Phytochemical and Phylogenetic Study of *Jatropha integerrema* Jacq. Cultivated in Iraq

Liqaa A. Jazaa , **Altifat F. Altaai *** , **Hadeel M. Habeeb**

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

*Correspondence email: Altifat.f@csw.uobaghdad.edu.iq

Received: 22/10/2024
Accepted: 17/6/2025
Online: 17/5/2026

ABSTRACT

Background: *Jatropha integerrema* Jacq. is cultivated in Iraq and recorded as an exotic genus. The leaves and flower extracts have medicinal properties for treating conditions such as HIV, ringworm, tumors, ophthalmia, malaria, bronchitis, and asthma. **Objective:** Quantitative analysis of Jatropha leaf and flower extract, estimation of total phenolics and terpenoids, and molecular analysis. **Methodology:** This research conducted a quantitative comparison of bioactive chemical constituents between leaf and flower extracts, and a phylogenetic analysis of two plant organs of *J. integerrema Jacq.*, leaves and flower petals, using nrDNA (ITS) and two plastid genes (*matK* and *trnT-trnL*). **Results:** Phenols, terpenoids, alkaloids, and steroids in different concentrations were present in leaves and flowers. The phenolic compounds had the highest values, 498.8 and 305.98 mg/g, whereas the steroids had the lowest concentrations, 2.00 and 1.25 in leaf and flower extract, respectively. The plant exhibited higher free radical scavenging activity compared with ascorbic acid. The antioxidant activity of leaves was higher than that of flower extracts in scavenging the free radicals (85.33% for leaves and 75.13% for flowers at 200 µg/mL). **Conclusions**: This study estimated that the various chemical concentrations were derived from the leaves and flowers of J. integerrema, which contains many phytochemical components with beneficial properties. So it has significant potential as a novel source of medication. The nrDNA and plastid genes are effective tools for comparing plant organs and improving agronomic traits related to plastid genes.

Keywords: *J. integerrema* Jacq., phylogenetic analysis, antioxidant activity, ITS, *matK*, *trnT-trnL*.

https://doi.org/10.24126/jobrc.2026.20.1.897

INTRODUCTION

Euphorbiaceae exhibit a diversified growth form (trees, shrubs, and herbs) with economically important species that provide food, drugs, rubber, and oil (1). *Jatropha integerrema Jacq*. was initially identified as an exotic genus in Iraq, and has been grown for ornamental purposes in gardens. It has been successful in growing and flowering because it has adapted to its surroundings (2). The plant is an evergreen shrub with shiny leaves and clusters of bright red or vermilion blooms shaped like stars, found throughout tropical regions (3). The *Jatropha spp*. plant has long been used in folk remedies to treat eczema, pruritus, and skin warts in India and Bangladesh (4). The flowers are edible with strong antioxidant activity among 51 wild species in China. The leaves and flower extracts have medicinal properties for treating conditions such as HIV, ringworm, tumors, ophthalmia, malaria, bronchitis, asthma, and as an aphrodisiac. This plant is grown in several African and Southeast Asian nations, including Zimbabwe, China, India, Mauritania, and the Philippines, and is significant for the manufacture of biodiesel. (5, 6, and 7). It has also been used

