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RESEARCH ARTICLE

Isolation, Formulation and Assessment of Anti-inflammatory Properties of Ursolic Acid from *Nerium oleander*

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Abstract

Purpose: The purpose of the research work was the extraction and isolation of Ursolic Acid (URA) from the leaves of *Nerium oleander* (*N. oleander*) and the assessment of its anti-inflammatory activity using an *in-vitro* model. **Methods:** Ursolic Acid (URA) is a bioactive molecule. It is a key component of *N. oleander*. The hydroalcoholic maceration method was used for extract preparation and was used to isolate the bioactive components of URA. The prepared extract, isolated URA were characterize and analyse by using pharmacognostic parameters, Fourier Transform Infrared (FTIR) and Thin Layer Chromatography (TLC) method. The carrageenan-induced inflammation rat paw oedema test in-vitro model was used for the assessment of anti-inflammatory properties of isolated bioactive compound URA. **Results:** The results of evaluation and characterization indicated that the extract's ash value and extractive values were within the parameters specified in the Indian Ayurvedic Pharmacopoeia. The prepared hydroalcohalic extract has potential bioactive components such as flavonoids, saponins, and triterpinoids. The isolated compound was URA. The extract may be able to alleviate inflammation, according to the percentage inhibition. **Conclusion:** The URA was successfully removed from the leaves of *N. oleander*. The examination criteria revealed that the extract contained certain small contaminants, which may have anti-inflammatory effects.

Keywords: Bioactive Substance, Inflammatory Activity, *Nerium oleander*, Ursolic Acid

1. Introduction

Nerium oleander is most common plant which is found most region of India. It belongs to Apocynaceae family. It includes the evergreen shrub or small tree species. It is known as oleander because of its obvious resemblance to the unrelated plant olive olea. However, it is also known as *N. oleander* Mill and is having variant with white and crimson flowers[1]. *Nerium oleander* is native to the Indian and Pakistani subcontinent, but also grows in the Mediterranean region and subtropical Asia. The range extends across the Himalayas from Nepal west to Kashmir at altitudes up to 1950 metres and extends into Baluchistan, Afghanistan, and is found in gardens throughout India. *N. oleander* is compared to the variety with white and crimson flowers. The leaves, roots and root bark are used to treat a variety of diseases. Charka recommended topical application of the leaves of the white-flowered variety for leprosy and other persistent, severe skin diseases. Sushruta applied *Karavira* in a therapeutic paste to treat alopecia. To treat epilepsy, leaf powder was snorted. The roots were considered extremely deadly when taken internally. The flower tincture had cardiotonic, CNS-active root, and spasmolytic effects. Externally, the root showed healing abilities for ulcers and haemorrhoids. Treatment of leprosy with oil from the root bark was successful. In homoeopathy, under strict medical supervision, a tincture of the leaves of *Nerium oleander* (red laurel)

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is used for diseases such as hemiplegia and paralysis. The characteristics of URA, its pharmacokinetics, and its therapeutic uses for the treatment of a variety of illnesses, including cancer, cardiovascular disease, anti-diabetic, immunomodulatory, hepatoprotective, and anti-microbial capabilities, were all summarized[2,3].

Many fruits and vegetables contain URA, a naturally occurring triterpene molecule. Interest is growing in URA's positive effects, which include anti-inflammatory, antioxidant, anti-apoptotic, and anti-carcinogenic qualities. It affects a variety of tissues and organs in the following ways: it suppresses nuclear factor kappa B signalling in cancer cells, boosts insulin signalling in adipose tissue, reduces cardiac damage indicators in the heart, reduces inflammation, and raises adiponectin levels. It inhibits atrophy, oxidative stress, and apoptotic signals in the liver, and enhances the expression of adenosine monophosphate-activated protein kinase and irisin in skeletal muscle. Additionally, URA is a different type of treatment and preventative approach for sarcopenia, cancer, diabetes, obesity, and heart, brain, and liver illnesses[3].

The various derivatives of URA have been isolated and names of URA derivates are 1,8-dihydroxy-3,7-dimethoxyxanthone, 1,5,8-trihydroxyxanthone. The other bioactive chemicals have been found which had significant antioxidant activity in the plant's aerial portions[4,5]. As a result, we decided to use *N. oleander* leaves in our current research as a potential source of URA. The purpose of the research work was the extraction and isolation of Ursolic Acid (URA) from the leaves of *Nerium oleander* (*N. oleander*) and the assessment of its anti-inflammatory activity using an in-vitro model. The URA's chemical structure is shown in Figure 1 below.

