The Antileshmaniasis Effect of *Brassica juncea* Seeds Fatty Acids on the Growth of *Leishmania tropica* Promastigotes Isolated Culture *in vitro*

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ABSTRACT

Background: *Brassica juncea* has many important phytochemical compounds like phenols, tannins, saponin, flavonoids, alkaloids, carbohydrates, proteins and important non-polar metabolites that possessed biological and nutrient importance.

Methods: The study employed extraction of fatty non polar components with petroleum ether from *B. juncea* (mustered)seeds and applying chemical analysis of mustard seeds extract with HPLC technique to investigate the chemical composition of the oil seeds that the plant rich with. Besides; study the effect of *B. juncea* non-polar seeds extract on the growth of *Leishmania tropica* promastigotes *in vitro*.

Result: Results showed that the mustard seeds were rich with important fatty acid such as Erucic acid, -Oleic acid and Palmatic acid as matched the retention times of the standard used in the study. These compounds had been found to be toxic for cutaneous leishmaniasis culture that, affected the parasites with prolonged treatment periods.

Key words: *Brassica juncea*, Erucic acid, *Leishmania tropica*, Mustard seeds.

INTRODUCTION

Natural plant sources have been and still are the focus of researchers and scholars in order to investigate natural and effective alternatives to solve various diseases and disorders (Hasan *et al.,* 2022; Alyasiri *et al.,* 2025), especially those materials that have biological effectiveness as anti-inflammatory, anti-fungal and bacterial infections and even those that are efficient in treating parasites such as leishmaniasis and others (Al-Timimi *et al.,* 2022; Hmeed *et al.,* 2025) and compounds that increase immunity and prevent tumors and cancer and in treating them as well (Zainab *et al.,* 2022). Study the effects of herbs and medicinal plants as major sources for new drug discovery and economical supplier of active components in the pharmaceutical industry and their importance had been increased with scientific progress all over the world (Oyi *et al.,* 2002). The world health organization (WHO) gave significant importance to expanding the use of drugs derived from plant sources instead of chemically manufactured medicines declaring that Modern medicine has built on what nature has to offer and has drawn upon traditional systems of knowledge of how these medicinal plants, herbs, roots and bark were wielded to cure diseases across civilizations. Around 40% of pharmaceutical products today draw from nature and traditional knowledge, including landmark drugs: aspirin, artemisinin and childhood cancer treatments. A closer look at these drugs reveals that the scientists behind them built off traditional knowledge to achieve their breakthrough discoveries (WHO, 2019).

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One of these medicinal plants is *Brassica juncea*, which belongs to the Brassicaceae family (Cruciferea) and is recognized for its medicinal and nutritional possessions. The most commonly used parts are the seeds, which are brown in color (Nkiruka *et al.,* 2019). Mustard (*Brassica juncea*) is used worldwide in folk medicine and as food spice. The plant contains chlorophylls, vitamins, glucosinolates, minerals and dietary fiber besides different amounts and kinds of polyphenols components and the volatile

components. Thus mustard has various biological with potent antioxidant activities such as anti-inflammatory, antibacterial, anti-virus, anti-depression. treatment of diabetes and cataracts prevention, anti-obesity and anti-cancer (Yan and Fangming, 2020; Hameed *et al.,* 2025).

Leishmaniasis, a vector-borne protozoal disease is widely distributed in tropics and neotropics, which regarded global disease burden that ranks 9th among the world infectious diseases. Human and mammalian leishmaniasis is characterized by a myriad of clinical spectra ranging from localized and ulcerative skin lesions (cutaneous leishmaniasis) to non-ulcerative nodules (diffused cutaneous leishmaniasis) to the inflammation of mucus membranes (muco-cutaneous leishmaniasis) to infection of visceral organs (visceral leishmaniasis; the fatal form of the disease causing maximum deaths) (Qutoof *et al.,* 2024). *Leishmania tropica* or Baghdad ulcer, is a type of parasite widespread around the world that infect human skin cells. *Leishmania*, possesses a heteroxenous life cycle, with has two forms amastigote form in host and promastigote in sand fly and in culture and swimming between flagellated promastigote and aflagellated amastigote form occurring respectively in invertebrate (sandfly gut) and vertebrate (a variety of lizards and mammals including human) macrophages (Schwarz *et al.,* 2017). Leishmania Life cycle, after feeding infected mammalian host amastigotes are ingested by sandy fly, engorgement is quickly followed by production of peritrophic membrane which is secreted by the epithelial cell lining the mid gut of the fly. Promastigotes develop from amastigote within 24 hours under rapid division period to their transformation in 2-5 days (Miranda *et al.,* 2021). The parasites are transmitted from human to human by bite of Phledotamine sand flies. The promastigote form has been demonstrated to show morphologically and functionally different morphotypes (Bordbar and Parvizi, 2014). In general, the parasite exists in two forms: The amastigote form, which is found in the vertebrate host and the promastigote form, which is found in the insect vector and artificial culture media (Qaisar *et al.,* 2020).