to treat stomachaches, toothaches, swelling, inflammation, leprosy, diarrhea, dyscrasia, vertigo, anemia, and diabetes (8). Numerous bioactive substances, including terpenes, tannins, alkaloids, flavonoids, and saponins, are present in the plant (2). Nowadays, it is very important to use genetic markers to evaluate phylogenetic relationships among different *Jatropha sp. germplasm* to determine the most desired traits (seed oil production) in this genus, as well as vegetative and floral growth, and improve it due to some species having low yield of seeds as compared with others (9,10). Despite their short size (± 700 bp), the internal transcribed spacers (ITS) of nuclear ribosomal DNA are often used as target genes in nuclear genomes for phylogenetic studies of distinct plant groupings. There are several copies of this gene in plant genomes. (11,12). The use of plastid genes, such as *matK* and *trnL* genes, as barcodes for plant DNA was proposed by the Consortium for the Barcode of Life (CBOL) (13). Although the *matK* gene (*Maturase K*) is a rapidly evolving plastid gene with high resolution, making it useful for comparing plant species, the gene can occasionally be challenging to amplify (14,12). The chloroplast *trnL* (UAA) intron primer is highly conserved but has low resolution, with a very robust amplification system (15,16) and may exhibit a series limitation at the intraspecific level, with a low evolutionary ratio for this molecule (17). The current study was conducted to investigate the phytochemical and phylogenetic analysis of two organs of *J. integerrema Jacq.* cultivated in Iraq, the leaves and flowers.

METHODOLOGY

Plant Collection and Extraction

The plant leaves and flowers were collected from the University of Baghdad/ College of Science for Women, then washed. The dried leaves and the fresh flowers are used. The leaf and flower were ground and macerated with 75% methanol at a (1:10) ratio (plant: solvent) for 7 days at 25°C in the dark, then filtered and dried (18).

Quantitative Analysis of The Leaves and Flowers Extract

Total phenolic estimation

The total phenolic compounds present in the alcoholic extract were marked using a standard Folin-Ciocalteu reagent. One milliliter of the extract, five milliliters of the Folin-Ciocalteu reagent (Merck, Germany), and one milliliter of 20% sodium carbonate made up the reaction mixture. The sample was mixed in a vortex mixer and then diluted with distilled water to a final volume of 10 mL. Following a 2-hour reaction, the absorbance at 765 nm was measured, and the phenolic content was estimated from the calibration curve created using gallic acid (Sigma-Aldrich, Germany). The total amount of phenolic compounds was expressed in gallic acid equivalent (GAE) mg/g (19).

Total alkaloids estimation

Using Dragendroff's approach, the presence of alkaloids was determined. After dissolving 1mL of the extract in diluted HCL and adding 3 drops of Dragon Drops, the crystalline precipitate indicated the presence of alkaloids. The positive alkaloid sample was subjected to quantitative evaluation (20).

Alkaloid Separation

The amount of alkaloids was determined in accordance with (21). The alkaloids were separated from the extracts (leaves and flowers). Then, the alkaloids were dissolved in 2N HCl, and the mixture was placed in a funnel and washed with 10 mL of chloroform three times. The mixture's pH was then adjusted to neutral with 0.1 N NaOH, and 5 mL of phosphate buffer (pH 7), 5 mL of Bromocresol Green (BCG) solution were added.

Standard curve

The Atropine alkaloids standard curve, as shown in Figure 1, was prepared by adding (0.4, 0.6, 0.8, 1, and 1.2 mL) of 1 mg/10 g Atropine solution to the extracts. The extracts are then shaken for a minute with 1, 2, 3, or 4 mL of chloroform until the final solution volume reaches 10 mL. The absorbance is then measured at 470 nm and compared with that of the solution prepared from the above solution without Atropine, mg/g, as a blank.

