





Comparative Evaluation of the Phytochemical and Morphological Analysis and Anti-Inflammatory Effect of *Lantana camara* on Mice Animal Model

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ABSTRACT

The aim of the study is to evaluate the phytochemical and morphological characterization between Iraqi and America Lanata camara extracts and to evaluate their inhibition effect on the egg albumin-induced paw inflammation. Successive maceration was used to eliminate the leaves of L. camara. The bioactive substance components present in the extract were identified using gas chromatography-mass spectrometry (GC-MS) analysis. In addition, HPLC analysis was performed for the detection of active compounds. The paw edema induced by injecting egg albumin (0.1ml) in both right and left hind-paw and the percentage inhibition as well as cell count, and skin thickness was measured. The initial phytochemical screening yielded positive test results for phenolic compounds, glycosides, tannins, saponins, anthraquinones, polysaccharides, and terpenoids. The results of GC-MS analysis of Iraqi and American leaves showed oleic acid (22.65%, 36.69%), octadecanoic acid (26.58%, 13.49%) and hexadecanoic acid (7.06%,14.52%), respectively, were the main compound in both extracts. The HPLC analysis indicated the presence of vitamin K in Iraqi and American leaves (9.52%, 9.77%) respectively, while there are no other research publications indicated to such results. The concentration 0.05% of Iraqi and America L. camara showed a significant inhibition effect (99.7%) of inflammation. The cell counts and skin thickness showed a significant decreased when treated with both extracts of L. camara. In conclusion, this study showed that the phytochemical composition of lantana camara leaf extract varies depending on the geographical region. The findings also suggest that this extract, contains various bioactive compounds that could be a valuable resource for developing pharmaceuticals to treat different diseases. The study also found that the extract has a significant anti-inflammatory effect and could be a promising option for inhibition of inflammation.

 $\mathbf{K}_{\mathbf{eywords}}$: Lantana camara, oleic acid, chromatography, maceration, vitamin K

INTRODUCTION

Natural plant remedies have historically plentiful among the plant kingdom and have functioned as significant models for the development of enhanced pharmaceuticals (1). Plants are known to contain a variety of chemicals. With a specific focus on their importance in the field of medicine, they possess the capacity to generate a diverse range of chemical compounds with varying biological effects (2). Throughout history, the natural world has served as a significant reservoir of vital medicinal compounds, with numerous plant species exhibiting advantageous effects on human health (3). *Lantana camara* is a flowering ornamental plant belonging to the Verbenaceae family. The annual flowering plant called lantana which is found in the tropical regions of the

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Americas and Africa, as observed by Linn (4) as well as, in Iraq (5). Furthermore, apart from its well-documented occurrence in the botanical universe, a variety of phytochemicals including phenols, flavonoids, saponins, tannins, glycosides, and resins have been discovered to possess analgesic, anti-inflammatory, antibacterial, and antipyretic attributes (6). L. camara demonstrates antiinflammatory qualities, making it potentially advantageous in the therapeutic management of illnesses such as psoriasis. Inflammation is seen as the immune system's reaction to a range of harmful stimuli, encompassing both exogenous and endogenous factors such as noxious agents, infections, cellular injury, and exposure to radiation (7). Inflammation is clearly a complex process involving many different aspects of both the immune and circulatory systems and serves to protect the host from invasion by potential pathogens (8). The aim of the study is to determine the characterization of Iraqi and America Lanata camara and to evaluate the anti-inflammatory effect of Lantana camara extracts.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the decision of the Ethics Committee of the Faculty of Veterinary Medicine of University of Baghdad, with approval number 1605, dated 26/7/2023. All institutional and national guidelines for the care and use of study animals were followed.

Plant Materials and Extraction

The leaves of Iraqi *L. camara* were collected from local center in Baghdad, Iraq and classified by the Ministry of Health, National Center for Herbal Medicine, Al-Razi Center for Medical Herbs. The leaves part of the American *L. camara* were collected from a special garden in Iraq. The plant's leaves were collected after flowering, which occurred from late spring to early fall (9).