Sulfur-containing glucosinolates, terpenoids, polyphenols, S-methyl cysteine sulfoxide, omega-3-fatty acids, beside the trace-elements, all these molecules that are produced in the plant have been recently play importance in food industries and pharmaceutical manufacturing (Idrees, *et al.,* 2019; Jasim *et al.,* 2025).

So that the current study relates the uses of Brassicaceae oil seeds as mustards bioactive molecules for possible antileshmaniasis investigation and provides an overview of the current knowledge of mustards benefits, as one of new of their biological activities. At present, the processing of extracting oil from its seeds to get the functional components as antiparacetic agent is not well utilized. Thus the current work highlighted such project.

MATERIALS AND METHODS

Collection and classification of *Brassica juncea* seeds

Mustard seeds of brown color were obtained from the local market at Tikrit city and sent for botanical classification at College of Science laboratories/University of Tikrit. The seeds were cleaned of dirt and ground with aid of electric grinder to increase the surface area towards the extraction fluid. The powdered seeds were stored in paper bags in a dry place until extraction.

Extraction the non-polar components of seeds with petroleum ether

The petroleum ether extract was prepared according to the method in (Grand *et al.,* 1988), by mixing 40 grams of powdered mustard seed (*B. juncea*) in 500 ml of petroleum ether in a tightly sealed flask, allowing the mixture to soak for 24 hours. The mixture was then placed on a magnetic stirrer for another 27 hours. Afterward, the extract was filtered with Whatman No. 1 filter paper. The non-polar crude extract was then concentrated using rotary evaporator, until dryness. The resulted residue was oily in texture to be stored in special bottles in a cool dry place until use.

Identification of fatty acids in mustard seed oil (*Brassica juncea*) residue by HPLC technique

Identification of fatty acids in the extracted residue was carried out at the laboratories of the Environmental and Water Directorate/Ministry of Science and Technology/Iraq according to (Beare-Rogers *et al.,* 2001). The following condition for the high performance liquid chromatography (HPLC) was applied, Table 1.

Anti-leishmaniasis activity

For this assay stock solutions (100%) from the oil residue was used to prepare four another dilutions with aid of 1% DMSO solution to get (1, 2, 3 and 4)/10 ml in 1% DMSO. All solutions were sterile with 0.22 mm Millipore filter.

Isolation of cutaneous leishmaniasis

Stock cultures of the cutaneous leishmaniasis parasite, isolated from infected individuals in Iraqi territories in the

Table 1: HPLC condition for fatty acids in non-polar seeds residue.

System	Conditions
Instrument	SYKAM/Germany
Samples	1 ml/ml plant residue
Mobile phase	A = (Methanol: D.W.: Acetic acid) (85:13:2).
	B = (Methanol: D.W.: acetic acid) (25:70:5).
Column	ODSC18 (250 × 4.6 Id) mm, 5 μm partical size
Flow rate	1.0 ml/min
Injection volume	20 μl
Detection wavelength	UV-Vis at λ 280 nm
Column temperature	Room temperature
Standards used	Erucic acid
	Oleic acid
	Palmitic acid
	Stearic acid

promastigote stage, were got from the Medical Research Center at the College of Medicine, Al-Nahrain University. The cultured medium was a ready-made RPMI 1640 culture liquid solution**.** Fetal calf serum was added besides the Antibiotic: Streptomycin, Pencillin and Gentamycin as follow: 9 ml of RPMI 1640 + 1 ml of Fetal calf serum + 10 microliters of Streptomycin and Penicillin antibiotic or alternatively, 3 microliters of Gentamycin according to (Anju *et al.,* 2020; Razooki *et al.,* 2020).

