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Chemical Composition and Ursolic Acid Quantification in Plumeria Rubra along the Syrian Coast

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Abstract: Plumeria rubra is an ornamental plant cultivated in several countries, including Syria. This plant is also used in traditional Indian medicine to treat diarrhea, asthma, bronchitis, and cough. This study aims to investigate the active components in the flower oil and quantify ursolic acid in different parts of P. rubra found along the Syrian coast. Samples were collected from various parts of P. rubra plants along the Syrian coast. Flower oil was extracted and analyzed using gas chromatography-mass spectrometry (GC-MS). Ursolic acid was detected and quantified in the methanolic extracts of different plant parts using high-performance thin-layer chromatography (HPTLC). Then, free radical scavenging was determined in the leaves' methanolic extract. The results revealed the presence of 30 compounds in the flower essential oil, with the highest percentage belonging to Nerolidalol at 14.11%, followed by Linalol at 13.86% and Geraniol at 10.24%. The study showed the presence of ursolic acid in leaves (0.029%), but the flowers and stem bark were not detected. In interaction with diphenyl picryl hydroxyl, the inhibitory concentrations of half of free radicals were 300µg/mL. These findings contribute to understanding the chemical characteristics of P. rubra and potential medical uses.

Keywords: Plumeria Rubra; Flower Oil; Ursolic Acid; Gas Chromatography-Mass Spectrometry (GC-MS); High-Performance Thin-Layer Chromatography (HPTLC); Chemical Composition; Ursolic Acid Quantification; Bronchitis and Cough.

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1. Introduction

Plumeria rubra (Apocynaceae) is found in tropical and subtropical regions and naturally grows in South America and India. The plant requires high temperature, humidity, and well-drained, slightly acidic soil for optimal growth [1]. In India, Plumeria species are widely used to treat diseases such as diarrhea, asthma, bronchitis, cough, and tumors [2]. *P. rubra* is a spreading shrub with a sturdy stem that branches into cylindrical, smooth branches with a 2-3 cm diameter. The leaves are concentrated at the branch tips and are characterized by their shiny dark green color, smooth edges, or pointed tips. The leaves are cylindrical and alternate, measuring approximately 15-30 * 5-10 cm, with sharp apexes and a smooth upper surface covered with a thick cuticle. The lower surface is somewhat covered with hairs. The main leaf vein is thick and prominent, with several lateral veins branching from it [1]-[3].

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The flowers are gathered in terminal cymes and have a peduncle length of 12.5 cm. They are white with a yellow center, circular with a diameter of 5 cm. The calyx is small with short lobes, while the corolla is funnel-shaped and has five petals. The fruit is pod-shaped, measuring 12 cm in length, and contains winged seeds. The flowering season occurs between May and September [1].

The major compounds of P. rubra are volatile oil [3] and iridoid glucosides, with one of the important compounds being Plumieride and Flavanoinds [4]. The plant also contains penta-cyclic triterpenes, including Ursolic and Oleanolic acids [5]. Studies have demonstrated various physiological effects of Plumeria species, such as anti-inflammatory activity, antipyretic and analgesic activity, anti-microbial activity, anticancer and antitumor activity, and antiviral activity [6]-[14].

Ursolic acid is considered a promising compound for developing multi-target bioactive agents. With relatively low toxicity, these compounds have multiple effects, including wound healing, anti-inflammatory, antibacterial, antiviral, hepatoprotective, and antitumor properties [15].

High-performance thin-layer chromatography (HPTLC) is a modern separation technique and an advanced version of TLC. It involves precise sample application and provides standardized and reproducible chromatograms. The instrument is connected to a program that analyzes and evaluates the results [16]-[18].

The cultivation of P. rubra as an ornamental plant has spread to the coastal areas of Syria, where the coastal climate is ideal for its growth. Despite the importance of the volatile oil extracted from its flowers in the cosmetics and creams industry, it has not been fully utilized in Syria. Therefore, the research aims to extract the volatile oil from the flowers of P. rubra grown along the Syrian coast and study its chemical composition. As well as to the quantification of ursolic acid, which is a promising compound in the treatment of various diseases, will be investigated in different parts of the plant to determine the 2,2-diphenyl-1picryl hydroxyl (DPPH) scavenging activity.

2. Materials and Methods Materials

Ursolic acid (>90%) was purchased from Sigma. HPTLC plates Silica gel 60 F254 and solvents (99% purity) were purchased from Merk. Di Phenyl Picryl Hydrazyl DPPH (Tokyo Chemical Industry). Butylated Hydroxy Toluene BHT (Titan Biotech).