2. Materials and Methods

2.1 Materials

Nerium oleander leaves were gathered from the university's medicinal garden in Ayodhya, Uttar Pradesh, India, and verified by Dr. Awadesh Kumar Shukla, an assistant professor in the Department of Botany at K. S. Saket P. G. University Ayodhya. The Institutional Animal Ethics Committee examined and approved the experimental protocol before the animals were gathered per CPCSEA, New Delhi, guidelines, and they were then treated with the utmost respect.

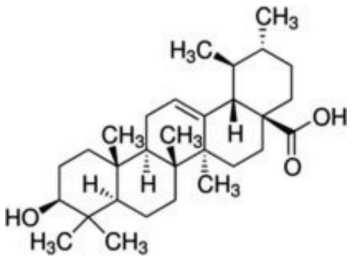

Figure 1. The structure of Ursolic Acid.

Animal testing was done at Bhupal Nobles' University in Udaipur, Rajasthan, India[6-8].

2.2 Methodology

2.2.1 Physicochemical Analysis

Physicochemical analysis of the crude drug was performed by using official methods such as total ash, acid insoluble ash, water soluble ash and determination of extractive values[9-12].

2.2.2 Extraction and isolation of URA from Nerium oleander Leaves

For this reason, the leaves of the plant *N. oleander* were collected, washed, dried and pulverized. The coarse powder of *N. oleander* leaves dipped in hydroalcoholic solvent for 15 days. The extract changed into concentrated to dryness using a rotary evaporator. The focused extract was freed from fatty fabric by the use of hexane, the extract turned into dissolved in hexane and the hexane soluble fabric turned into discarded, and the insoluble phase filtered and dried. The insoluble liquid phase becomes dissolved in ethanol at a temperature of 40°C and allowed to face for 24 hours. The induced cloth became recrystallized and purified with ethanol. The isolated natural bioactive compound was stored for in addition studies[13] (Figure 2).

2.2.3 Phytochemical Screening

Phytochemical screening was performed by using official methods[12,13].

2.2.4 pH Test

pH was determined by using digital pH meter. The test sample was taken and dissolved in 10 cm^3 of distilled water. Further, upon inserting calibrated electrodes of virtual pH meters and analyzing them, the pH price was determined three times.

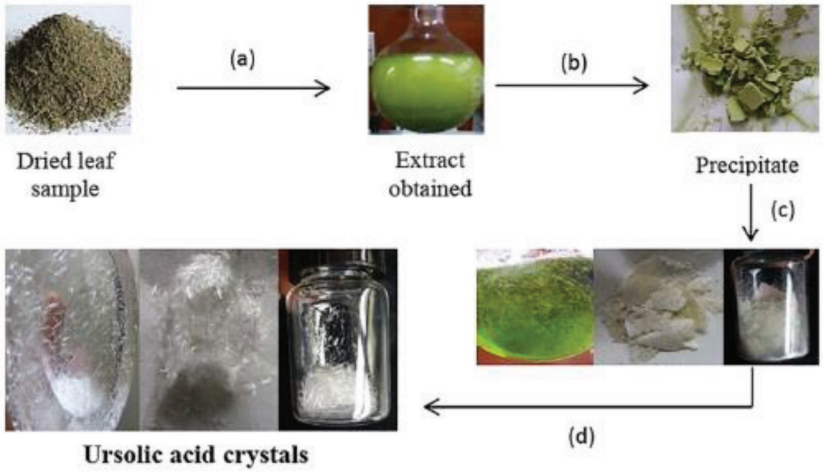

Figure 2. The process of isolation URA from plant material.

2.2.5 Analysis by Thin Layer Chromatography (TLC)

A standard solution, a developing solution gadget, and a TLC test answer were prepared. Methanol and water (7:3) had been mixed with *N. oleander* extract to put them together. After heating it in a water bath for five minutes, the solution was cooled and filtered. Five grammes of popular URA had been dissolved in 1 millilitre of a methanol-water method to put together the standard answer (7:3). Toluene, acetone and formic acid (7.8: 2.2:0. zero.15, v/v/v) are the growing solvent device. TLC plates have been prepared with the usage of a silica gel answer and the Retention Factor (Rf.) calculated. The plates have been tested with UV light at 254 nm while they have been kept in a developing solvent gadget[14] (Figure 3).

2.2.6 Melting Point Range

The capillary method was used to determine the melting factor of the remote compound through the plant extract. The capillary tube was changed into packed with the drug and sealed at one stop. The virtual melting factor tool recorded the reading in 3 times. The common readings of melting factor were cited.