The assay procedure for anti-cutaneous leishmaniasis

The assay was proceeded on *Leishmania tropica* for 24,48.72 and 96 hours interval time for exposure. The inhibition of the promastigote growth assay was performed as previously described by (Mayara *et al.,* 2023; Razooki *et al.,* 2019).

Firstly, promastigote (23×10^4 cells per well) were grown in four tissue culture plate of 96-well of 200 µl in RPMI growth medium final volume in the presence and absence of different sterile oil concentrations (1, 2, 3 and 4 ml /10 mL). The plate was incubated at 26°C for the fourth time intervals in a biological oxygen demand (B.O.D) incubator. The inhibitory effect was evaluated by adding 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT dye (5 mg/mL). before 4 hours from the end of exposure time of each plate. Then 50 µL sodium dodecyl sulfate (SDS) was added to dissolve the formazan crystals. The absorbance for all plate was read at 620 nm using Elisa plate reader.

Statistical analysis

The statistical analysis system- SAS 2018- programs used to analyze all received data. The program was used to detect the effect of difference factors in study parameters for least significant difference-LSD used to significant at level α=0.05 compare between means (ANOVA/Two way) in this study (SAS, 2018).

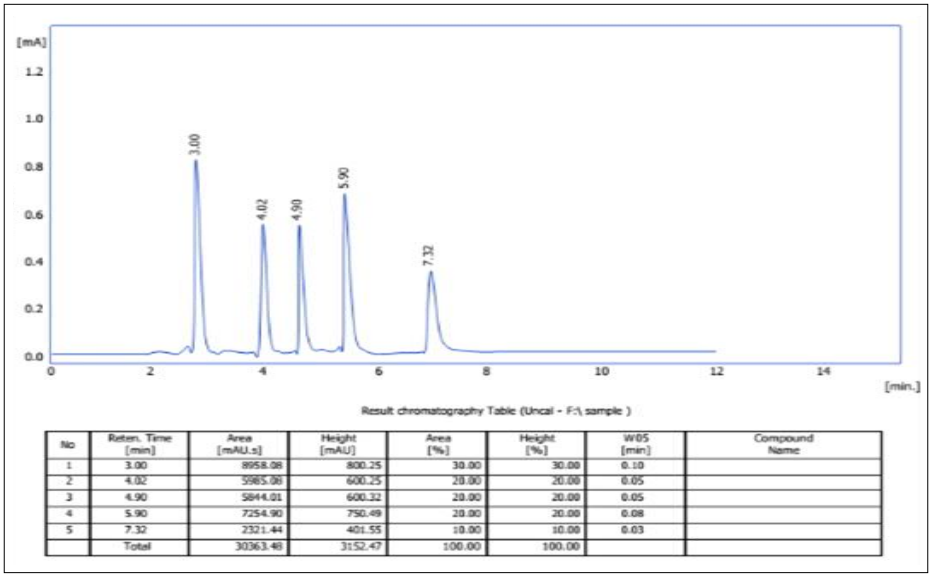
RESULTS AND DISCUSSION

Detection of fatty acids in mustard seed oil (*Brassica juncea*) residue by HPLC technique

The chemical analysis of mustard seed oil usage HPLC technology presented that the oil sample contain a number of fatty acids such as Erucic acid ($C_{22}H_{42}O_2$) of peak appeared at a retention time (Rt) (3.00) minutes, corresponding to the standard sample at a retention time (3.08), Oleic acid appeared at Rt (4.02) minutes aganist standard peak Rt of (4.08) minutes, palmatic acid with Rt of (4.90) minutes in corresponding to standard Rt of (4.95) minutes and Stearic acid at a retention time of (7.32) minutes consistent, the standard retention time was of (7.36) minutes (Fig 1). All sample peaks matched the retention times of the standard (Fig 2, 3, 4, 5).