The L. camara leaves were cleaned with distilled water to get free of the dust, then dried for two weeks at room temperature with a fan. The dry sample was grinded into a powder and run through a 40 Sieve. The dry powder was stored in a tightly sealed amber glass container, protected from direct exposure to sunlight. In the present investigation, the control variables included the duration of maceration and the temperature of extraction (10). The powdered extract was subsequently subjected to the maceration procedure. The process of maceration is conducted at ambient temperature, utilizing distilled water as the solvent for extraction. This extraction process was carried out for a week, with periodic hand agitation occurring every 24 h. Subsequently, the combination passed filtration, and the resulting extract was subjected to concentration through the application of heat over a water bath until it reached a semi-solid state. The extract was

stored in a sealed container in a cool and dry environment to inhibit the growth of microorganisms (11).

Phytochemical Screening

The objective of the phytochemical analysis was to determine the biochemical constituents present in both extracts of Iraqi and American *L. camara* by observing their color reactions with various reagents. The usual methodology was employed to analyze each extract for the presence of alkaloids, glycosides, tannins, diterpenes, flavonoids, carbohydrates, proteins, steroids, and saponins (12-14).

Gas Chromatography-Mass Spectrometry (GC-MS)

The active compounds were identified and quantified by the GC-MS device type (Agilent Technologies,7820A GC system) American-made (15). The paragraph discusses a GC-MS analysis using an HP-5ms fused silica capillary column and an Agilent Technologies 7820A GC system with 5975C Series GC/MSD equipment. The analysis lasted for 55.583 minutes. The split injector had a temperature setting of 240°C with a split ratio of 10. A single injection of the essential oil was used, and helium was the carrier gas at a flow rate of 1 mL/min. The ionization energy was 70 eV, and the interface temperature was 280°C. The mass spectrometer operated in full-scan mode, and components were identified by comparing retention lengths and mass spectra with the NIST mass spectra database. The relative abundance (% area) of each compound was calculated based on peak area ratios (16).

High Performance Liquid Chromatography (HPLC)

HPLC with Ultraviolet (UV) detection, specifically utilizing the Shimadzu LC-10ATVP model manufactured by Shimadzu Corporation in Kyoto, Japan, was applied to quantify vitamin K in aqueous extract of Lantana camara. Chromatographic separation was facilitated by a C18 column, with isopropanol and acetonitrile comprising the mobile phase. Detection of the analyte was conducted at 220 nm. The quantification of vitamin K was based on the comparison of peak areas in HPLC chromatograms of the samples against a standard (17) with a known vitamin K concentration of 10 ppm. The concentration of vitamin K in the sample was determined using the formula:

 $Concentration = \frac{Peak Area\% Sample}{Peak Area\% Standard} \times Concentration of Standard$

Gel Preparation

The gel formula was prepared using Carbopol polymer, a non-sensitizing and inert stabilizer and suspender used in pharmaceutical products. A 1% concentration was created by dispersing Carbopol powder in deionized water, then adding triethanolamine to neutralize the pH (18).

Induced Edema by Egg Albumin (Acute inflammation)

Twenty five male mice were randomly divided into five equal groups (five mice in each group) both types of the extract used for one mouse as following: Group (A) as a positive control application topical with pure gel only, while treated groups (B, C, D, and E) as following: treated based on the experimental protocol with some modification by topical application of different concentration of *L. camara* gel (0.01%, 0.03%, 0.05%, and 0.07%) on subplantar surface of the right hind-paw (Iraqi *L. camara*) and left hind-paw (America *L. camara*) of mouse prior the 30 min of acute inflammation induction (19). Acute inflammation method was performed through the injection fresh egg albumin (0.1 mL) that induced edema in mice model (20).

The measurements of paw edema were according to the volume displacement by use of verina caliper once prior the injection of egg albumin. The original paw volume (Vo) of right and left hind-paw of each mouse was calculated, and the post injection paw volume (Vt) was obtained after injection of egg albumin for each mouse at one hour (19).

Inhibition (%) = $\frac{(Vt - V0) \text{ control} - (Vt - V0) \text{ treated}}{(Vt - V0) \text{ control}} \times 10$

Skin Thickness and Cell Count

The tissue samples were preserved using a 10% formalin for a duration of two days. Afterward, they were processed for paraffin embedding procedure and cut into sections of 6μ m thickness using a rotary microtome. The tissue sections have been stained with hematoxylin and eosin (21).

