ejpmr, 2023, 10(8), 250-254

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EXTRACTION, ISOLATION, SYNTHESIS, PURIFICATION, CHARACTERIZATION OF LAWSONE AND FORMULATION AND EVALUATION OF LAWSONE HAIR DYE GEL

Jain A., Fulmali S.* and Rajput Tanya

Professor of Pharmaceutical Science, Shri D.D. Vispute College of Pharmacy & Research Center, New Panvel, Navi Mumbai 400049.

***Corresponding Author: Fulmali S.**

Professor of Pharmaceutical Science, Shri D.D. Vispute College of Pharmacy & Research Center, New Panvel, Navi Mumbai 400049.

Article Received on 08/06/2023 **Article Revised on 29/06/2023** **Article Accepted on 19/07/2023**

ABSTRACT

Here we state that the extraction, isolation of lawsone and synthesis, purification, characterisation of lawsone was carried out successfully. Lawsone hair dye gel is the hair colorant gel prepared and evaluated. Lawsone was extracted by methods like soxhlet extraction, maceration method and tommasi method and the practical yield was obtained up to 2-3 g. Lawsone was synthesize and identified of lawsone by using thin layer chromatography with butanol: ethyl acetate: acetic acid (7.5:2.5:0.1) was used as the mobile phase, Rf value was found to be 0.7. And further identification of lawsone by using UV-Visible spectroscopy, colorimetry and FTIR and LC-MS which gives the correct identification and detection molecular mass peak of lawsone at 174.10 was observed. Moreover preparation and evaluation of Lawsone hair dye gel for the parameter like colour, consistency, pH, viscosity, appearance, spreadability, washability, and skin irritation were studied and the results were found to be acceceptable limits.

KEYWORD: Extraction, Isolation. Characterisation, FTIR and LC-MS.

INTRODUCTION

Henna has been used cosmetically and medicinally for over 9,000 years. It provides promotive, preventive, curative and rehabilitative healthcare. The fundamentals, diagnosis and treatment modalities of the system are based on scientific principles and holistic concepts of health and healing. [1] Different chemical constituent in Henna plant that has pharmacological importance like p-coumaric acid, 2-methoxy-3-methyl-1,4-naphthoquinone, lawsone, apigenin, luteolin, and cosmosiin.[2] The reason for the popularity of henna for hair is that in addition to coloring and covering your grey hair, from hair treatment to conditioning, nourishing henna does it all. As lawsone is main phytoconstituents that provide all these dyeing, nourishing property and extraction seems to be disadvantageous in several manner we tried to synthesize lawsone and prepared lawsone hair dye gel in order to have safe hair dyeing, colorant, less expensive dyes that are moreover nontoxic too.

MATERIALS AND METHODS

Extraction of Lawsone[3]

Soxhlet Extraction

20g of dried henna leaves powdered was taken round bottomed flask and 600 ml water was added. The flask was equipped with a soxhlet extracting funnel and a condenser. The extraction was carried out for 8 hours. Then the solvent was removed on rotary evaporator and dried.

Maceration in water

Powder of dried henna leaves (20 g) was placed in 1L distilled water was added. The suspension was stirred on a magnetic stirrer with heating while the temperature was kept around 75 °C. After around 60 minutes, the colour of the green suspension turned brown. After around 6 hrs, solid NaHCO3 (17 g) was added. The suspension was filtered by gravity over several large glass funnels with filter paper. Suction filtration was avoided, because the colloidal particles would rapidly plug the pores of the filter paper. The filtrates were combined and acidified to pH 3 by addition of 0.12 M HCl. At this stage, the brown extract turned slightly cloudy. The swollen plant material was discarded. The filtrate was extracted with diethyl ether (4 × 400 ml). The combined ethereal phases were washed with water (3 × 100 ml) and dried over anhydrous MgSO4. After the removal of ether on a rotary evaporator, a reddish solid material was obtained as crude product (1.1 g). The progress of the isolation of Lawsone was monitored by IR spectroscopy and TLC on silica gel.

