



Original Research Article (Experimental)

Anti-inflammatory activity of crude and detoxified leaves of *Daphne oleoides* Schreb. on carrageenan-induced paw edema in wistar rats

Sayeedur Rahman ^a, Najeeb Jahan ^{a, *}^a Dept. of Ilmul Advia (Pharmacology), National Institute of Unani Medicine, Bangalore, India

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ABSTRACT

Background: *Mazaryun* (*Daphne oleoides* Schreb.) is used as an anti-inflammatory drug in Unani medicine after detoxification, as it is defined under fourth-degree drugs.

Objective(s): To evaluate and compare the anti-inflammatory activity of crude and detoxified *Mazaryun* in maximum and minimum doses.

Materials and methods: Anti-inflammatory activity was carried out by carrageenan-induced paw edema test. Wistar rats of either sex, weighing 150–200 gm, were divided into seven groups (I, II, IIIA, IIIB, IVA, IVB, and V) of six animals in each. Group I - plain control, administered with 1 ml of 1% CarboxyMethyl Cellulose (CMC); Group II - standard control, given Diclofenac Sodium (6 mg/kg); Group III - crude *Mazaryun* and Group IV - detoxified *Mazaryun*, A and B are maximum and minimum doses of test drug, respectively; and V group - positive control was not treated with any other drugs. The data was statistically analyzed by ANOVA repeated for inter-group analysis and ANOVA one-way for intra-group analysis with post hoc Tukey Kramer multiple comparison test. The GC–MS analysis of crude and detoxified leaves of *Mazaryun* was also carried out in continuation of study to determine the phytochemical changes before and after detoxification.

Results: Maximum dose of detoxified *Mazaryun* and standard control groups showed significant anti-inflammatory activity at $p < 0.001$, and detoxified *Mazaryun* showed dose-dependent activity. The GC–MS fingerprints showed totally eight different chemical constituents in its crude and detoxified form.

Conclusion: The study standardised the concept of detoxification in Unani medicine, as the detoxified *Mazaryun* showed significant anti-inflammatory activity and present of totally different chemicals constituents.

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1. Introduction

The word "inflammation" is derived from a Latin word "inflammo", means "I set alight; I ignite, or to set on fire". In Greek, inflammation is known as a hot thing. The Greek word, phlegmon has been used to define inflammatory lesions within [1,2].

Inflammation is defined as a local protective response of living mammalian or vascularised tissues to injury or infection due to any agent. It is a normal response to disturbed homeostasis caused by infection, injury, and trauma [2–5]. According to Unani

concept, *Waram* (inflammation), is used for a broad term, and refers to any abnormal swelling, be it due to accumulation of blood, pus, water, and flatus [6]. *Waram* is a swelling that is produced because of absorption (inflow) of some abnormal matter in any organ [7,8].

In Unani medicine, *Mazaryun* (*Daphne oleoides* Schreb.) is mentioned under fourth-degree drugs [9] and detoxification is recommended before use [10] as all parts of the plant are poisonous. Skin contact with the sap can cause dermatitis in some people [6]. The leaves of *Mazaryun* are traditionally used for the treatment of inflammatory disorders [6,11,12]. It has hot and dry temperament in fourth-degree [6,7,13]. Therefore, Unani physicians have advocated some detoxification methods before using *Mazaryun* for medicinal purpose [6,7,13–17]. If *Mazaryun* is used without detoxification it may produce severe complications; it may also lead to vomiting and/or diarrhoea [13,14]. *Daphne* is a genus of

* Corresponding author.

E-mail: drnajeeljahan@gmail.com

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around 70–95 species of deciduous and evergreen shrubs in the family Thymelaeaceae, native to Asia, Europe, and North Africa. It is a small multi-branched shrub found in the Western Himalayas. The bark contains diterpenes including mezerein and daphnetoxin (0.02%) [18,19] of which mezerein is anti-inflammatory and anti-carcinogenic [19]. Previous research studies have reported wound-healing [20], antimicrobial [21] and antioxidant properties [20] of *D. oleoides* Schreb., but till date no scientific study has been carried out on its crude and detoxified forms to document the comparative pharmacological effect of *Mazaryun* before and after detoxification. Therefore, the present study was envisaged to evaluate the anti-inflammatory activity of the crude and detoxified leaves of *D. oleoides* on carrageenan-induced paw edema in Wistar rats.