Anti-leishmaniasis activity

The current study data presented an important effects ($p<0.05$) between different doses of the non-polar residue of *B. juncea* seeds oil with different times of exposure. The highest concentration showed (4 ml/10 ml) showed the highest activity inhibition against *Leishmania tropica* at 24 hour (24.50±0.96), 48 hour (44.25±1.70), 72 hour (72.25±1.25) and for 96 hour exposure inhibition result was (104.25±1.44), while the least dose inhibition was (1 mg/10 ml) in the following manner of treatment periods: at 24 hour (62.25±1.55), at 48 hour (94.00±1.73), at 72 hour (135.50±1.32) and at 96 hour (191.00±1.29), compared to controls (74.00±1.58, 148.50±2.72, 260.00±7.16 and 375.00±5.80) respectively (Table 2).



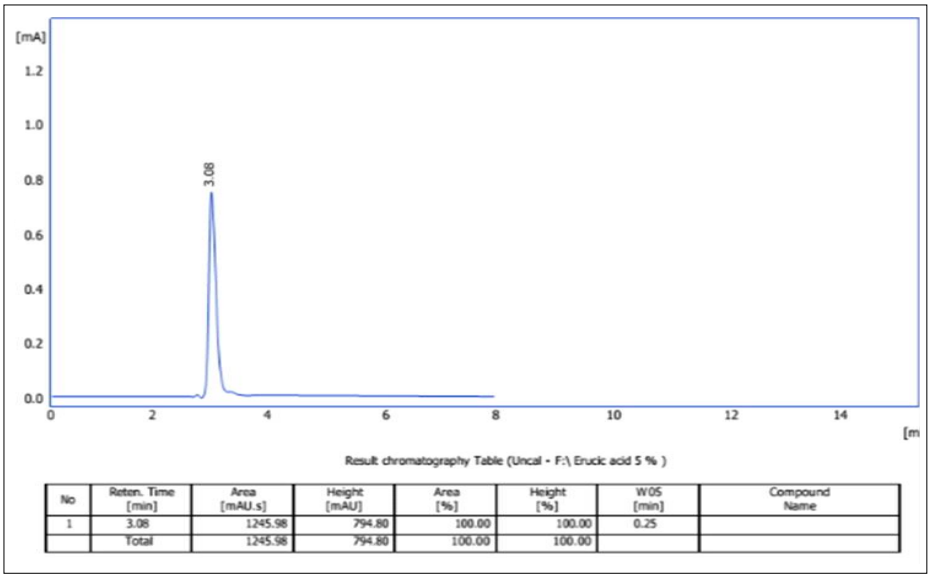
Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.00	8958.08	800.25	30.00	30.00	0.10	
2	4.02	5985.08	600.25	20.00	20.00	0.05	
3	4.90	5844.01	600.32	20.00	20.00	0.05	
4	5.90	7254.90	750.49	20.00	20.00	0.08	
5	7.32	2321.44	401.55	10.00	10.00	0.03	
	Total	30363.48	3152.47	100.00	100.00		

Fig 1: Chromatographic analysis of fatty acids in mustard seed oil using HPLC technique.

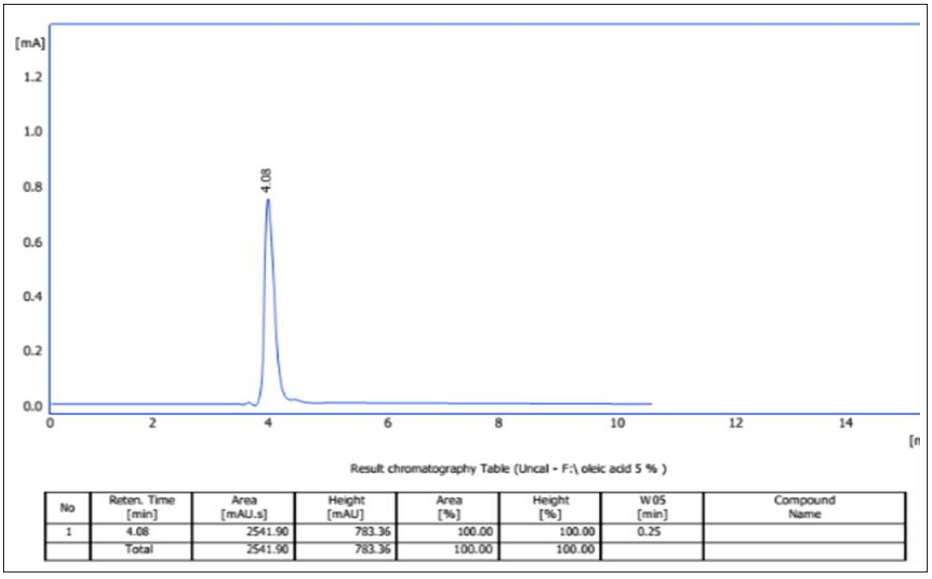
Despite the obtainability of many chemical compounds to treat cutaneous leishmaniasis, they have been found to have toxic side effects due to prolonged treatment periods. Additionally, the high cost of several chemical drugs necessitates the search for novel effective substance that predicted to be safer and effective.