2.1. Instruments

Gas Chromatography Instrument: GC-plus MS2010 (Shimadzu) Sensitive electronic balance of Shimadzu AX200 (Japan). High-Performance Thin Layer Chromatography (HPTLC) instrument of CAMAG.

2.2. Methods

2.2.1. Plant Collection and Extraction

Flowers, leaves, and stems bark of *P. rubra* were collected from the Sheikh Badr area, Tartous Governorate, between July and October. The plant was identified and classified by Imad Al-Qadi, Professor of Plant Taxonomy at Damascus University, based on the Flora of Pakistan [1]. The samples were dried immediately after collection by air drying at normal room temperature, away from sunlight.

2.3. Extraction

The Essential oil was extracted from 500 grams of plant material using steam distillation. Plant extracts were prepared using the ultrasound device according to the study by En et al., [19] The flowers, leaves, and stems bark samples were ground to 40 meshes. Two grams of flower and stem bark samples were extracted with 40 ml of 90% methanol at 40°C for 10 minutes. The leaf sample was extracted three times with 40 ml of petroleum ether solution at 40°C, followed by 40 ml of 90% methanol at 40°C for 10 minutes in each extraction process. The extracts were dried using a rotary evaporator.

3. Analytical Methods

3.1. Gas Chromatography Analysis

The volatile oil components were analyzed using a gas chromatography instrument with a mass spectrometer detector (GC-MS), GCMS-QP2010 plus (SHIMADZU, Kyoto, Japan). Column: OPTIMA 5 (100% dimethylpolysiloxane USP G1, G2, G38) with a film thickness of 0.25 µm, a column length of 25 m, and a diameter of 0.25 mm. Carrier gas is Helium at 54 kilopascals

(K.pa) pressure. Split ratio: 1:30. Injector: Automatic, injector temperature set at 250°C, injection mode is split, and the injected sample volume is 1 µl. Thermal program: Oven temperature from 240 to 80°C with a three °C/minute rate. Total program time: 62 minutes. Mass spectrometer: Ion source temperature of 200°C, ionization voltage of 70 eV. Identification of volatile oil components was performed using the Wiley electronic library.

High-performance thin Layer Chromatography (HPTLC) was used for qualitative and quantitative detection of Ursolic acid in different parts of the plant (flowers, leaves, stem bark). The spotting device was an automatic CAMAG device, allowing precise sample application. The syringe used for injection was a Hamilton syringe with a maximum capacity of 100 µL. The TLC chamber, used for chromatographic separation, was also made of CAMAG. The scanner device was connected to the CATS4 software, facilitating data analysis.

Mobile phase: The mobile phase consisted of a mixture of hexane, ethylacetate, and methanol (v/v/v 0.5:1.8:8.2). The mixture was allowed to equilibrate in the developing tank for 45 minutes to achieve a saturated atmosphere. Detection wavelength: Spectrophotometric detection was performed at 540 nm [20].

The stock solution was prepared by dissolving 2 mg of Ursolic acid in a 10 mL volumetric flask and diluted with methanol to the calibration mark. Then, a solution with a concentration of 40 µg was prepared.

The extracts were dissolved in 10 mL of methanol. The extracts were then filtered using a 0.45 µm filter.

The HPTLC plate was placed in a glass chamber containing a 1% iodine solution in chloroform. After development, the plate was kept dark for 10 minutes for a complete reaction. Subsequently, the remaining iodine was removed by drying the aluminum-coated region with hot air.

3.2. Calibration of Ursolic acid

3 µL of the extracts from the three plant parts (leaves, stem bark, and flowers) were applied on the TLC plate (35, 30, 25, 20, 15, and 10 µL). The distance between each point was 6 mm. A calibration curve was constructed based on the applied concentrations and the corresponding absorption for each spot. After development, the HPTLC plate was sprayed with 1% Methanolinc sulphuric acid solution and heated in an oven at 110°C for 5 minutes. The absorption was measured at a wavelength of 540 nm using a tungsten lamp. A calibration curve was plotted for Ursolic acid based on the applied concentrations and the corresponding peak areas.

The scanner device measured the absorption for both sample spots and standard spots. A calibration curve was constructed, representing the relationship between the concentrations of Ursolic acid standards and their corresponding peak areas.