2. Material and methods

2.1. Animals

The study was carried out on healthy Wistar rats weighing 150–200 g of either sex. The animals were procured from a registered breeder and allowed to acclimatize for one week. They were housed in clean polypropylene cages at room temperature (25 ± 2 °C), humidity 45–55% with 12 h light–12 h dark cycle throughout the experimental period and were provided with standard diet and water *ad libitum* unless stated otherwise. The animal care procedures and experimental protocol were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The study was conducted after obtaining the ethical clearance by the Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine (NIUM), vide Reg. no. IAEC/06/17/IA/03.

2.2. Chemicals and reagents

All the chemicals used were of analytical grade. Diclofenac Sodium was purchased from Cipla Drug Company and carrageenan from Sigma Aldrich Chemicals Pvt. Limited, Bangalore, India.

2.3. Plant materials and preparation of powder

The leaves of *Mazaryun* (*D. oleoids* Schreb.) were purchased from Dr. Mohd. Afsahul Kalaam, Research Officer Unani RRIUM, Habak, Naseumbagh Campus, Kashmir University, Srinagar, 190006, India. Acetic acid and *Roghan-e-Badam* (almond oil) were purchased from an authentic herbal supplier from a local market in Bangalore, India. The leaves of *Mazaryun* were identified by Dr. S. Noorunnisa Begum, Associate Professor, Centre for Repository of Medicinal Resources (C-RPR) at TransDisciplinary University (TDU) 74/2, Bangalore-64, vide authentication number (FRLHT Acc. No. 5355). A voucher specimen (Ref. no. 77/IA/Res/2020) was deposited in the department of *Ilmul Advia* (pharmacology), drug museum, NIUM, Bangalore, for future reference. The leaves of *Mazaryun* were divided into two equal parts - one part was kept crude and another part was detoxified. The leaves selected for detoxification were kept in an earthen pot, and soaked in acetic acid for three consecutive days and nights (72 h) and the acetic acid was changed daily as mentioned in Unani classical literature [6,13]. After completion of 72 h, the *Mazaryun* was taken out from acetic acid and washed with fresh water and then dried in an oven at 45 °C. Then, the dried leaves were powdered and *charb* (anointed) with almond oil [6,12–15]. The half crude undetoxified leaves of *Mazaryun* were simply ground into fine powder at the laboratory of department of *Ilmul Advia*, NIUM.

2.3.1. Dosage of the drug

The human therapeutic dose of *Mazaryun* mentioned in Unani classical literature is 3–5 g [13,14,16]. The dose for rats was calculated by dividing it by adult human weight of 60 kg and multiplying it with the conversion factor of 7 to accommodate the surface area of animal [22] and was found to be 0.35 mg/kg for low dose and 0.58 mg/kg for high dose. The dose of the test drug powder for each rat was dissolved in 1 ml of freshly prepared 1% CMC, daily before each administration.

2.4. Carrageenan-induced paw oedema test

This test was carried out by the method of Vogel [23]. Inflammation was induced in animals by carrageenan-induced edema test to find out activity against acute inflammation. Animals were divided into 7 groups of 6 animals in each.

- Group I** - Animals served as plain control and each animal was administered with 1 ml of 1% CMC.
- Group II** - Animals served as standard control and were administered standard drug, Diclofenac Sodium, in the dose of 6 mg/kg BW [24].
- Group III A** - Animals were treated with low dose of crude *Mazaryun*, 0.35 gm/kg BW.
- Group III B** - Animals were treated with crude *Mazaryun* in high dose, 0.58 gm/kg BW.
- Group IVA** - Animal were treated with detoxified *Mazaryun* in the low dose, 0.35 gm/kg BW.
- Group IV B** - Animals were treated with detoxified *Mazaryun* in high dose, 0.58 gm/kg BW.
- Group V** - Animals served as positive control and were not treated with any drugs.