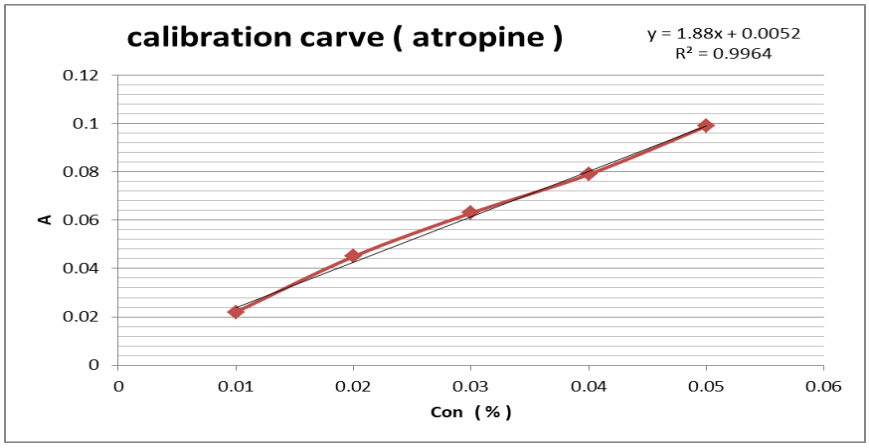

Figure 1: Atropine alkaloids standard curve

Total estimation of steroids

The samples were reacted with ferric chloride in H_2SO_4 to ascertain the total amount of steroids. Proportionate amounts of steroids are then determined based on the development of red color. In order to set up the reaction, ferric chloride and cholesterol reagent were prepared. First, 840 mg of ferric chloride was dissolved in 100 mL of glacial acetic acid Figure 2. Then, 10 mL of this solution was added to 100 mL of glacial acetic acid, and 8.5 mL of the solution was diluted into another 100 mL of glacial acetic acid. Next, a standard working solution at a concentration of 100µg/mL was prepared. Finally, 10 mL of cholesterol solution was combined with 0.85 mL of ferric chloride stock reagent, bringing the volume to 100 mL of glacial acetic acid. This was established as a standard working solution at a concentration of 100µg/mL. After applying a triple-acidic extract to the samples, approximately 0.1 and 0.2 milliliters were extracted. 0.5 to 2.5 milliliters of a standard solution (10 milliliters of cholesterol, 0.85 milliliters of ferric chloride stock reagent /100 glacial acetic acid) were added to each tube, and the volume was increased to 5 milliliters using the final ferric chloride reagent. The blank was created by combining 5 milliliters of diluted ferric chloride with 4 milliliters of concentrated H_2SO_{**} in each tube. The tubes were then incubated for 30 minutes, allowing the color intensity to develop and the absorbance to be measured at 540 nm (22).

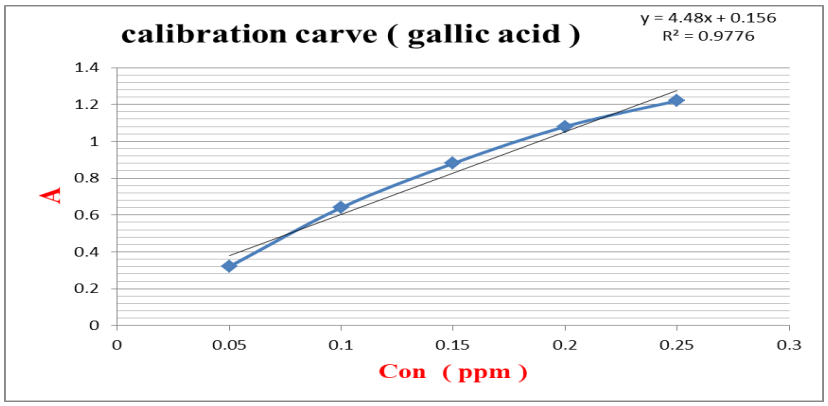

Figure 2: Glacial acetic acid standard curve

Total estimation of terpenoids

The amount of terpenoids was determined using the method of (23) . The procedure was as follows: 1.5 g of each extract was dissolved in 7mL of methanol and acetonitrile, shaken for 30 minutes, and then incubated for 24 hours at room temperature in the dark. The mixture was then centrifuged for 15 minutes at 6000 rpm/min to yield 5mL of supernatant, after which 1.5mL of chloroform and 0.5mL of concentrated H_2SO_4 were added. After one minute of mixing the solution and adding methanol to reach a final volume of 10 mL, various concentrations of linalool were employed as standards, as shown in Figure 3. The absorbance at 538 nm was measured, and the proportion of terpenoids was determined (23).