This is what prompted the entire world to turn and call for a return to nature since the year 2000. Accordingly, the attention of researchers and scientists turned to finding natural alternatives, especially in the field of health and treatment of many modern diseases. Medicinal plants and their active components had the greatest share of researchers' interest and investigation of their compounds as alternatives to solve many diseases and health crises in societies (Mina *et al.,* 2024; Zeena, 2024; Hasan *et al*., 2022; Mansoor *et al.,* 2024; Hasan *et al*., 2023; Ogaili *et al.,* 2023; Al-Halbosiy *et al.,* 2020; Mutter *et al*., 2024; Hasan *et al.,* 2024; Razooki *et al.,* 2025). The high oil content in the seeds of the mustard family has given industrial importance to the plants of this family as *Brassica juncea*, in addition to their use as flavorings and spices, especially the plant seeds and leaves. Researchers attribute the biological effectiveness of this plant to its containing major secondary metabolite compounds such as: alkaloids, saponins, phynolic and flavonoid components in addition to the nutritional compounds in the plant, such as proteins



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.08	1245.98	794.80	100.00	100.00	0.25	
	Total	1245.98	794.80	100.00	100.00		

Fig 2: Chromatographic analysis of the standard fatty acids, erucic acid using HPLC technique.



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.08	2541.90	783.36	100.00	100.00	0.25	
	Total	2541.90	783.36	100.00	100.00		

Fig 3: Chromatographic analysis of the standard fatty acids, oleic acid using HPLC technique.

and carbohydrates. As this plant contained such active constituents, the extracts of different parts of the plant showed various biological activities as antimicrobial, antifungal, Antiviral, anti-inflammatory properties. The plant also possessed antioxidant activity and showed beneficial effects as anti-diabetic, anti-obesity, anticonvulsant and anticancer activity, (Jignesh *et al*., 2021). Therefore, *Brassica juncea* seed oil, identify its fatty acids and determine their types and assess the inhibitory effect of the oil on the biological activity of the *Leishmania* parasite.

The current study showed that the seeds is rich with major bioactive fatty acid components like Erucic acid (C_{22} $H_{42}O_2$). It is an omega-9 unsaturated fatty acid originate in mustard seed oil and rapeseed oil. It features a carbon chain composed of 22 carbon atoms with a double bond at the ninth carbon. It appears as a pale yellow liquid at room temperature, inexplicable in water but soluble in organic thinners. Historically, it has been used in lubricants, plastics and surfactants. It was previously used in cooking oils, but its use in food is now limited due to health concerns related to heart health. Biologically, it serves as an energy source and a structural component in plant cells, with ongoing regulatory oversight em phasizing health considerations in its various applications (Acharya *et al*., 2024; Al-Maliki *et al.,* 2025).