The tissue samples were analyzed using light microscopy and microphotography to measure the thickness of the epidermis and the number of keratinocytes. The images were captured using a Future Win Joe microscopic camera and analyzed using the Fiji image analyzer system (22). The histometric measurements consisted of analyzing five microscopic fields from each slide within each study group.

Statistical Analysis

Data were subjected to analysis using SAS, 9.11 and means were compared using Least Significant Differences (LSD) test, whereas proportions were assisted using chi square and Data on epidermis thickness and keratinocyte counts were subjected to a one-way analysis of variance (ANOVA) using the Standard Least Squares procedure of JMP Pro 16.0.0 software (SAS Institute Inc., Cary NC, USA). Significant means were separated using LSD at $P \le 0.05$ (23).

RESULTS

Characteristic Features

After flowering of the plant that last from the late spring to the early fall, the plant after seeds growth was

characterized by the following characteristics: (Table1) (Figure 1).

Table 1. Common characteristic features of Lantana camara

Feature	Iraqi <i>L. camara</i>	American L. camara
Height	Low growing plant with (3-6) feet	a spreading shrubby plant (2-3 feet).
Flower colors	Multi-colored with pink, and yellow	Multi-colored with red, orange, and yellow
Leaves	Dark green leaves and ovate with large teeth on the edge.	Pale green and ovate with a flat base and small teeth on the edge.



Figure 1. Common characteristic features of (A) Iraqi and (B) American Lantana camara

Phytochemical Screening

The results of the phytochemical screening, as shown in Table 2, indicate a significant concentration of alkaloids, phenolics, and flavonoids and moderate concentration of tannin, saponin, terpenoids, anthraquinones, Cardiac glycosides and absence of coumarin and steroid.

Table 2. Qualitative phytochemical screening of leaves extracts of *L. camara*

Phytochemical	Iraqi <i>L. camara</i>	American L. camara		
Alkaloid (Dragondorff's test)	-	-		
Alkaloid Mayer's test	+++	+++		
Phenolic	+++	++		
Flavonoids	++	+++		
Tannin	++	++		
Saponin	++	++		
Anthraquinones	+	+		
Cardiac glycosides	+	+		
Terpenoids	+	+		
Coumarins	-	-		
Steroids	-	-		

+++: Strongly positive, ++: Moderately positive, +: Weakly positive, - Negative

GC-MS

The GC-MS analysis of *L. camara* was performed and bioactive compounds were identified. The results of GC-MS

analysis of Iraq cultivated show octadecanoic acid up to 26.58% followed by oleic acid, n-Hexadecanoic acid, Hexadecanoic acid, octadecanoic acid. while the result of

American *L. camara* show oleic acid up to 36.69% followed by n-Hexadecanoic acid, octadecenoic acid, methyl ester, Hexadecanoic acid methyl ester (Table 3).

Table 3. Comparison between phytochemical of Iraqi and American Lantana camara extract

	Iraqi L. camara			American L. camara		
Compounds	Peak No.	Rt (min)	Area %	Peak No.	Rt (min)	Area %
Oleic acid	11	22.834	22.56	13	22.834	36.69
Octadecanoic acid	10	22.327	26.58	11	22.316	13.49
n-Hexadecanoic acid	8	20.687	14.52	10	20.687	7.06
Hexadecanoic acid, methyl ester	7	20.169	7.79	9	20.169	10.65
9,12-Octadecadienoic acid	9	22.219	2.06	15	23.384	3.98
Diphenyl sulfide	5	14.807	1.27	7	15.649	2.11
4-Fluorodiphenylmethane	3	15.282	5.59	_	_	-
(R)-(-)-14-Methyl-8-hexadecyn-1-ol	13	23.395	2.47	_	_	-
Ethyl formate	-	_	_	2	4.375	2.60
Oxirane, (ethoxymethyl)	_	_	-	1	4.126	2.39
Benzene, 1-fluoro-3-(phenylmethyl)	4	15.541	2.03	_	_	-
Carbazole, 1,6-dimethyl-	1	4.266	1.90	_	_	-
Tetradecanoic acid	6	18.087	1.62	_	_	-
1,3-Cyclopentanedione	-	_		4	9.122	2.33
Phenol, 2,4-bis(1,1-dimethylethyl)	-	_		6	14.376	2.25
13-Octadecenal, (Z)-	14	23.967	1.24	_	_	-
26,26-Dimethyl-5,23-ergostadien-3. betaol	15	28.476	1.21	_	_	_
D-Mannitol, 2,4-di-O-methyl-, tetraacetate	-	_	_	8	16.307	2.08
Ethanamine, N-(ethoxymethyl)-N-ethyl	-	_	_	3	8.873	2.02
D-erythro-Pentose, 2-deoxy-	_	-	_	5	13.664	1.82