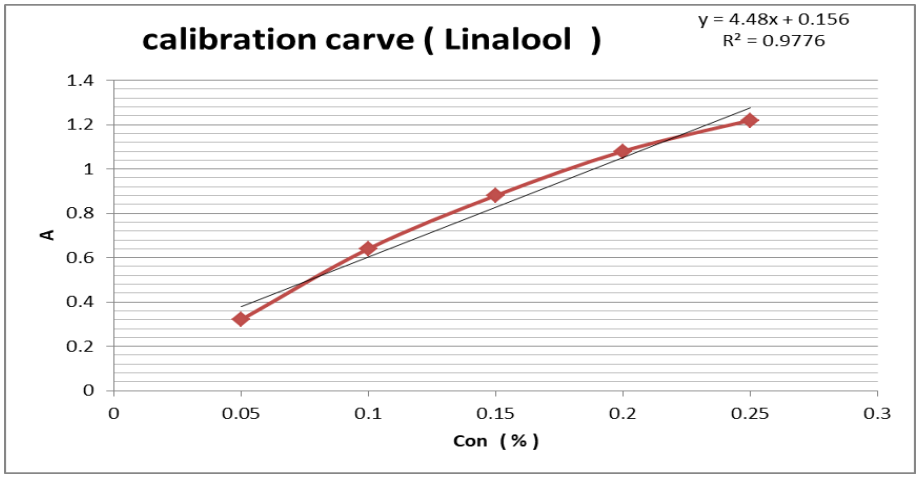

Figure 3: Linalool standard curve

Antioxidant activity

According to (24). By scavenging 1,1'-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant potential of leaves and flowers was investigated. A stock solution of 2 mL was prepared by combining 2 mL of leaf or flower extract with 100 mM DPPH in methanol. As a standard, ascorbic acid was employed. The mixture was mixed and then incubated for 20 minutes at 35 °C in the dark. After that, the procedure was repeated three times, and the absorbance was recoded at 512 nm. The IC50, or the amount of sample needed to scavenge half of the DPPH radicals, based on the following equation, the ability of DPPH scavenging was determined:

% DPPH radical scavenging activity = [1-(B sample/B control) x 100]

B control = absorbencies of control

B sample = absorbencies of the sample

Molecular analysis

The present study is based on fresh young leaves and flowers of *J. integerrema Jacq.* for molecular analysis. DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) procedure with some modifications. (25) using the Genomic DNA Mini Kit (plant) of Geneaid. The PCR amplification was performed for ITS, *matK* and *trnT-trnL* (26). Primers were supplied by Bioneer Company.Amplifications were conducted using AccuPower PCR PreMix (Bioneer) in a MyGenie96/384 Thermal Block (Bioneer) . The ITS region was amplified using the following thermal cycling program: 95 ◦C for 2 min, 34 cycles of 95 ◦C for 45 s, 58 ◦C for 45 s, and 72 ◦C for 90 s, with a final extension at 72 ◦C for 5 min. The *matK* gene was subjected to the following thermal cycling program: 95 ◦C for 3 min, 40 cycles of 94 ◦C for 30 s, 49 ◦C for 1 min, and 72 ◦C for 1 min, and a final extension at 72 ◦C for 10 min, that for the trnT-trnL region was as follows: 95 ◦C for 5 min, 15 cycles of 95 ◦C for 45 s and 60 ◦C for 1 min, with an extension at 72 ◦C for 2 min, subsequently 20 cycles of 45 s at 95 °C and 1 minute at 54 °C, and a final extension of 2 minutes at 72 °C. The three primers used in this study are elucidated in Table 1.

Table (1): Primers for PCR amplification F, forward; R, reverse .