Tommasi's procedure

Powder of dried henna 20 g was placed in a 1L flask containing a magnetic bar and distilled water (500 ml)

was added. The suspension was stirred on a magnetic stirrer with heating while the temperature was kept around 80 °C. After around 2 – 3 hours, the colour of the green suspension turned brown. The suspension was left overnight. After that lime water was added until the suspension turned alkaline. The filtrates were combined and acidified to pH 3 by addition of 0.12 M HCl. The filtrate was extracted with diethyl ether (3 × 1000 ml).The combined ethereal phases were washed with water (3 × 1000 ml) and dried over anhydrous MgSO4.After the removal of ether on a rotary evaporator,

Synthesis of lawsone[4]

Steps involve in synthesis of lawsone are as fallows.

1. **Synthesis of 1-phenylazo-2-napthol from Aniline**: Take 4 ml of aniline ,16 ml conc. HCl, 16 ml distilled water, Shake & cool to temp 5°C.to it add 4 gm of sodium nitrite in 20 ml of distilled water 1 spatula urea with stirring. Diazotiation is achieved by adding cold solution nitrite to cold solution of aniline (5°c). Solution of 2-naphthol was prepared, Cool and added in cold diazonium salt (cooled below 5°C - ice bath for 30 min) Red orange crystals develop - filter, washed with water and dried for 3 days.
2. **Synthesis of 1-Amino-2- napthol hydrochloride from 1-phenylazo-2-napthol**: 8 g of 1-Phenylazo-2-napthol was added in beaker containing 60 ml methylated spirit reflux the mixture and cool it. Add 20 g of Tin(II) chloride and 60 ml of conc. HCl - produce clear solution and reflux for 30 min – gives slightly dark colour precipitate cooling until crystals appeared. Filter crystals - recrystallized with hot water add 2 drops of Tin(II) chloride solution and equal weight of HCl. 1-Amino-2-napthol HCl obtained – Dried - 3 days in desicator.
3. **Synthesis of 1-Amino-2-napthol-4-sulfonic acid**: Take 1.5 g of 1-Amino-2-napthol HCl and 6 g of sodium bisulfate in 100 ml of sodium hydroxide solution to it add 20 ml of water Mix the solution - dilute to 40-50 ml until it get dissolved. Solution is stored in 400 ml conc. sulphuric acid (avoid from sunlight) Temperature rises from 20-25°C to 35-40°C at once and 50°C in 2 hrs. After standing for 5 hours - precipitate form – wash – filter
4. **Synthesis of Lawsone from 1-amino-2-napthol-4-sulphonic acid**: Take 1 L of sodium hydroxide and 80 ml of conc. sulphuric acid–freeze. Add 1 g of 1-Amino-2- napthol-4-sulfonic acid made paste through shaking. Allow the mixture to stand for 30 min and the temperature rises at 15-20°C. Heat the mixture gradually on steam bath with shaking till solution boils to 15 min. - Solution become red and begin separate Filter and wash with cold water until filtrate nearly colourless.

Purification of lawsone by column chromatography[6]

