33.58	33.24	0.15	
2	6.43	1485.20	356.22	15.22	16.08	0.09	
3	7.40	5879.25	897.90	36.90	36.45	0.24	
4	10.36	958.65	247.19	13.58	13.34	0.11	
	Total	12018.35	2090.56	100.00	100.00		

Figure 1. Chromatogram obtained in analysis of Alcia kurdica roots organic extract by preparative HPLC.

Three sterols were identified by comparison with the chromatograms (Figure 2A, B, C) of the standard sterol compounds obtained under similar conditions (Figures 2 D, E, F) shown the chromatograms of stigmasterol (R_t =5.6 min), campesterol (R_t= 6.43) and beta-sitosterol (R_t =7.13 min) respectively.

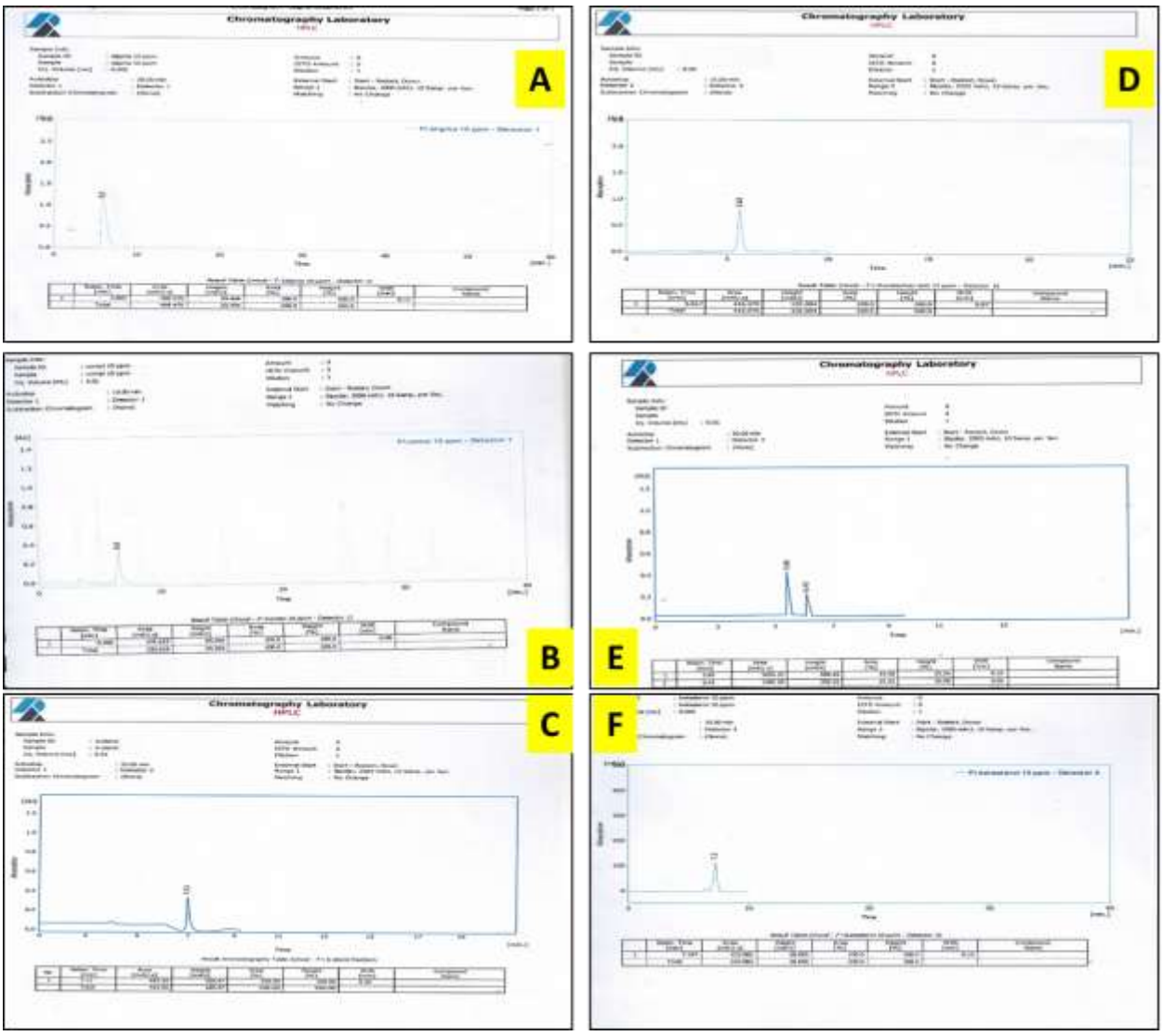


Figure 2. Chromatogram of isolated products (A) stigmasterol standard, (B) campesetrol standard, (C) beta-sitosterol standard, (D) isolated stigmasterol, (E) isolated campesterol, (F) isolated beta-sitosterol.