The resulting line equation was: $Y = 8.119x + 422.4$.

The free radical scavenging capacity of methanolic extract of leaves against DPPH was measured [21]. 3mL of each extract concentration was mixed with 1 ml of 01mM ethanolic solution containing DPPH radicals. The samples were mixed and then left in the dark for 30 min, and the absorbance was measured at 518 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The calibration curve was produced within BHT's 10-100 µg/ml concentration range [29].

Results were calculated by preparing a series of concentrations (100 - 200- 300- 500- 700- 900 µg/mL) from each extract and showed as IC_{50} (the inhibitory concentration of half of free radicals) [29]. The percentage of scavenging activity was calculated using the following equation:

DPPH scavenging activity

(%) = (Acontrol – Asample) / Acontrol × 100.

Where:
Acontrol: The absorbance of control
(Ethanol + DPPH)
Asample: The absorbance of sample
(Extract + DPPH).

4. Results

The study focused on investigating the chemical composition of Plumeria rubra and quantifying the presence of ursolic acid in different parts of the plant along the Syrian coast.

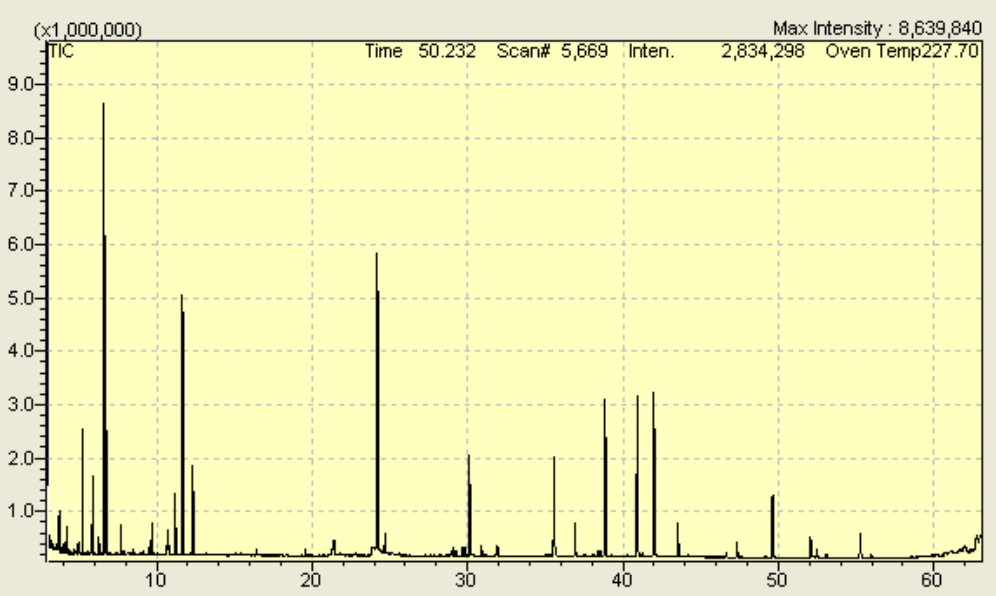

Figure 1: GC-MS chromatogram of the essential oil *P. rubra* flowers

Gas chromatography-mass spectrometry (GC-MS) analysis Figure 1 of the flower essential oil revealed the presence of 30 compounds Table 1. Notably, the highest percentage was attributed to the compound Nerolidalol, accounting for 14.11% of the essential oil. Linalool followed this at 13.86% and Geraniol at 10.24%.

Table 1: components of the essential oil compounds of P. rubra flowers

Peak no.	Compound	Peak area %	Retention time	Peak no.	Compound	Peak area %	Retention time
1	Furaneol	0.82	3.067	16	Nerolidalol	14.11	24.2
2	Benzaldehyde	0.65	3.694	17	Trans farmizole	0.7	24.585
3	1،3،5، trimethyl	0.69	3.784	18	Nonadecane	0.41	29.689
4	Decane	0.74	4.249	19	Farnesol	4.32	30.127
5	Phenylacetaldehyde	3.1	4.249	20	Trans-Trans-Farnesol	0.42	30.904
6	O-tolualdehyde	2.02	5.244	21	Benzyl-Salcylate	4.77	35.565
7	Trans Linalol oxide	0.41	5.876	22	Nonadecane	1.37	36.924
8	Linalol-L	13.86	6.625	23	Linalyl Benzoate	7.32	38.853
9	Nonanal	3.61	6.755	24	Geranyl Linalol isomer	7.56	40.919
10	3،8 Nonadien-2-on	0.98	7.718	25	Geranzyl Propionate	7.68	41.988
11	1-alpha-Terpineol	1.05	9.688	26	Heneicosane	1.44	43.534