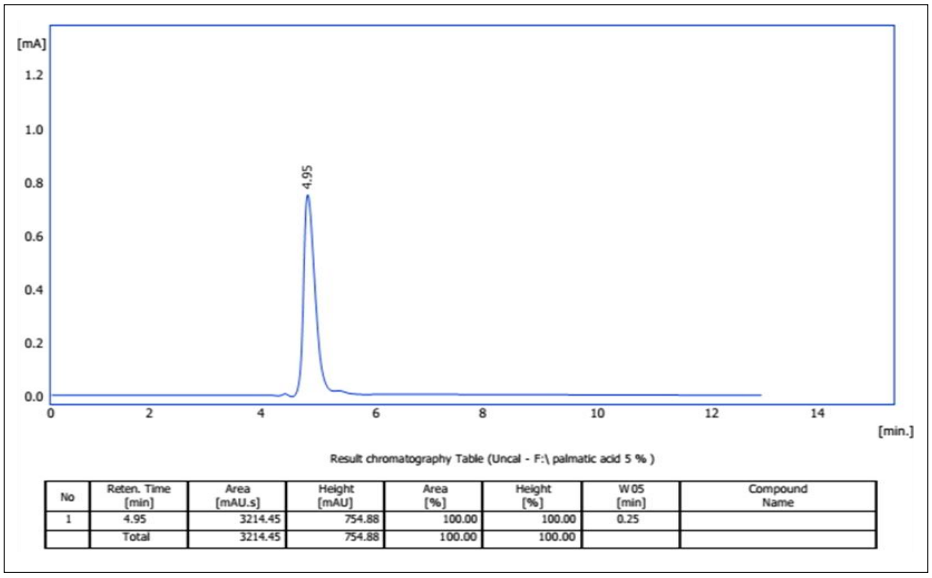


Fig 4: Chromatographic analysis of the standard fatty acids, oleic acid using HPLC technique.

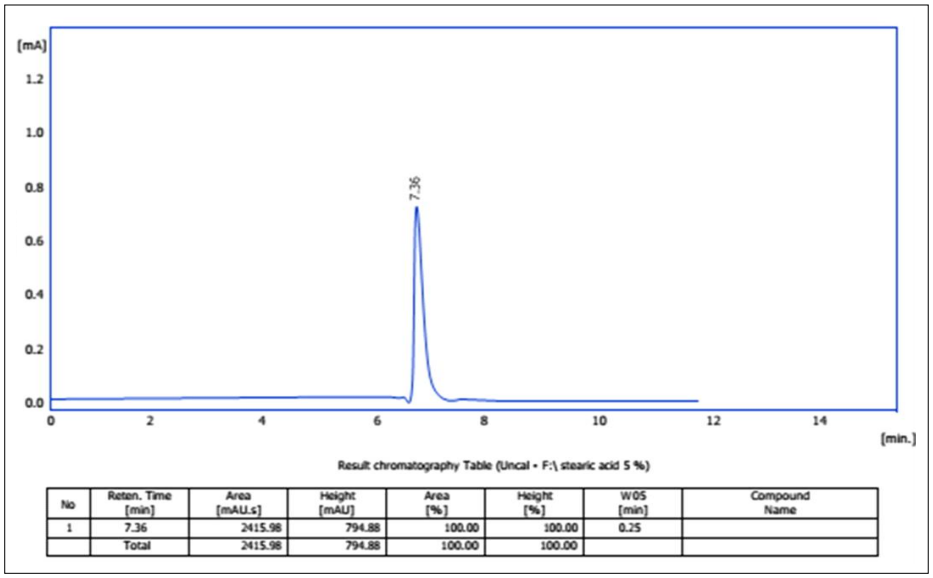
Table 2: Inhibition effect of oil *Brassica juncea* extraction on *Leishmania tropicalis* with different concentration and expoure time.

Conc./10 ml	Oil *Brassica juncea* extraction							
	% Mean inhibition rate							
	Time exposure (hour)							
	After 24 hour		After 48 hour		After 72 hour		After 96 hour	
	Mean	Error	Mean	Error	Mean	Error	Mean	Error
1 mg	62.25^{d}	1.55	94.00^{c}	1.73	135.50^{c}	1.32	191.00^{d}	1.29
	A		B		C		D	
2 mg	44.25^{c}	1.11	75.00^{b}	1.08	110.50^{b}	0.96	158.25^{c}	2.95
	A		B		C		D	
3 mg	34.00^{b}	1.22	62.25^{b}	1.65	96.25^{b}	1.55	132.75^{b}	1.80
	A		B		C		D	
4 mg	24.50^{a}	0.96	44.25^{a}	1.70	72.25^{a}	1.25	104.25^{a}	1.44
	A		B		C		D	
Controls	74.00^{e}	1.58	148.50^{d}	2.72	260.00^{d}	7.16	375.00^{e}	5.80
	A		B		C		D	

Small letters compare with vertical view.