2.2.7 FTIR Spectroscopy

The FTIR evaluation was performed by using Agilent Cary 630 FTIR spectrometer. The isolated bioactive compound was taken and scanned. Spectrum peaks of the FTIR were noted and interpreted. These spectrum peaks were used for the characterization of the isolated bioactive compound[15].

2.2.8 Formulation

In the melting method, all the composition of formulation of simple ointment was taken in a melting crucible and heated to 70°C. After melting, the aggregate become gently mixed at a temperature of 70°C for about 5 minutes after which cooled to 40°C with consistent stirring. The ointments were then swirled to achieve a smooth consistency, saved at room temperature (25°C), and used to prepare URA ointment. To put together an ointment method containing 2% Ursolic Acid, 10g of ointment base was added and the mixture became triturated to a hundred g using a spatula. Primarily based on the assessment standards, the quality ointment system was decided on. Table 1 indicates the composition of the ointment base[16].

2.2.9 In-vivo Anti-Inflammatory Activity

2.2.9.1 Animals

Albino Wistar rats (180-two hundred g body weight) had been used for the existing examination. The

Table 1. Composition of simple ointment base

Name of Ingredients	Quantity in Percentage (%)
White petrolatum	94
White bees wax	5
Wool fat	5
Hard paraffin	5
Cetostearyl alcohol	5

animals have been kept in normal surroundings and fed with a conventional pellet weight-reduction plan. The animals have been given seven days to acclimate to the laboratory environment before the test. They have not been given any food earlier than the 18-hour test. Afterwards, they were taken to a test. The animals had been properly cared for as consistent with the requirements of CPCSEA New Delhi. The approval of experimental protocol was acquired via the Institutional Animal Ethics Committee.

2.2.10 Carrageenan-induced Rat Paw Edema Method

Three businesses of albino Wistar rats (each containing six animals; n = 6) had been formed: the positive manage institution, the standard remedy organization, and the take a look at the organization. The same old institution, along with apigenin ointment and diclofenac sodium gel 1.0%, become in comparison with the wonderful manipulates institution (check group). Carrageenan (0.1 ml, 1% w/v in ordinary saline) was injected into the subplantar tissue of the right hind paw of each animal in each organization to induce oedema. A digital screw gauze is used to degree linear paw circumference. Measurements of paw circumference were taken each before and 4 hours after the oedema changed into delivered. The manage group acquired no therapy in any respect. The subplantar tissue of the animal's right hind paw turned into dealt with with each the usual and takes a look at formulations with the aid of gently rubbing it 50 times with the index finger[17]. The following formula was used to determine the % value of edema inhibition:

$$\% \text{ inhibition} = 1\text{- } (y\text{-}x \,/\, b\text{-}a) \times 100$$

Wherein x represents the initial paw thickness of the test group animal, y represents the paw thickness following treatment; b represents the paw thickness following remedy, and represents the beginning paw thickness of the manage group animal[18,19].

3. Results

3.1 Physicochemical Screening

The results of physicochemical analysis parameters such as total ash value 4.91%, acid insoluble 1.98%, water-soluble extractive value 2.23%, and alcohol soluble extractive value 2.07%, respectively, were obtained for the additives that were insoluble in acid, soluble in water, and soluble in alcohol. This demonstrates that more additives are soluble in water than in alcohol (Table 2).

3.2 Phytochemical Screening Test

A phytochemical screening test for test sample was performed and was found to be *N. oleander* hydroalcoholic extract containing triterpenoids, saponins, and flavonoids, the results are shown in Table 3.

After drying for 6 hours, 6.5% of the fabric was lost (Table 4). In triplicate, the extract's pH became decided, and the common value turned into 5.717±10 (Table 5). Primarily based on parameters such as pH, melting

Table 2. Results of physicochemical analysis

S. N.	Tests	Observations (%)	Standard % (API)
1	Total ash	4.91	Not more than 10
2	Acid-insoluble ash	1.98	Not more than 2.5
3	Water soluble extractive value	2.23	Not more than 20
4	Alcohol soluble extractive value	2.07	Not more than 10

Table 3. Results of phytochemical screening test

S. N.	Tests	Observations
1	Fehling test	+
2	Test for Flavonoids	+
3	Test for Saponins Foam Test	+
4	Test for terpinoids	+

Note: A plus sign (+) denotes the presence of a compound.

Table 4. Loss on drying observations at different time intervals

S. N.	Time (h)	Weight
0	0	72.82mg
1	1	72.82mg
2	2	72.82mg
3	3	72.82mg
4	4	72.82mg
5	5	72.82mg
6	6	72.82mg

Table 5. pH values of extract

S.N.	pH	Mean ± S.D
1	5.71	5.717±10
2	5.70	
3	5.70	

Figure 3. TLC of URA shown in visible and ultra 254nm violet light.

point, and drying loss, the extract was assessed. The temperature at which the substance melted turned into discovered to be 240 °C, that is identical to the 238 °C claimed melting factor of UA.