In this model, acute inflammation was induced by sub-plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in rat hind paw of all the following groups: II, IIIA, IIIB, IVA, IVB and V. The test drugs and standard drug were administered 30 min prior to carrageenan injection. The thickness of hind paw was measured by plethysmometric method. Inflammation was observed in animal after 1, 2, 3, 4 and 5 h of carrageenan injection. The percentage inhibition of inflammatory edema in test and standard groups animals was calculated by the formula described by Newbould (1963), $i = 100\{1-(a-x)/(b-y)\}$. [26] The mean paw volume/thickness of test/standard groups was also analysed statistically in comparison with positive control group.

2.5. Collection of data

Paw thickness was measured just before the carrageenan injection, that is, at “0 h” and then carrageenan sub-plantar injection was given, and paw thickness was measured at 1, 2, 3, 4, and 5 h. Increase in paw thickness was measured as the difference in paw thickness at “0 h” and paw thickness at respective hours [25].

The percentage inhibition of inflammatory edema in test and standard control group animals was calculated by the formula described by Newbould (1963), $i = 100\{1-(a-x)/(b-y)\}$, where a = Mean hind paw volume of test/standard group animals after carrageenan injection, b = Mean hind paw volume of positive control animals after carrageenan injection, x = Mean hind paw volume of test/standard group animals before carrageenan injection, y = Mean hind paw volume of positive control animals before carrageenan injection [26]. The mean paw volume/thickness of test/standard groups was analysed statistically in comparison with positive control group by ANOVA test followed by post hoc multiple comparison test [27].

2.6. Statistical analysis

The data was analysed by ANOVA repeated for intergroup analysis with post hoc Tukey Kramer multiple comparison test and ANOVA one way for intra-group analysis with post hoc Tukey Kramer multiple comparison test.

2.7. GC–MS analyses

GC–MS analyses were carried out to detect the phytochemical changes in its crude and detoxified forms.

3. Results

The results of the study showed that the mean hind paw thickness of positive control group animals was 0.980 ± 0.013ml at t = 0. After injecting carrageenan into hind paw of these animals, maximum mean hind paw edema was observed with, 1.993 ± 0.013 ml, at the end of 1 h and at the end of 2, 3, 4 and 5 h it was 1.981 ± 0.006, 1.971 ± 0.006, 1.963 ± 0.004, and 1.950 ± 0.007 respectively (Table 1).

In plain control group animals, the paw thickness at t = 0 was 0.965 ± 0.018 ml and this remained almost constant at the end of 5 h (Table 1).

The mean hind paw thickness at t = 0 in the animals of standard control group was 0.985 ± 0.014 ml, and it was shown to increase, 1.491 ± 0.008 ml after injection of carrageenan at the end of 1 h and then gradually decreased to 1.396 ± 0.004, 1.336 ± 0.008, 1.306 ± 0.006, and 1.290 ± 0.005 ml at the end of 2, 3, 4 and 5 h; respectively. The percentage of inhibition in standard control group was 50.9%, 59.6%, 65.2%, 67.9%, and 69.1% at the end of 1, 2, 3, 4, and 5 h respectively. The percentage of inhibition was maximum at the end of 5 h, which was significant at p < 0.001 (Table 1).

In low dose of crude Mazaryun, group IIIA animals, the mean hind paw thickness at t = 0 was 0.960 ± 0.017 and after injection of carrageenan, the mean of hind paw thickness was maximum, 1.886 ± 0.014 ml, at the end of 1 h after which the mean hind paw thickness gradually reduced to 1.863 ± 0.015, 1.823 ± 0.013, 1.781 ± 0.013, and 1.758 ± 0.009 at the end of 2, 3, 4 and 5 h respectively. The percentage of inhibition of edema in this group

was found to be 10%, 11.2%, 14.3%, 17.8%, and 19% at the end of 1, 2, 3, 4, and 5 h respectively (Table 1).

In high dose of crude Mazaryun, group IIIB animals, the mean hind paw thickness at t = 0 was 0.834 ± 0.150 ml and after injection of carrageenan, it was maximum, 1.746 ± 0.016, at the end of 1 h after which the mean hind paw thickness gradually reduced to 1.695 ± 0.018, 1.636 ± 0.010, 1.606 ± 0.008, and 1.591 ± 0.007 ml at the end of 2, 3, 4 and 5 h respectively. The percentage inhibition of edema in this group was found to be 11.3%, 15.3%, 20.3%, 22.7%, and 23.2% at the end of 1, 2, 3, 4 and 5 h respectively (Table 1).