Capital letters compare with horizontal view.

Different letters refer to significant different ($p<0.05$).



Result chromatography Table (Uncal - F:\ stearic acid 5 %)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.36	2415.98	794.88	100.00	100.00	0.25	
	Total	2415.98	794.88	100.00	100.00		

Fig 5: Chromatographic analysis of the standard fatty acids, palmatic acid using HPLC technique.

Also the seeds contain (Oleic acid). It is an unsaturated fatty acid consisting of 18 carbon atoms with a single double bond at the ninth position. Its chemical formulation is $C_{18}H_{34}O_2$. It is originate in many vegetable oils *e.g.* canola oil, olive oil and sunflower oil, in addition to in animal fats in smaller amounts. It is used in the food industry to improve cholesterol levels and heart health and in the chemical industry in products like cosmetics and soap. Mustard oil contains smaller amounts of oleic acid compared to other oils. Oleic acid assistances lower bad cholesterol (LDL) and increase good cholesterol (HDL) and has numerous health benefits (Teres *et al*., 2008; Abd El-Rahmana *et al.,* 2024). Palmitic acid also appeared in the oil seeds. It is a soaked fatty acid found in plants and animals, consisting of 16 carbon atoms with the chemical formula $C_{16}H_{32}O_2$. It is present in coconut oil, palm oil and animal fats. Used in the food manufacturing such as margarine and in the chemical industry in soap and cosmetics, it serves as an energy source in the body. Despite its benefits, excessive consumption may increase the risk of cardiovascular diseases (Niloy *et al*., 2024; Al-Khuzaay *et al.,* 2024; Yahya *et al.,* 2024; Hameed *et al.,* 2025). Moreover; Stearic acid is a soaked fatty acid containing of a long carbon chain with 18 carbon atoms. It naturally occurs in various plant and animal fats such as cocoa butter, shea lard and animal fat. Usaged in the food and chemical industries as a whitening and emulsifying agent, it is involved in the production of soaps, detergents and cosmetics. It is generally considered safe for use in foods and skincare products, but excessive consumption should be avoided as it may increase levels of harmful cholesterol (Hunter *et al*., 2009; Kareem *et al.,* 2023; Altemeemi *et al.,* 2021).

Amongst vegetable oils of this lipid profile which is considered as the best of favorable human health benefits in decreasing blood total cholesterol and low-density cholesterol, lowering cardiovascular risk, with an increasing insulin sensitivity and, inflammation reducing, all theses factors might lead in cancer cells growth reduction (Ostrikov *et al*., 2021; Singh *et al*., 2014; Li *et al*., 2023; Obaid *et al.,* 2020).

In cases of treating topical fungal infections, extract of mustard seed could be useful. In addition the benefit usage of this plant extracts in treating fungal and bacterial infections as well as their preservative activity that could be used in herbal and food preparations (Bajpai *et al*., 2023; Román *et al*., 2024; Rahman *et al.,* 2020; Yaseen *et al.,* 2024; Obaid *et al.,* 2020).

Mustard seeds steroids were found to possess substantial anti-viral properties against some pathogenic viruses such as the measles virus, RNA viruses and even herpes simplex virus type 1 (Rahman *et al*., 2024; Yaseen *et al.,* 2025; Obaid *et al.,* 2025).

CONCLUSION

The current study represented an attempt for new approach in parasitic treatment epically topical leishmaniasis from herbs sources even the non-polar components in the mustard seeds not yet had been studied as new strategies to be in considered one of the crucial components with antileshmaniasis effects. Thus the current study lightened the effeteness rule of herbs in management leshmaniasis emerged or re-emerged from the obscurity and became considerable threats to the global health especially in our country.

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Author contribution

All authors confirm their contribution to the paper including study conception**;** the study design; data collection, analysis and interpretation of results. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest regarding the publication of this manuscript.

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