3.3 TLC Profile of Test Sample

Using TLC analysis, the Rf value was obtained by dividing the solute path length by the solvent path length. The measured Rf value was 0.53 cm. The TLC plate was seen under UV fluorescent light and showed a purple colour. URA can be used with the mobile phase toluene: acetone: formic acid (7.8: 2.2: 0.15, v/v/v) to successfully separate, similar to the standard product[1].

3.4 FTIR Study of Isolated Compound

FTIR spectra interpretation of isolated compounds are shown in Table 6.

4. Discussion

Based on the physicochemical evaluation effects, there exists a huge disparity between the entire ash and acid-insoluble ash, suggesting the presence of an extraordinary awareness of acid-soluble inorganic radicals like calcium oxalate within the ash. The phytochemical analysis indicated the presence of triterpenoids, flavonoids, and saponins in the extract. The extract tested a lower level of infection compared to the usual remedy, albeit still showing a big diploma of inflammatory activity as decided to employ *in-vivo* anti-inflammatory evaluation, with an inhibition charge of 28.71%. Each experimental organization consisted of six animal subjects, and the consequences had been presented the ointment containing Ursolic Acid exhibited inhibition of inflammation, with the maximum observed inhibition rate reaching 61.17%. Compared to the control group, a significant reduction in paw oedema inflammation was observed three hours after carrageenan injection. The bioactive compound Ursolic Acid demonstrates potential antibacterial properties and plays a key role in conferring anti-inflammatory effects. The potential anti-inflammatory impact is attributed to the likely inhibition of cyclooxygenase, lipoxygenase, and nitric oxide synthase activities, as well as the presence of antioxidants. Additionally, the gel formulation has shown potential efficacy in treating skin conditions such

Table 6. FTIR peaks of isolated compound URA

A peak at 3559 cm^{-1} corresponds to the stretching vibration of the O-H bond present in an alcohol functional group.
At 3483 cm-1, a peak is observed, signifying the stretching vibration of the O-H bond.
A peak at 3324 cm^{-1} represents the stretching vibration of the O-H bonds within an aromatic compound.
At 3339 cm^{-1}, a peak is observed, indicating the O-H stretching in a carboxylic acid.
The peak observed at 2957 cm^{-1} corresponds to the C-H stretching vibration in the CH_3 and CH_2 groups of an aromatic compound.
At 1749 cm^{-1}, a peak is observed, which can be attributed to the stretching vibration of the C=O bond in carboxyl groups.
A peak at 1591 cm^{-1} represents the stretching vibration of the C=C bond.
At 1350 cm^{-1}, a peak corresponds to the C-H deformation in a gem dimethyl group.
A peak at 1432 cm^{-1} represents the C-H bending in the alkane methyl group of an aromatic compound.
Within the spectrum, a medium intensity peak at 1344 cm^{-1} indicates the O-H bending in an alcohol.
In the range of 1210-1344 cm^{-1}, a strong peak suggests the C-O stretching in the aromatic ester of an aromatic compound.
At 990 cm-1, a peak is observed, corresponding to the C=C bending in an alkene.
Peaks at 1052 cm^{-1} and 990 cm-1 are observed, which can be attributed to the C-O bond and the C=C-H group, respectively

Table 7. Results of carrageenan-induced paw edema volume in rats

Treatment	Paw volume (ml)a (Percentage inhibition of edema)			
	1h	2h	3h	4h
Control	1.20±0.01	1.21±0.17	1.22±0.02	1.08±0.01
Declofanac Sodium gel 1.0%	0.71±0.06** (42.05)	0.53±0.01** (56.16)	0.37±0.02** (70.14)	0.27±0.07** (75.02)
Ursolic acid-F6 Formulation	0.92±0.03** (28.71)	0.76±0.02** (39.62)	0.52±0.02** (61.17)	0.41±0.01** (49.68)

as boils, abscesses, burns, and eczema. Consequently, the bioactive constituents, particularly Ursolic Acid, are suitable for topical application.

5. Conclusion

URA was isolated from the leaves of *N. oleander*, which was then studied for its physiochemical and phytochemical properties and extraction value. According to the results of the evaluation parameters, the isolated URA contains a lower-than-acceptable number of impurities. Using *in-vitro* analysis, URA was also tested for its anti-inflammatory properties, and the results showed that it is capable of inhibiting inflammatory action.

6. References

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