In group IVA animals of low dose of detoxified Mazaryun, the mean hind paw thickness was 0.968 ± 0.016 ml at t = 0 and after injection of carrageenan, the mean thickness was found to be maximum, 1.866 ± 0.019 ml, at 1 h and which gradually reduced to 1.833 ± 0.016, 1.815 ± 0.016, 1.785 ± 0.012, and 1.763 ± 0.014 ml at the end of 2, 3, 4, and 5 h respectively. The percentage inhibition of edema in this group was found to be 12.7%, 14.9%, 15.9%, 18.2%, and 19.3% at the end of 1, 2, 3, 4 and 5 h respectively (Table 1).

In group IVB animals of high dose of detoxified Mazaryun, the mean hind paw thickness was 0.973 ± 0.019 ml at t = 0 and after injection of carrageenan, the mean hind paw thickness was found to be maximum, 1.746 ± 0.016 ml, at 1 h and gradually reduced to 1.700 ± 0.016, 1.641 ± 0.011, 1.603 ± 0.005, and 1.570 ± 0.012 ml at the end of 2, 3, 4, and 5 h respectively. The percentage of inhibition of edema in this group was found to be 24.9%, 28.5%, 33.6%, 36.9%, and 39.4% at the end of 1, 2, 3, 4 and 5 h respectively (Table 1).

When the results of all the groups were compared, it was found that the mean hind paw thickness was maximum in positive control group animals at 1 h. When the percentage inhibition of all the groups were compared at the end of 1, 2, 3, 4, and 5 h, it was found that the Mazaryun in high dose showed significant percentage inhibition than the other groups, except standard control group, as the percentage inhibition of edema in standard control group was found to be highly significant p < 0.001. When the mean hind paw thickness was compared with plain control among the group, it was found that the mean hind paw thickness of high dose of detoxified Mazaryun and standard control group were towards plain control group, and standard control group showed more significant reduction in edema when compared to all the groups. The mean hind paw thickness of group IVB showed significant reduction in edema when compared to IIIA, IIIB, IVA, and V groups (Table 1).

Table 1
Effect of crude and detoxified leaves of Mazaryun on carrageenan-induced paw edema in Wistar rats.

Groups	Dose mg/kg	Rat paw edema volume at different time interval (ml)						P value
		0min	After carrageenan injection					
			1h (Inhibit.%)	2 h (Inhibit.%)	3h (Inhibit.%)	4h (Inhibit.%)	5h (Inhibit.%)	
Group I Plain Control	1 ml of 1%CMC	0.965 ± 0.018	0.970 ± 0.012	0.970 ± 0.012	0.968 ± 0.013	0.965 ± 0.008	0.965 ± 0.018	
Group II Standard Control Diclofenac Sodium	6 mg/kg BW	0.985 ± 0.014	1.491 ± 0.008 (50.9%)	1.396 ± 0.004 (59.6%)	1.336 ± 0.008 (65.2%)	1.306 ± 0.006 (67.9%)	1.290 ± 0.005 (69.1%)	<0.001
Group IIIA Crude Mazaryun Low dose	0.35 mg/kg BW	0.960 ± 0.017	1.886 ± 0.014 (10%)	1.863 ± 0.015 (11.2%)	1.823 ± 0.013 (14.3%)	1.781 ± 0.013 (17.8%)	1.758 ± 0.009 (19%)	<0.001
Group IIIB Crude Mazaryun High dose	0.58 mg/kg BW	0.834 ± 0.150	1.746 ± 0.016 (11.3%)	1.695 ± 0.018 (15.3%)	1.636 ± 0.010 (20.3%)	1.606 ± 0.008 (22.7%)	1.591 ± 0.007 (23.2%)	<0.001
Group IVA Detoxified Mazaryun Low dose	0.35 mg/kg BW	0.968 ± 0.016	1.866 ± 0.019 (12.7%)	1.833 ± 0.016 (14.9%)	1.815 ± 0.016 (15.9%)	1.785 ± 0.012 (18.2%)	1.763 ± 0.014 (19.3%)	<0.001
Group IVB Detoxified Mazaryun High dose	0.58 mg/kg BW	0.973 ± 0.019	1.746 ± 0.016 (24.9%)	1.700 ± 0.016 (28.5%)	1.641 ± 0.011 (33.6%)	1.603 ± 0.005 (36.9%)	1.570 ± 0.012 (39.4%)	<0.001
GroupV Positive Control	No Treatment	0.980 ± 0.013	1.993 ± 0.008	1.981 ± 0.006	1.971 ± 0.006	1.963 ± 0.004	1.950 ± 0.007	
P value		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	