DISCUSSION

The advancement of isolation and separation chromatographic techniques has enabled the investigators to identify the exact components of the Alcea Kurdica extract especially HPLC and GC techniques. HPLC separates components under low temperatures permitting separation of heat-sensitive components of Alcea Kurdica without or with a minimum destruction especially the separation of sterols[11]. Preparative HPLC was used in this investigation to extract three phytosterols from plants in a very pure form because the chemical and pharmaceutical industries are seeing an increase in the need for manufacture of extremely pure value chemicals in various amounts[12].

The primary phytosterol is -sitosterol, which can oxidize just like cholesterol to produce -sitosterol oxides. Due to the presence of sitosterol oxides, this makes the isolation of pure -sitosterol difficult [13]. Its IUPAC designation is 17(5-Ethyl-6-methylheptane-2-yl)-10,13-dimethyl-2,3,4-,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]. phenanthren-3-ol.

Cinchol, cupreol, rhamnol, quebrachol, and 22:23 dihydro stigmasterol are other names for stigmasterol, along with sitosterin and 3)stigmast-5-en-3-ol[14]. With a lengthy history of use in pharmaceutical products, beta-sitosterol is widely regarded as safe and a potential nutritional complement with no harmful side effects[15]. Known herbal medicines for the treatment of benign prostatic hyperplasia and prostate cancer have -sitosterol in their formula. Moreover, the substance increased cellular levels of enzymatic and non-enzymatic antioxidants, making it a potent anti-diabetic, neuroprotective, and chemoprotective agent[16].

Campesterol is abundant in seeds, nuts, cereals, beans, legumes and vegetable oils . campesterol (24-methylsterol) is similar in structure to cholesterol and similarly metabolized by intestinal bacteria. Due to structural similarity to cholesterol, plant sterols including campesterol have cholesterol-lowering effects[17].

Campesterol can regulate carrier proteins, intestinal cells, and lipid metabolism, including the synthesis and esterification of cholesterol and assembly of lipoproteins[18]. Campesterol was extracted with hexane from Lannea humilis stem bark shows antimicrobial and antioxidant activities[19]. Its IUPAC name is (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol[20].

Although structurally identical to sitosterol, stigmasterol varies from this owing to a double bond that is created at position C-22 by the sterol C-22 desaturase[21]. Chemically, stigmasterol is (3S, 8S, 9S, 10R, 13R, 14S, 17R) -17-[(E, 2R, 5S) (E, 2R, 5S) [-5-ethyl-6-methylhept-3- en-2-yl] 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, and 17 -10, 13-dimethyl -dodecahydro-1Hcyclopenta[a] After being isolated, phenanthren-3-ol was detected using reactions such as the Salkowski and Liebermann Burchard reaction[22].

The antioxidant properties of the stigmasterol found in the bark of Butea monosperma were demonstrated by a reduction in hepatic lipid peroxidation and an increase in catalase, superoxide dismutase, and glutathione activities[23].

This method gave a quick and accurate analysis of the sterols present in the organic roots extract of A. kurdica. Different members of Malvaceae family gave positive results when tested for the presence of sterols including Abelmoschus cailei, Hibiscus asper, H. rosa-sinensis, H. sabdariffa, H. schizopetalus, Malvaviscus arboreus, Sida acuta and Sida rhombifolia[24]. However, no data found about the nature of sterols in Alcea kurdica species , we were able to detect β-sitosterol, campesterol and stigmasterol in the roots of A. kurdica. The results shared similarities with other species of Alcea genus as in Althaea rosea which contains β-sitosterol and stigmasterol in its flowers, leaves and seeds[25]. Other Alcea species containing sterols are Althaea armeniaca & Althaea nudiflora[26]**.**

CONCLUSION

HPLC techniques has revealed an important method for separation of the beta-sitosterol, campesterol and stigmasterol from petroleum extract of the roots of Alcea kurdica.