Gene	Primer F/R	Primer sequence (5' -3')	References
ITS	F / KRC	**5'**- GCACGCGCGCTACACTGA- **3'**	**27**
	R/ AB102	**5'** -TAGAATTCCCCGGTTCGCTCGCCGTTAC- **3'**	**28**
MatK	F / K1R	**5'** -ACCCAGTCCATCTGGAAATCTTGGTTC- **3'**	**29**
	R/ K3F	**5'**- CGTACAGTACTTTTGTGTTTACGAG- **3'**	**29**
trnT-trnL	F / trn-b	**5'**- TCTACCGATTTCGCCATATC- **3'**	**30**
	R/ trn-a	**5'** - CATTACAAATGCGATGCTCT- **3'**	**30**

In order to verify the PCR results, the amplified fragments were separated by gel electrophoresis on 2% (w/v) agarose . After dissolving 2 grams of agarose in 100 mL of 0.5X TBE buffer (pH=8), the mixture was heated while stirring to melt the agarose. The agarose was left to cool at 60°C, and then ethidium bromide was added at 10 mg/mL (5 µl) of DNA ladder 100bp (Bioneer), and then visualized under UV light after ethidium bromide (2µl) staining to elucidate the amplified fragment of the primers under consideration.

Statistical Analysis

The Statistical Analysis System (31) was used to determine how variation in circumstances affected the study's parameters. The least significant difference (LSD, at $P \leq 0.05$) test (Analysis of Variance, ANOVA) was used to compare the study's means.

RESULTS

Quantitative analysis of leaf and flower extract of J. integerrema Jacq

Results shown in Table 2 comprise the amounts of several bioactive compounds, determined by spectrophotometric methods, which are simple techniques for quantifying total phenols, terpenoids, alkaloids, and steroids in the methanolic extracts of the leaves and flowers. The results indicate that phenolic compounds have the highest value, 498.8 and 305.98 mg/g in leaves and flowers, respectively. Whereas the steroids had the lowest concentrations in the leaves and flower extract (2 and 1.25, respectively). All compounds were higher in the leaves than in the flowers,

except the terpenoid which was higher in the flowers (8.15 units) than in the leaves (4.58 units). Flavonoids, tannins, alkaloids, saponins, terpenes, and sterols were detected in *J. integerrema* leaf methanolic extract, with the same results reported by (18).

Table (2): The total active compounds (unit) in leaves and flowers of J. integerrema Jacq.

No	The constituent	Leaves	Flower
1	Total phenolic content (mg / 100 g)	498.8	305.98
2	Total terpenoid content	4.58	8.15
3	Total alkaloid content	5.11	3.58
4	Total steroid content	2.00	1.25

Antioxidant activity of Jatropha leaves and flower extract

Antioxidant ability of the *J. integerrema Jacq* leaves and flower extracts was accentuated by the DPPH assay, and the results are shown in Table 3. According to the results of this study, both extracts have the capacity to scavenge free radicals. It was revealed that the leaf and flower extracts showed high values compared with ascorbic acid. The antioxidant activity of the leaves was higher than that of the flowers at all concentrations, except at a concentration of 12.5µ g/mL, which was higher in the flowers than in the leaves. The highest scavenging was 85.33 and 75.13 % at 200 µg/mL, while the lowest values were (30.96, and 34.01) % at 12.5 µg/mL for leaf and flower, respectively, which differed significantly between them. The antioxidant capacity increased with the increasing concentration of leaf and flower extracts. Interestingly, the greater quantity of phytochemicals found in the leaf and flower extracts obtained may be the cause of this result.

Table(3): The percentage (%) of antioxidant activity in leaves and flowers of *J. integerrema Jacq* .