Peak No.= peak number, Rt=retention time

HPLC

HPLC analysis revealed that the Iraqi *L. camara* extract contained approximately 1.11 ppm of vitamin K, while the American variant had about 1.14 ppm. These

concentrations were estimated based on the assumption of a linear relationship between the chromatogram peak areas and the vitamin K concentrations, a common and generally accepted approach in chromatographic analyses of this nature.

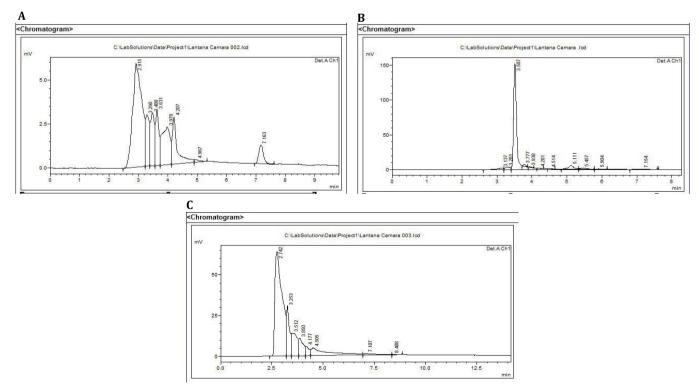


Figure 2. HPLC analysis results, (A) Standard vitamin k, (B) Iraq L. camara, (C), America L. camara (The x axis represents retention time, and on the y axis represents absorbance units)

The HPLC analysis results showed the presence and abundance of vitamin K in both Iraqi and American extracts (9.527 % and 9.770%, respectively) by comparing with the standard vitamin K (85.578%) as shown in Figure 2A, B, C and Table 4. These results have not been revealed by any previous research's publication, while another study indicate the presence of other vitamins such as A, C, E (24).

In addition, the position of the peaks of vitamin K in the standard, Iraqi *L. camara* and America *L. camara* was the same at position number 3, with a retention time 3.507 min, 3.480 min and 3.512 min, respectively, as shown in Table 4.

Table 4. HPLC analysis

Sample	Peak No.	Rt (min)	Area %
Standard	3	3.507	85.578
Iraqi <i>L. camara</i>	3	3.480	9.5270
American L. camara	3	3.512	9.770

Anti-inflammatory Effect of L. camara extract

The evaluation of the anti-inflammatory effect of *L. camara* was conducted using the egg albumin prompted paw oedema in male mice. The obtained results exhibited statistical significance when compared to the positive control. A statistically significant decrease (P<0.05) in paw edema generated by egg albumin was seen in the treated groups (Table 5). The maximum thickness of the paw was observed one hour after subplantar injection in the positive control group. The *L. camara* treated mice prevented paw edema induced by egg albumin to varying degree according to concentration which measured after one hour. The effect was significant (P<0.05) when compared between different concentrations, while 0.05% and 0.07% no different significant between them. Figure 3.