Financial Support and Sponsorship: Nil

Conflict of Interest: None

REFERENCES

1. Badhai S, Barik D, Mallick BC. (2020); Anticancer efficacy of β-Sitosterol Loaded Hydroxyapatite-Alginate on Colon Cancer Cell in Vivo. *Research Journal of Pharmacy and Technology*. 13(3):1147-51.
2. Mohammed M, Mahdi MF, Talib B, Abaas IS. (2021); Identification and isolation of lupeol and p-sitosterol from Iraqi Bauhinia variegata and determination the cytotoxic activity of the hexane extract of its leaves, stems and flowers. *Research Journal of Pharmacy and Technology*. 14(11):5703-8.
3. Hana R. Bajes, Sawsan A. Oran, Yasser K. Bustanji. (2021); Chemical Composition and Antiproliferative and Antioxidant Activities of Methanolic Extract of Alcea setosa A. Malvaceae. *Research Journal of Pharmacy and Technology*. 14(12):6447-4.
4. Mahajan NS, Manikrao AM, Shinde PN, Jawarkar RD, Khatale PN, Dhawale SC. (2012); A review: Biological importance of mercapto substituted 1, 2, 4-triazole derivatives. *Research Journal of Pharmacy and Technology*. 5(7):863-76.
5. Agrawal S, Kochar N, Chandewar A. (2018); Review on Costus speciosus a Medicinal Plant. *Research Journal of Pharmacy and Technology*. 11(4):1697-702.
6. Tariq NP, Ifham S. (2013); Ethnobotanical survey of medicinal plants in Yelagiri Hills of Tamil Nadu. *Research Journal of Pharmacy and Technology*. 6(6):652-4.
7. Mansour O, Darwish M, Ismail G. (2018); Phytochemical Study of Stryax officinalis Lin Syria. *Research Journal of Pharmacy and Technology*. 11(3):1214-6.
8. Azab A. (2016). *Alcea: Traditional Medicine, Current Research and Future Opportunities*. Eur Chem Bull, 5: 505-14.
9. Fahamiya N, Shiffa M, Aslam M. (2016); A comprehensive review on Althaea rosea Linn. *Journal of Pharmaceutical Research*. 6(11).
10. Tesevic V, Vajs V, Lekic S, Dordevic I, Novakovic M, Vujisic L, Todosijevic M. (2012); Lipid composition and antioxidant activities of the seed oil from three Mlvaceae species. *Archives of biological sciences*. 64(1):221-7.
11. Kasthuri KT, Radha R, N Jayshree, Anoop Austin, P Thirugnanasambantham. (Jul-Sep 2010); Standardization and In Vitro Anti Inflammatory Studies of A Poly Herbal Oil Formulation – Megni. *Research J. Pharm. and Tech*.3 (3): Page 792-794.
12. Nagele E, Huber U. (2002); Isolation of formononetin and other phytoestrogens from red clover with the Agilent 1100 Series purification system. *Agilent Technologies Application*.
13. McCarthy FO, Chopra J, Ford A, Hogan SA, Kerry JP, O'Brien NM, et al. (2005); Synthesis, isolation and characterisation of beta-sitosterol and beta-sitosterol oxide derivatives. *Org Biomol Chem.* 3:3059-65.
14. Bin Sayeed MS, Karim SM, Sharmin T, Morshed MM. (15 Nov 2016); Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: a plant-derived orphan phytosterol. *Medicines.* 3(4):29.
15. Paniagua-Perez R, Madrigal-Bujaidar E, Reyes-Cadena S, Molina-Jasso D, Gallaga JP, Silva-Miranda A, Velazco O, Hernandez N, Chamorro G. (25 Aug 2005); Genotoxic and cytotoxic studies of beta-sitosterol and pteropodine in mouse. *Journal of Biomedicine and Biotechnology*. 2005(3):242-7.
16. Saeidnia S, Manayi A, Gohari AR, Abdollahi M. (1 May 2014); The story of beta-sitosterol-a review. *European journal of medicinal plants*. 4(5):590.
17. Choi JM, Lee EO, Lee HJ, Kim KH, Ahn KS, Shim BS, Kim NI, Song MC, Baek NI, Kim SH. (2007); Identification of Campesterol from Chrysanthemum coronarium L. and its Antiangiogenic Activities. *Phytother. Res*;21:954-9.
18. Calpe-Berdiel L, Escolà-Gil JC, Blanco-Vaca F. (1 Mar 2009); New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. *Atherosclerosis*. 203(1):18-31.

19. Tekeshwar Kumar, Amber Vyas, Vishal Jain. (2020); Phytochemical and pharmacological screening of leaf of Lannea coromandelica Linn. *Research J. Pharm. and Tech*; 13(6): 2815-2818.
20. Abdulameer Ali Yaseen Akhlass Mery Kadim. (2018); Impact of nano Chelated Iron, GA3 and Organic Fertilizer (Acadian) in Moringa leaves Content of α-Tocopherol and Phytosterols. *Research J. Pharm. and Tech*; 11(5):1840-1846.
21. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C, Berenbaum F. (1 Jan 2010); Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthritis and cartilage*. 18(1):106-16.
22. Kaur N, Chaudhary J, Jain A, Kishore L. (1 Sep 2011); Stigmasterol: a comprehensive review. *International Journal of Pharmaceutical Sciences and Research*. 2(9):2259.
23. Panda S, Jafri M, Kar A, Meheta BK (2009); Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. *Fitoterapia*; 80 (2): 123- 126.
24. Focho DA, Nkeng EA, Lucha CF, Ndam WT, Afegenui A. (30 April 2009); Ethnobotanical survey of plants used to treat diseases of the reproductive system and preliminary phytochemical screening of some species of malvaceae in Ndop Central Sub-division, *Cameroon. Journal of Medicinal Plants Research*. 3(4):301-14.
25. Fahamiya N, Shiffa M, Aslam M. (2016); A comprehensive review on Althaea rosea Linn. *Journal of Pharmaceutical Research*. 6(11).
26. Egamberdieva D, Mamedov N, Ovidi E, Tiezzi A, Craker L. (2017); Phytochemical and pharmacological properties of medicinal plants from Uzbekistan: A review. *Journal of Medicinally Active Plants*. 5(2):59-75.