12	Citronellol	0.83	10.453	27	Hexadecimal Palmatic Aldehyde	0.65	47.341
13	Neral	2.17	11.205	28	Hexacosane	2.72	49.615
14	Geraniol	10.24	11.665	29	Hexanedioic acid	0.92	52.064
15	Citral	3.19	12.33	30	Hexacosane	1.11	55.266

The standard of Ursolic acid exhibited a UV absorption spectrum. It appeared on the plate with a retention factor (Rf) 0.3. Upon measuring the absorption of the three samples, a spot with an Rf value of 0.3 was found in the leaf sample, exhibiting the same absorption spectrum as the Ursolic acid standard Figures 2 to 3.

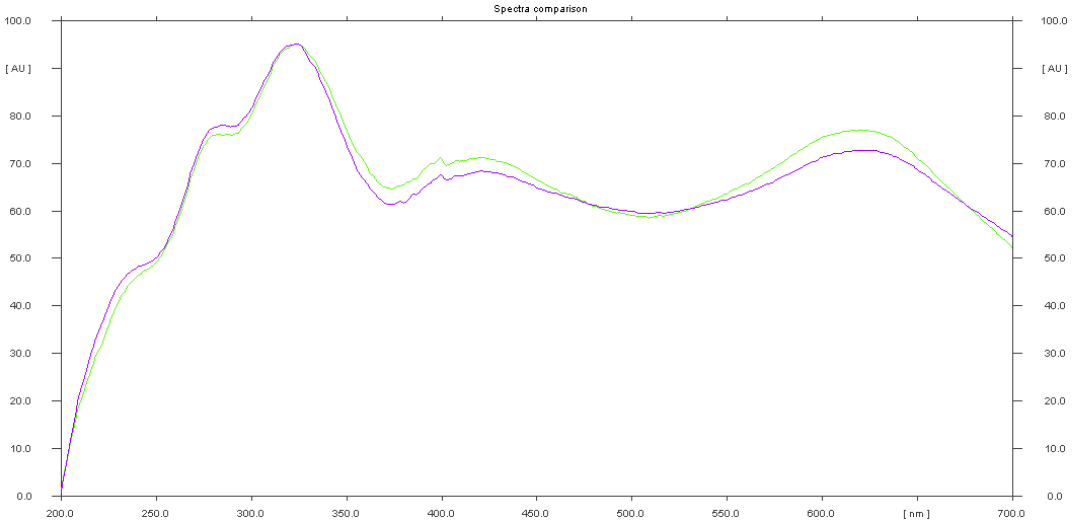

Figure 2: Spectral comparison of Ursolic acid between standard and leaves extract