Table 5. Effect of different concentration of Lcamara on mean increase paw edema induced by egg albumin in mice after one hour

	Concentration of <i>L. camara</i>					
Treatment	0.01%	0.03%	0.05%	0.07%		
Positive Control		4.58±0.09 A				
Iraqi L. camara	2.11±0.07 ^{Ba}	1.14±0.07 ^{Bb}	0.01±0.07 ^{BC}	0.01±0.18 ^{BC}		
American L. camara	2.08±0.06 ^{Ba}	1.12±0.07 ^{Bb}	0.01±0.03 ^{Bc}	0.01±0.15 ^{BC}		
LSD	0.204					

Values are means±SEM, n= 5. Means with the different capital letters in same column and small letters in same row differed significantly, (P≤0.05)



Figure 3. Anti-inflammatory effect of *L. camara* at paw oedema induced by egg albumin in mice after one hour. A: Positive control, B: treated with 0.01%, C: treated with 0.03%, D: treated with 0.05%

Table 6. Effect of L. camara extracts on the percent of inhibition in paw oedema induced by egg albumin in mice after one hour

		Concentration of L. camara			
Treatment	0.01%	0.03%	0.05%	0.07%	Chi- Square
Positive Control		4.58			
Iraqi <i>L. camara</i>	53.9	75.7	99.7	99.7	9.42
America <i>L. camara</i>	54.4	75.7	99.7	99.7	6.58
Chi-Square	7.08	9.14	8.12	10.2	

Measurement of Skin Cells and Thickness

The America lantana extract revealed a higher reduction in skin thickness and the number of keratinocytes at 10 days of treatment compare with Iraqi extract that compare with inflammatory induce group. In addition, retinoid showed lower incidence of reduction in thickness and the keratinocyte numbers in compared to both America and Iraqi *L. camara* (Table 7).

Table 7. Thickness of epidermis and numbers of keratinocytes of mice

 induced inflamation and treated with L. camara and Retinoid

Groups	Day	Thickness (µm)	Keratinocytes count
Control Negative		30.5 ± 10.8 ^b	7.50 ± 4.70 ^b
Control Positive		94.1 ± 4.26 ª	28.2 ± 0.48 ^a
Iraqi <i>L. camara</i>	5	26.1 ± 0.95 bc	7.00 ± 1.55 bc
	10	17.8 ± 0.73 bc	1.40 ± 0.40 °
American L. camara	5	24.1 ± 0.83 bc	4.40 ± 0.25 bc
	10	16.6 ± 0.26 °	1.20 ± 0.37 °
Retinoid	5	28.4 ± 0.88 bc	6.00 ± 0.55 bc
	10	$19.2 \pm 0.57 \ ^{\rm bc}$	3.40 ± 0.25 bc
P-value		< 0.001	< 0.001

DISCUSSION

Lantanas exhibit an extensive variety of species, each with distinct genetic compositions, flower colors, seed properties, leaf structures, heights, and other physical attributes, hence resulting in a multitude of morphological variances. In the realm of genetics, it is observed that different Lantana camara species exhibit diverse gene sequences and expression patterns, which might potentially influence their growth, development, and response to environmental stimuli (25). Differences in the quantity of components between Iraqi and American Lantana camara species might arise due to several variables, such as genetic variation and developmental stage. Hence, the study observed variations in the types and quantities of secondary metabolites acquired, contingent upon the genetic composition of the species (26). The chemical composition of Lantana species can also be influenced by environmental influences. Quantitative assessment is employed to evaluate the impact of environmental factors on plant metabolic processes and phytochemical substances. Various factors exert an influence on the rate of growth, development, and allocation of assimilates towards crucial metabolites. Various factors, including temperature and moisture, have been recognized as significant in modifying both the quality and quantitative production of phytochemicals in plants (27). The process of phytochemical screening was employed to enable the identification of the principal chemical constituents present within the extract. In contrast, phenolic compounds represent a significant and extensively prevalent category of secondary metabolites found in various plant species (28). An additional study has demonstrated the impact of Phenolic chemicals, namely flavonoids, tannins, cardiac glycosides, and saponins, which

have been documented to possess anti-inflammatory properties (29). The composition of *L. camara* can be influenced by various factors, including but not limited to the kind of soil, the environment, and the growth conditions. Furthermore, the chemical compositions of *L. camara* show differences in both quantity and quality across different organs during the plant's life cycle. The observed variances may be ascribed to ecological factors, such as the plant's age, life cycle stage, harvest timing, or genetic variability among plants in the same geographic area (30).