Values are expressed as Mean ± SEM (n = 6). Test used ANOVA repeated for intergroup analysis and ANOVA one way for intragroup analysis. Inhibitory rate (%) was listed in the bracket. The "p" value of all intragroup comparison is highly significant = p < 0.001, but comparison between group IIIA vs group IVA and group IIIB vs group IVB the "p" value is not significant = p > 0.05.

The results of GC–MS fingerprints showed a total of eight different chemical constituents in its crude and detoxified forms (Table 2 and 3) The β -Amyrin and α -Amyrin were found to be present only in detoxified form and their concentration was more than the other chemical constituents, i.e., 29.123% and 47.946% respectively (Table- 3). The details of GC-MS analysis with structural elucidation of components is under the process of publication.

4. Discussion

Unani medicine offers many anti-inflammatory drugs, and the test drug, *Mazaryun* is one of them, mentioned under the fourth-degree drugs [9] and detoxification is recommended before use [10]. It possesses pharmacological properties like purgative [6,13,28,29], anti-inflammatory [6,7,16,28], anti-pyretic [20], anti-helmenthic., [16], *Mudirr* (diuretic) [16], corrosive [6,14], detergent [16,30], and *Mujaffif* (siccative) [6]. This drug has been investigated for various pharmacological actions like, wound-healing, antimicrobial, and antioxidant, but scientific data on its anti-inflammatory property is lacking. Therefore, the present study was envisaged to validate the concept of detoxification by evaluating the inflammatory properties of crude and detoxified leaves of *Mazaryun*, in animal model. The results of the study were compared between the plain and standard control with the minimum and maximum doses of test drug, to find out the dose-dependant effect if any.

In contemporary medicine, *Waram* or *Iltehab* may be correlated with the concept of inflammation; however, if the comprehensive concept of *Waram* is considered, the contemporary concept of inflammation may be a sub-type of *Waram*. In contemporary medicine, inflammation can be defined as a defensive mechanism of the body to prevent spread of the disease throughout the body. Inflammation is caused by biological agents like bacteria, viruses, parasites, fungi etc.; chemical agents like strong acids, alkalis, poisons, and any chemicals which may result in tissue damage; physical agents like soft tissue injuries, fractures, heat including burns, and cold; and mechanical agents like trauma, presence of foreign body, ligature, and dead tissues [2,31].

The results of this test in plain control (Group I), showed that the paw thickness at $t = 0$ was 0.965 ± 0.018 ml and that remained almost constant at the end of 5 h. However, in positive control animals (Group V), at $t = 0$, the mean hind paw thickness was 0.980 ± 0.013 ml, after injecting carrageenan into the hind paw of these animals, and showed maximum mean hind paw edema at the end of 1 h, 1.993 ± 0.013 ml, and at the end of 2, 3, 4, and 5 h it was 1.981 ± 0.006 , 1.971 ± 0.006 , 1.963 ± 0.004 , and 1.950 ± 0.007 respectively. In positive control, induction of edema was maximum because carrageenan is a strong chemical used for the release of inflammatory and pro-inflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF- α , etc.) [27]. The edema formation in the paw may be the result of synergism between these inflammatory mediators that increased vascular permeability and/or the mediators that increased the blood flow [32]. The course of

acute inflammation is biphasic; the first phase starts with the release of histamine, serotonin, and kinins after the injection of phlogistic agent in the first few hours [33], while the second phase is related to the release of prostaglandins-like substances in 2–3 h. Prostaglandins are the main culprit responsible for acute inflammation [34].