Concentration of sample µg/ml	Mean ±SE		
	Ascorbic Acid µg/ml	Leaves µg/ml	Flowers µg/ml
12.5	27.16 ±1.06 B c	30.96 ±1.45 B d	34.01 ±1.51 A d
25	28.97 ±1.27 C c	52.20 ±2.09 A c	40.16 ±2.14 B d
50	40.63 ±2.52 B b	57.91 ±2.57 A c	56.94 ±2.64 A c
100	46.10 ±2.61 B b	65.20 ±3.07 A b	65.90 ±2.91 A b
200	54.78 ±2.86 C a	85.33 ±4.19 A a	75.13 ±3.02 B a
LSD value	6.33	7.05	6.53
Means having with the different capital letters in same row and small letters in same column differed significantly at level (P≤0.05).			

Molecular Analysis

In this study, a comparison between two plant organs, *J. integerrema Jacq* leaves and flower petals, via the detection of the presence of nrDNA ITS and two plastid genes *matK* and *trnT-trnL* was conducted. As shown in Figure 4, amplification of DNA with ITS, matK, and trnT-trnL primers resulted in different band patterns, indicating distinct presence between nuclear and plastid genes. The nrDNA ITS was present in both leaves and flowers with highly productive, very clear bands of 1200 bp. while *matK* and *trnT-trnL* genes were amplified and existed in leaves only ,not in flowers. Both regions were characterized by lighter visible bands about 500 bp. The presence of ITS barcodes in both organs is expected, as this region is well conserved and is the most consistently recommended nrDNA region (32).

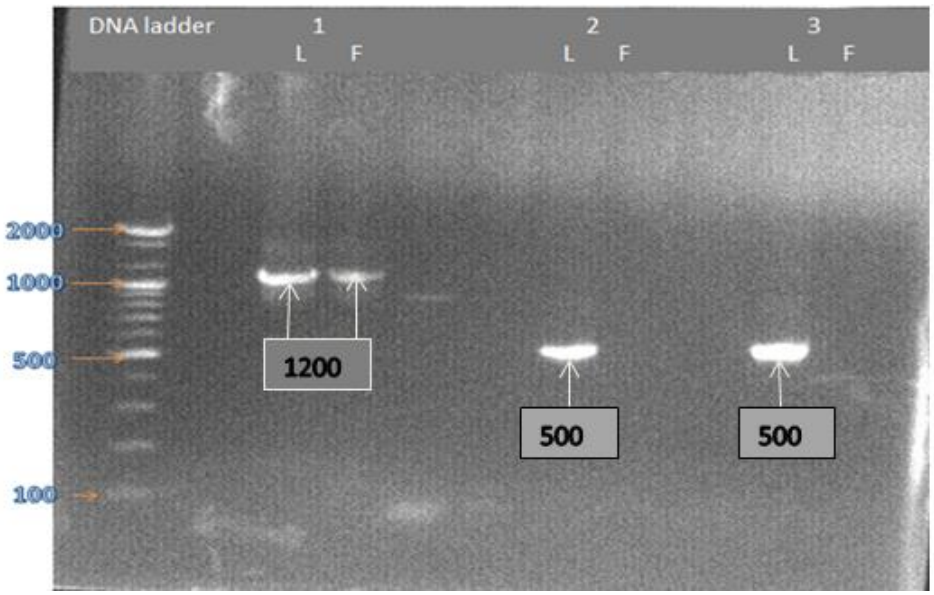

Figure 4: Agarose (2%) gel electrophoresis of PCR products run at 135 v/cm² for 45 minutes , 1=ITS, 2= matK, 3= trnT-trnL , L= leaves, and F= flowers. DNA ladder (100bp.) ,