These results are consistent with a study that found oleic acid and stearic acid in L. camara plants, among other places (31). Oleic acid, a monounsaturated fatty acid from the omega-9 family, is widely recognized for its potential to enhance cardiovascular well-being, mitigate inflammatory processes, and promote weight reduction. Olive oil, avocados, and nuts are frequently utilized as primary sources of this substance. Multiple investigations have revealed the presence of oleic acid in L. camara plants across diverse geographical locations (32). Octadecanoic acid, often known as stearic acid, is classified as a saturated fatty acid and can be found in a diverse range of dietary lipids derived from both plant and animal sources. It is common for soaps and cosmetics to be manufactured utilizing it. Based on extant research, stearic acid has been found to potentially confer some health benefits, including but not limited to reducing inflammation, enhancing insulin sensitivity, and reducing cholesterol levels. Octadecanoic acid, akin to oleic acid, has been identified in L. camara specimens originating from several geographical regions (33). Fatty acids have a vital role in *L. camara*, serving as essential components for energy storage, membrane formation and functionality, signaling processes, as well as defense mechanisms against herbivores and pathogens. This study suggests that *L. camara* produces several fatty acids, such as oleic acid, octadecanoic acid, n-hexadecanoic acid, 9,12-octadecanoic acid, and tetradecanoic acid, which could potentially have biological applications. Research has provided evidence for the anti-inflammatory and anticancer characteristics of these fatty acids, along with their potential to improve skin health (34). The HPLC analysis indicated a disparity in the concentration of vitamin K between L. camara specimens from Iraq and America. One potential factor contributing to changes in vitamin K content is genetic variability. Variations in genetic characteristics may lead to variances in the ability of L. camara species to manufacture vitamin K. Various strains within a given species may possess genetic differences that might influence their capacity to efficiently produce vitamin (35). The findings of the study indicated that the extracts derived from L. camara leaves had inhibitory effects on inflammation during the early phase. This suggests that the extract may potentially inhibit the release of mediators associated with the early phase of

inflammation. Histamine, bradykinin, serotonin, and prostaglandins are endogenous inflammatory mediators that are generated by injured cells, contributing to the processes of inflammation and pain. Prostaglandins are synthesized by the metabolic conversion of arachidonic acid utilizing the cyclooxygenase pathway (36). L. camara contains several bioactive compounds such as oleic acid. Octadecenoic acid, methyl ester and n-Hexadecanoic acid and others as shown in Table 3. The America L. camara revealed higher concentration of oleic acid than Iraqi L. camara which may contribute to the higher reduction effect on number of keratinocytes and thickness. These compounds possessed anti-inflammatory properties. These properties were due to the plant's ability to reduce the number of keratinocytes in mice skin with psoriasis, which have been described in our earlier publication (40). The counting of the cells and thickness measurement was conducted and revealed that higher oleic acid content in Lantana camara has been shown to prevent the creation of pro-inflammatory cytokines such as TNF-alpha and interleukin 6, which have a role in the pathogenesis of inflammation. By reducing the levels of these cytokines, Lantana camara may help to decrease the proliferation of keratinocytes in the skin (37). Historically, various herbs and medicinal plants were used for customary treatments, health maintenance, disease management, and potential pharmaceutical advancement due to their medicinal properties (38). Plants function as a significant store of various essential medicinal chemicals. Specifically, plants that demonstrate a significant ability to produce secondary metabolites (39). In Conclusion, the comparative study of morphological and phytochemical analysis of Iraq and America L. camara provided valuable information about the varieties and similarity of the phytochemicals of the plant between the two species and its potential antiinflammatory and anti-proliferation effects.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

EDITORIAL TRANSPARENCY

Orooba MS Ibrahim serves as a member of the editorial board for The Iraqi Journal of Veterinary Medicine. Despite this role, the peer review process and the final publication decision were made independently and impartially, ensuring no influence from the author's editorial position.

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تقييم مقارن للتحليل الكيميائي النباتي والمور فولوجي والتأثير المضاد للالتهابات لانتانا كامارا على النموذج الحيواني للفئران

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