The results of the standard control (Group II) showed 0.985 ± 0.014 ml mean hind paw thickness at $t = 0$ and it increased to 1.491 ± 0.008 ml after injection of carrageenan, at the end of 1 h, and then gradually decreased to 1.396 ± 0.004 , 1.336 ± 0.008 , 1.306 ± 0.006 , and 1.290 ± 0.005 ml, at the end of 2, 3, 4, and 5 h respectively. The percentage inhibition in standard control group was 50.9%, 59.6%, 65.2%, 67.9%, and 69.1% at the end of 1, 2, 3, 4, and 5 h respectively. In this group of rats, the percentage inhibition of inflammatory edema was found to be maximum at the end of 5 h, which was significant at $p < 0.001$. The standard control group animals showed more significant reduction in edema compared to all the groups (Table- 1). This may be because the standard drug, Diclofenac Sodium, is a non-steroidal anti-inflammatory agent which may responsible for the blockage of prostaglandins and inflammatory pathway because the second phase of acute inflammation is sensitive to both the clinically useful steroidal and non-steroidal anti-inflammatory agent [35].

The results of minimum dose crude *Mazaryun* (Group IIIA) showed 10%, 11.2%, 14.3%, 17.8%, and 19% inhibition of edema at the end of 1, 2, 3, 4, and 5 h respectively whereas, the maximum dose crude *Mazaryun* (group IIIB) showed 11.3%, 15.3%, 20.3%, 22.7%, and 23.2% of inhibition at the end of 1, 2, 3, 4, and 5 h respectively. This result revealed that the leaves of crude *Mazaryun* in maximum dose possess better anti-inflammatory activity than in minimum dose.

The results of minimum dose detoxified *Mazaryun* (Group IV A) showed 12.7%, 14.9%, 15.9%, 18.2%, and 19.3% inhibition of edema at the end of 1, 2, 3, 4, and 5 h respectively, whereas the animals of maximum dose detoxified *Mazaryun* (Group IV B) showed 24.9%, 28.5%, 33.6%, 36.9%, and 39.4% inhibition of edema at the end of 1, 2, 3, 4, and 5 h respectively. This result revealed that the detoxified *Mazaryun* in maximum dose possesses significant anti-inflammatory activity than the detoxified *Mazaryun* in minimum dose.

Moreover, the study was carried out in Wistar rats of either sex to explore any difference in the anti-inflammatory effect of detoxified *Mazaryun* in both male and female rats; however, there was no such difference found, and the results were same in both the sex.

The GC–MS analysis of crude and detoxified leaves of *Mazaryun* was carried out in continuation of this study, and the GC–MS fingerprints showed a total of eight different chemical constituents in its crude and detoxified form; β -Amyrin and α -Amyrin were present in detoxified form in more concentration, 29.123% and 47.946% respectively, and presence of saponins was also detected in qualitative phytochemical analysis in its detoxified form [36]. These results are in accordance with many scientific studies which reported that the plants containing β -Amyrin and α -Amyrin possess

Table 2
GC–MS of crude *Mazaryun* (before detoxification).

Sl.No.	Retention Time	Name of the compound	Molecular formula	Molecular weight	% of compound
1	48.966	Thiohydroxylamine, S-benothiazol-2-yl-N,N-dicyclohexyl-	C ₁₉ H ₂₆ N ₂ S ₂	346	5.050%
2	49.480	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	11.106%
3	49.944	Cyclopropanamine, 2-phenyl-, trans-	C ₉ H ₁₁ N	133	9.349%
4	51.227	9,12-Hexadecadienoic acid, methyl ester	C ₁₇ H ₃₀ O ₂	266	9.730%
5	51.481	Oxacycloheptadec-8en-2-one, (8Z)	C ₁₆ H ₂₈ O ₂	252	6.088%
6	51.576	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	C ₁₈ H ₃₀ O ₂	278	35.580%
7	56.227	Sucrose Octa acetate	C ₂₈ H ₃₈ O ₁₉	678	14.866%
8	57.039	Heptasiloxane, hexadecamethyl-	C ₁₆ H ₄₈ O ₆ Si ₇	532	8.231%

Table 3
GC–MS of detoxified *Mazaryun* (after detoxification).