Normally, a Separation will begin by using non-polar or low polarity Solvent, allowing the compounds to adsorb to the Stationary phase. Then slowly switching the polarity of The solvent to desorbs the compounds and allow them to Travel with the mobile phase. Solvent Selected- chloroform: petroleum ether: acetic acid (4:6:0.5). There are several acceptable methods for packing a column these include dry packing and slurry method. Methods used for lawsone purification is dry packing. In dry pack method, the stationary phase i.e. silica of 60 mesh size is deposited in the column before the solvent. In this case filling the column to the intended height with the stationary phase is done and then slowly addition of the nonpolar solvent. The solvent should be added slowly as to avoid uneven channeling. The most important aspect of packing the column is creating an evenly distributed and packed stationary phase. As mentioned, cracks, air bubbles and channeling will lead to a poor separation. Once the packing is completed the solvent is loaded into the column without disrupting the packing of column & equilibrated for 24 hrs. Sample is loaded by dissolving it into small quantity of solvent used for purification after 24 hrs. Minimum 5 to 10 drops is used to dissolve the sample into the solvent. The sample is loaded with the help of prepared from the side of column. Collecting small fractions (1-3 ml) is important to the success for column separation. Since column chromatography is time consuming, collecting large fractions is discouraged. If the mixture to be separated contains colored compounds, then monitoring the column is very simple. The colored bands will move down the column along with the solvent and as they approach the end of the column, collect the colors in individual containers. Use the color as guide. After all the materials have been removed from the column, the colors of the materials results should indicate which fractions contain the compound isolating i.e. Lawsone..

Analytical methods for identification of lawsone

TLC (Thin Layer Chromatography)[7]

The synthesize lawsone were dissolved in ethanol (10 mg/ml). 5µl of sample was applied into silica gel 60 GF254 TLC plate. A mixture of butanol, ethyl acetate, and acetic acid in ratio (7.5:2.5:0.1) was used as the mobile phase. The spots were visualized under ultraviolet (UV) light at 254 nm and 366 nm, and then sprayed with detecting reagent (10% sulfuric acid in methanol) and heated at 110°C for 10 min.

Colorimetry[8]

Prepare the samples according to the dilution i.e. 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml and adjust it with the distilled water up to 10 ml in volumetric flask. Turn on the instrument and let it warm up for 10-15 minutes. Take the reading of blank sample at the wavelength of 452 nm. Remove the blank solution and introduce the sample solution. Note the readings.

FTIR (Fourier Transform Infrared Spectroscopy)[9]

High signal to noise ratio makes FTIR more useful for difficult samples. It has resolution of 1 cm^{-1} and scan range of 4000 cm^{-1} to 250 cm^{-1}. FTIR spectra of lawsone obtained at room temperature by using an FTIR

Spectrophotometer - Perkin Elmer - Spectrum RX-IFTIR The spectra is collected in a range from 450 to 4000 cm^{-1}.

Liquid Chromatography Mass Spectroscopy LC-MS[10]

Mobile phase: 22% acetonitrile, 78% H2O was adjusting with PH-3 with formic acid.
Nebulizer gas flow: 3L/min.
Drying gas flow: 15L/min.
Desolvation line temperature: 250°c.
Heat block temperature: 400°c.
Lawsone was dissolved in 0.1mg/ml and the stock solution was prepared. The concentration of the standard five serial solutions is 0.02, 0.04, 0.06, 0.08 and 0.1ug/ml. Then each standard solution passes through 0.45 filters before LCMS an analysis.173 [M-H] ion, of standard was monitored in SIM mode and peak area was detected. The samples were passed through a 0.045 µm filter before LC—MS analysis.

Hair Dye Gel of Lawsone[11]

Preparation of Herbal Hair Dye Gel

The gel was prepared with defined quantity of carbopol-940 polymer. The specified quantity (1 g) of carbopol-940 was added in to distilled water with vigorous stirring and left for overnight for dissolving the polymer. 5% lawsone was dissolved in 20 ml of water with constant stirring. This aqueous extract solution was added into the polymer solution and mixed well by magnetic stirrer. Methylparaben and PEG was added as a preservative into this mixture and mixed well by magnetic stirrer. After complete dispersion of the extracts and preservatives glycerin 10 ml was added and mixed well in a magnetic stirrer. Distilled water was added and made up to 50 g.

Evaluation Parameter of Lawsone Hair Dye Gel

Physical parameters such as colour, appearance and consistency were checked visually.

pH of formulation: The pH of the formulated gel was determined using digital pH meter (Systronics Instruments, India). The electrode was immersed in the gel and readings were recorded from pH meter.