DISSCUSION

In previous studies on the antioxidant ability of *J. integerima Jacq* leaves and flower extracts, strong relationships were found between antioxidant potential and the active components of the extracts, suggesting that polyphenolic compounds contributed to the antioxidant activity (33). These results were consistent with the current study's findings, the contents containing high phenolic, terpenoid, alkaloids, and steroids exhibited higher antioxidant activity. The highest antioxidant capacity suggests that *J. integerrima* leaves and flowers may be a valuable source of free radicals. *J. integerrima* contains a variety of natural metabolites, including phenols, steroids, and alkaloids, which can act as reducing agents and have positive biological effects. The Phylogenetic comparison between two plant organs, *J. integerrema* Jacq , and the existence and function of matk and trnT-trnL with the chloroplast genome have been clarified by numerous studies . The matK protein serves as a group II intron maturase in the chloroplast and is still a crucial gene in the evolutionary reconstruction of plants. (34,35) as well as the transfer RNAs (trns) genes that are mainly distributed and expressed through the chloroplast genome, to consider the trnT-trnL as chloroplast-specific universal primers (35,36). (37) Related plastid developmental patterns and abundance in plant organs with gene expression and their relative contributions of mRNA levels, plastid interconversion with relation to gene-specific molecular network may affect the availability of plastid genes between the petals and leaves (38).

CONCLUSION

This study estimated that the various chemical concentrations were derived from the leaves and flowers of J. integerrema, which contains many phytochemical components with beneficial properties. So it has significant potential as a novel source of medication. The nrDNA and plastid genes are effective tools for comparing plant organs and improving agronomic traits related to plastid genes.

ACKNOWLEDGMENT

The authors gratefully acknowledge the staff of the Department of Biology, College of Science for Women, University of Baghdad, for their technical and general support.

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دراسة كيميائية ووراثية لنبات الجاتروفا المزروع في العراق

لقاء علي جازع ، التفات فاضل الطائي ، هديل مكي حبيب

قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، العراق

الخلاصة

خلفية البحث: نبات الجاتروفا *Jatropha integerrema* Jacq المزروع في العراق حيث سجل كنوع دخيل على البيئة العراقية، مستخلصات الأوراق والزهور لها خصائص طبية لعلاج حالات مختلفة مثل فيروس نقص المناعة البشرية، والسعفة، والأورام، والتهاب العين، والملاريا، والتهاب الشعب الهوائية، والربو. **الهدف من البحث:** التحليل الكمي لمستخلص اوراق وأزهار نبات الجاتروفا ، تقدير الفينولات والتربينويدات الكلية واجرء التحليل الجزيئي. **المواد وطرائق البحث:** اجري في هذا البحث مقارنة كمية وتحليلية للمركبات الفعالة المتواجدة في مستخلص اوراق وازهار النبات و تحليل وراثي بأستعمال الDNA النووي بمنطقة ITS والحامض النووي في البلاستيدات بمنطقتي الجينات *matK* and *trnT-trnL* من خلال الترحيل الكهربائي . **النتائج :** اذ اظهرت النتائج وجود كل من الفينولات والتربينات و القلويدات و الستيرويدات وبتراكيز مختلفة بين الاوراق والازهار. كان اعلى تركيز للمركبات الفعالة هو الفينولات 498.8 و 305.98 واقل تركيز للستيرويدات هو 2.00 و 1.25 في الاوراق والازهار على التوالي. كما تميز النبات باحتوائه على نسبة عالية من المركبات الكاسحة للجذور الحرة مقارنة بحامض الاسكوربك (فيتامين C)، **الاستنتاجات:** وبينت النتائج ان تأثير الاوراق المضاد للاكسدة كان اعلى من تأثير مستخلص الازهار، اذ بلغت اعلى نسبة لكسح الجذور الحرة 85.33 للاوراق و 75.13للازهار بتركيز 200 µg/mL. أن نتائج التحليل الوراثي والمقارنة بين الأوراق والأزهار أظهرت أختلاف في تواجد المناطق الوراثية ونسبها حيث كانت الحزم لمنطقة ITS واضحة في الأوراق والأزهار، بينما تواجدت حزم المناطق الجينية للمادة الوراثية للبلاستيدات *matK* و *trnT-trnL* فقط في الأوراق ولم تظهر في الأزهار.

الكلمات المفتاحية: نبات الجاتروفا، تحليل وراثي، مضادات الاكسده ITS, *matK, trnT- trnL* .