	Retention Time	Name of the compound	Molecular formula	Molecular weight	% of compound
1	47.992	Phthalic acid, butyl isohexyl ester	C ₁₈ H ₂₆ O ₄	306	4.591%
2	49.474	3,7-Dimethyl-8-oxo-1,5-dioxo-spiro (5.5) undecane-3-carboxylic acid, methyl ester	C ₁₃ H ₂₀ O ₅	256	2.205%
3	50.087	Methyl 2,3,4-tri-O-acetyl-6-O-ethyl- α -D-mannopyranoside	C ₁₅ H ₂₄ O ₉	348	2.290%
4	50.320	4-(diethoxyphosphinoyl)-N-(prop-2'-enyl) butanamide	C ₁₁ H ₂₂ NO ₄ P	263	2.125%
5	51.525	Oleic anhydride	C ₃₆ H ₆₆ O ₃	546	8.333%
6	53.570	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy) trisiloxane	C ₁₁ H ₃₂ O ₄ Si ₄	340	3.586%
7	54.316	β -Amyrin	C ₃₀ H ₅₀ O	426	29.123%
8	57.863	α -Amyrin	C ₃₀ H ₅₀ O	426	47.946%

anti-inflammatory activity [37]. Many scientific studies have been reported that the saponins possess anti-inflammatory activity [38]. Therefore, the significant anti-inflammatory activity of detoxified *Mazaryun* than the crude *Mazaryun* may be due to the presence of its active components, β -Amyrin, α -Amyrin, and saponins, after its detoxification.

When the percentage inhibition of edema was compared in all the groups, at the end of 1, 2, 3, 4, and 5 h, it was found that the *Mazaryun* in maximum dose (Group IVB) possesses significant anti-inflammatory activity than IIIA, IIIB, IVA, and V groups but was found to be less significant when compared with the Group II. The mean hind paw thickness of maximum dose detoxified *Mazaryun* and standard control group were towards plain control group. If these results are analyzed according to the contemporary action of anti-inflammatory drugs, then it can be hypothesized that *Mazaryun* whether, detoxified in minimum dose or maximum dose may block the prostaglandins and inflammatory pathway, as that of standard drug Diclofenac Sodium, or may possess other actions including inhibition of lipoxygenase, superoxide radical production and superoxide scavenging, effects on neutrophil aggregation and adhesion, cytokine production, and cartilage metabolism [39]. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) of the phenylacetic acid class and similar to other NSAIDs, diclofenac is associated with serious dose-dependent gastrointestinal, cardiovascular, and renal adverse effects with the symptoms like headache, dizziness, stomach pain, diarrhoea, rashes, etc. [39] Therefore, research should be carried out on different doses of leaves of detoxified *Mazaryun* to developed as a safe anti-inflammatory drug for the treatment of patients with acute and chronic pain.

In Unani medicine, the *Haad auram* and soft *auram* can be correlated with contemporary concept of acute inflammation and are recommended to be treated with the drugs which possess *Mohallil* (resolving), *Mujaffif* (siccative), *Qabiz* (astringent), and *Rade* (excretory) drugs [15,40]. The drugs which possess hot and dry temperament are also strongly advised by Unani physicians, to be used in *Haad* and soft *auram* (acute inflammations) so as to remove the moisture present in soft *auram*. The test drug, *Mazaryun*, is said to possess the *Mohallil* (resolving) [6,39] and *Qabiz* (astringent) properties, and also belongs to the class of hot and dry fourth-degree drugs [12]. Therefore, it may resolve the morbid inflammatory matter by virtue of its hot temperament, and it produced *Tafarruq* (separation) in the morbid humours and removed away these matters in the form of vapours, as its dry temperament desiccates the moisture of morbid matter, or it removes the morbid inflammatory matter by *idrar* (excretion) or this action of the drug may be due to its specific form or structure which is known as *Surat-e-nawiya*. [41].

5. Conclusion

From the above results and discussion, it can be concluded that the leaves of *Mazaryun* in detoxified form exhibited significant anti-

inflammatory activity in a dose-dependent manner than its crude form, by virtue of its *Mohallil*, *Qabiz*, and *Rade* properties; hot and dry temperament in fourth-degree, and due to the presence of phytochemicals, especially β -Amyrin and α -Amyrin. The contemporary anti-inflammatory drugs like, NSAIDs are associated with many undesirable complications. Hence, there is a need to find out alternatives to current widely used NSAIDs and further research should be carried out at different doses of detoxified *Mazaryun* to have a cost-effective anti-inflammatory drug with reduced toxicity and side-effects to improve clinical utility.

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Conflict of interest

None.

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