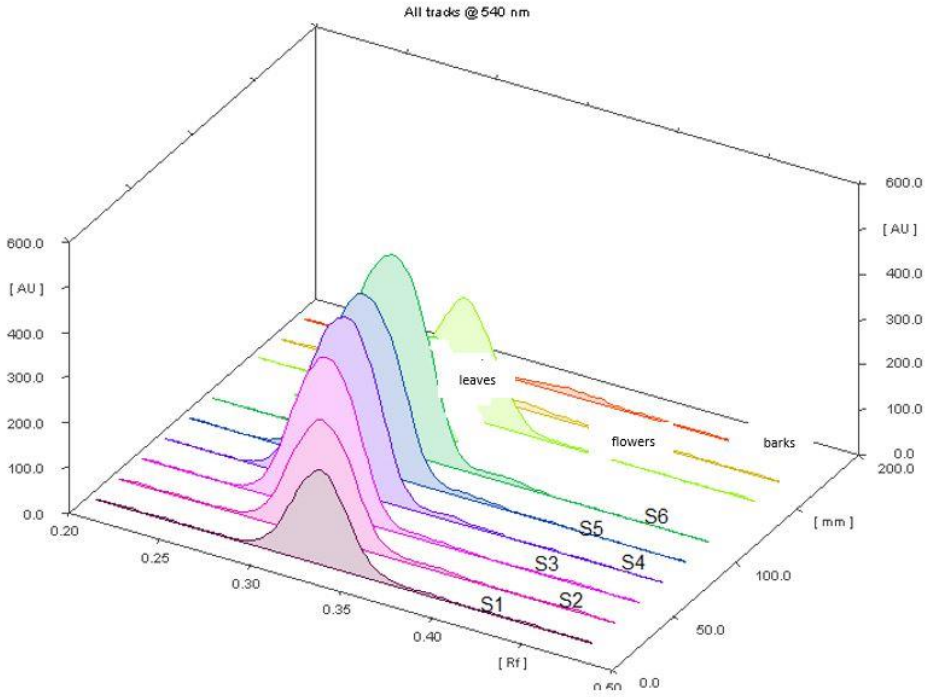

Figure 3: 3D spectra of all peaks at 540 nm

This indicates the presence of Ursolic acid in the leaf sample. However, the absorption spectrum of Ursolic acid was absent in the flower and stem bark samples, suggesting the absence of Ursolic acid in these parts.

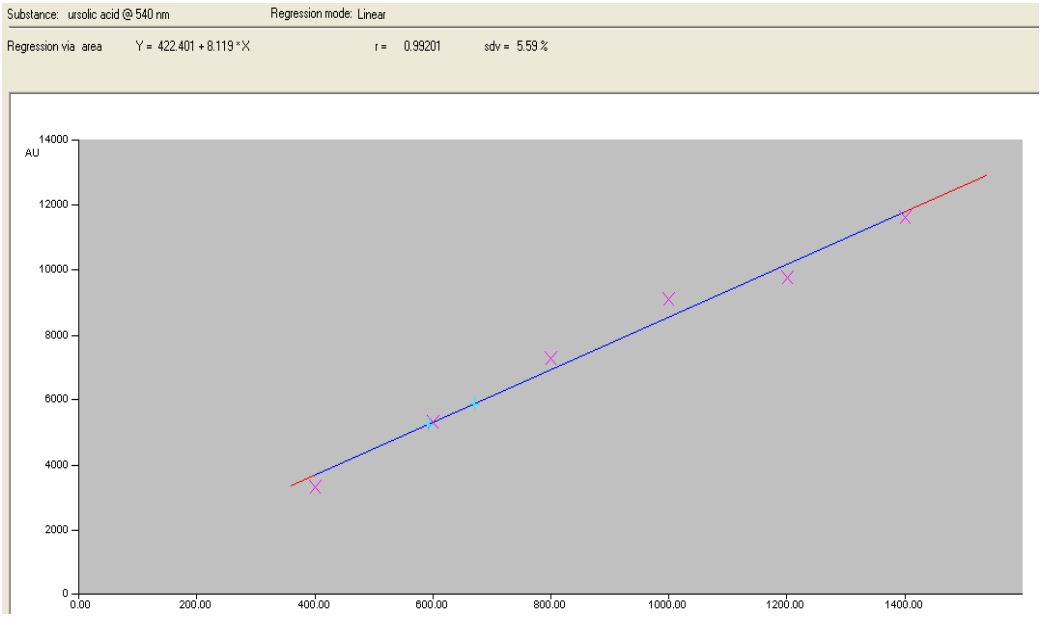

Figure 4: curve between the concentrations of Ursolic acid titers and the area of their absorption peaks.

From the calibration curve, the concentration of Ursolic acid in the spot of the leaf extract was calculated to be X = 595.97 ng in 3 µl of the leaf extract solution Figure 4 and Table 2. By calculation, the percentage of Ursolic acid in the leaves was 0.029%.

Table 2: concentrations of Ursolic acid in the standard series samples

Sample	Concentration (ng/spot)	Peak area
S1	400	3311.67
S2	600	5294.69
S3	800	7276.26
S4	1000	9096.37
S5	1200	9770.77
S6	1400	11629.07
Leaves extract spot	**X**	**5868.87**

These findings indicate that the flower oil of P. rubra has a complex composition, while ursolic acid is present in small amounts only in the leaves. These results enhance our understanding of the chemical characteristics of P. rubra and potential medical applications.

The methanolic leaves' inhibitory Concentration (IC50) was 300 µg/mL, while BHT had an IC_{50} value = 20 µg/mL.

5. Discussion

P. rubra is widely distributed in tropical and subtropical regions such as India, South America, and North America. It is used in traditional medicine for treating diseases such as diarrhea, asthma, bronchitis, cough, and tumors [2]. Consequently, numerous researchers have studied the chemical composition of different plant species and conducted various studies to determine the physiological effects of plant extracts [6]-[14]. Additionally, the essential oil of the plant's flowers is utilized to produce creams and cosmetic products.

The cultivation of P. rubra plants is prevalent in the coastal regions of Syria, where the coastal climate provides an ideal environment for its growth. This plant is characterized by its distinctive fragrant flowers, which have led to its cultivation as an ornamental plant.

Due to the widespread cultivation of the plant on the Syrian coast, its ease of propagation, and the abundance of global studies on the plant and its chemical compounds, this study focused on examining the cultivated plant on the Syrian coast. GC-MS analysis revealed three major components in the essential oil of the plant's flowers, namely Nerolidalol (14.11%), Linalol (13.8%), and Geraniol (10.24%). These results differed from the study conducted by Goswami et al. in India [22], where the major components of the plant's essential oil were Benzyl Salicylate (26.7%), Benzyl Benzoate (22.3%), Geraniol (trace amounts), Linalol (0.1%), and E. Nerolidalol (7%). On the other hand, the results of this study were in approximate agreement with a study conducted by Goswami et al. [22] on the cultivated P. rubra with orange flowers in Malaysia, where the percentages of Nerolidol and Geraniol in the essential oil were 14.4% and 4.1%, respectively. This difference in the composition of the essential oil of the flowers can be attributed to the influence of climate and soil on the components of the plant's essential oil, resulting in variations in fragrance and physiological effects.

Linalol is known for its soothing properties and is the main component of lavender essential oil. It is used in various compounds that aid insomnia patients in sleeping [23]. Nerolidol possesses anti-microbial, antifungal, and antiparasitic properties and calming effects [24]-[26].

On the other hand, Geraniol has antioxidant and anticancer properties and anti-microbial and antifungal effects. The concentration of ursolic acid in the leaves was 0.029%, while the stems and flowers of the plant did not contain any ursolic acid. In a study by Srivastava et al. [27] on the leaves and stems of P. rubra in India, the percentage of ursolic acid in the plant's leaves was 0.98%, and in the stems, it was 0.051%. The difference in the concentration of ursolic acid may be attributed to variations in the extraction methods used. This study obtained methanolic extracts using ultrasound waves, while Srivastava et al. [27] employed the Soxhlet extraction method.

The difference could also be due to variations in the surrounding environmental conditions, as ursolic acid is a secondary metabolite produced by the plant as a defensive mechanism against pathogens such as fungi and bacteria present in its surroundings.

Methanolic leaf extract had good free radical scavenging activity, and this may be due to the high content of antioxidants like Flavonoids glycoside and pentacyclic triterpenes, including Ursolic acid and Oleanolic acid. Free radicals scavenging activity results were higher than those calculated by Ruiz-Teran. et al. [28], in which the percentage of inhibition of free radicals was 2000µg/mL.

6. Conclusions

This study focused on exploring the chemical composition of P. rubra found along the Syrian coast and quantifying ursolic acid in different parts of the plant. The analysis revealed a complex composition of 30 compounds in the flower oil, with notable percentages of Nerolidalol, Linalol, and Geraniol. However, ursolic acid was only detected in the leaves, while it was absent in the flowers and stem bark. These findings contribute to our understanding of the chemical characteristics of P. rubra and potential medical uses. The research employed advanced techniques such as gas chromatography-mass spectrometry (GC-MS) to analyze the flower oil's composition and high-performance thin-layer chromatography (HPTLC) to quantify ursolic acid. HPTLC, in particular, proved to be a reliable method for detecting and evaluating the compound. The results emphasized the presence of ursolic acid in small amounts, exclusively in the leaves. Although further investigations are necessary to determine the exact medicinal potential of ursolic acid in P. rubra, its multi-target bioactive properties, along with other triterpenoid compounds, suggest promising applications in wound healing, anti-inflammatory treatments, antibacterial and antiviral therapies, hepatoprotection, and antitumor activities. The cultivation of P. rubra along the Syrian coast benefits from the ideal coastal climate, yet the utilization of its flower oil remains underutilized in Syria's cosmetics and creams industry. The findings of this study shed light on the chemical profile of P. rubra P. rubra, providing valuable insights for potential applications in medicine and industry. Further research can explore the specific therapeutic properties of the identified compounds and optimize extraction methods to harness the full potential of this versatile plant.

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Ethics and Consent Statement: This research adheres to ethical guidelines, obtaining informed consent from all participants. Confidentiality measures were implemented to safeguard participant privacy.

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