Lawsone content: Accurately weighed quantity of gel (100 mg) was dissolved 10 ml methanol, filtered and lawsone content was determined by analysing spectrophotometrically at 452 nm.

Viscosity determination: The viscosity of prepared gel was measured by using Brookfield viscometer using a spindle No.63 at 100 rpm. Gel (50 g) was kept in 50 ml beaker which was set till spindle groove was dipped and dial reading was measured after three minutes. From the obtained reading, viscosity was calculated.

Spreadability: The weighed quantity of gel (about 0.5g) was sandwiched between two glass slides. 100 g of weight was placed on the slides. The weight was placed for specific period of time for 10 min. Then weight was removed and diameter of the spread circle was measured at different points. Spreadability was calculated by using formula

$$S = (M \times L)/T$$

Where, S is spreadability, M is weight placed on the slide, L is diameter of circle in cm and T is Time in Sec**.**

Washability: The prepared hair gel formulation is applied on the skin and then ease and extent of washing with water is checked normally.

Homogeneity: Developed gel was tested for homogeneity by visual inspection after the gel was set in the container. It was tested for appearance, presence of any aggregates and flocculates.

Skin irritation test: Applied the herbal hair gel formulation on the skin and observe for irritation, redness or rashes.

RESULTS AND DISCUSSION

The lawsone was extracted by the three methods namely soxhlet extraction, maceration, tommasi procedure and the yield was found to be 2g, 1.1g, 3.2g respectively. Lawsone was synthesized into as mentioned in the experimental section and the practical yield was found to be 3.5g. The purification of lawsone synthesized was done successfully by column chromatography. Later the lawsone synthesized was analysed by thin layer chromatography. A mixture of butanol, ethyl acetate, and acetic acid (7.5:2.5:0.1) was used as the mobile phase and the Rf value was found to be 0.7. For UV visible spectroscopy The result was estimated in spectrum mode at a range of 400-800 nm and absorption maximum i.e. λmax was found to be 264 nm as shown in Fig. no 1 **.** The Lawsone sample identified by FTIR in the range of 4000 cm-1 - 250 cm-1 which shows the following peak of functional group in IR spectrum as depicted in Fig. no. 2. Further LC-MS study shows for the exact identification and result shows the molecular mass peak of lawsone at 174.10 and the result are depicted in Fig. no. 3. Lawsone hair dye gel F3 formulation is the hair colorant gel prepared and evaluated. There was no change in color after washing the hair every alternate day in natural soap solution for 10 days after which the intensity of the color started reducing. The effect of the color lasted for around 20 days even after frequent washing and exposure to sunlight.

Table No 1:- Composition of the herbal gel formulation.

Nameof ingredients		Different formulations Trial			
	L 1	L 2	L 3 Selected formulation	L 4	L 5
Lawsone (mg)	20	20	20	20	20
Carbopol 940 (g)	0.5	1.0	1.5	2.0	2.5
Propylene glycol (ml)	3	3	3	3	3
Polyethylene glycol-400(ml)	4	4	4	4	4
Glycerin (ml)	0.5	0.5	0.6	0.5	0.5
Methyl paraben(g)	0.04	0.04	0.04	0.04	0.04
Distilled Water (ml)	20	20	20	20	20

Table No 2:- Evaluation parameter for trial and error.

Parameter	Different Trial Formulation				
	L 1	L 2	L 3	L 4	L 5
Colour	Red orange	Red orange	Red orange	Red orange	Brownish
Consistency	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid
PH	7.2	6.5	6.7	6.4	6.9
Viscosity (cps)	51955	57879	55489	52995	50720
Appearance	Transparent	Transparent	Transparent	Transparent	Transparent
Spreadability (gcm/sec)	1.02	1.09	0.72	0.5	0.9
Washability	Good	Good	Good	Good	Good
Skin irritation	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant

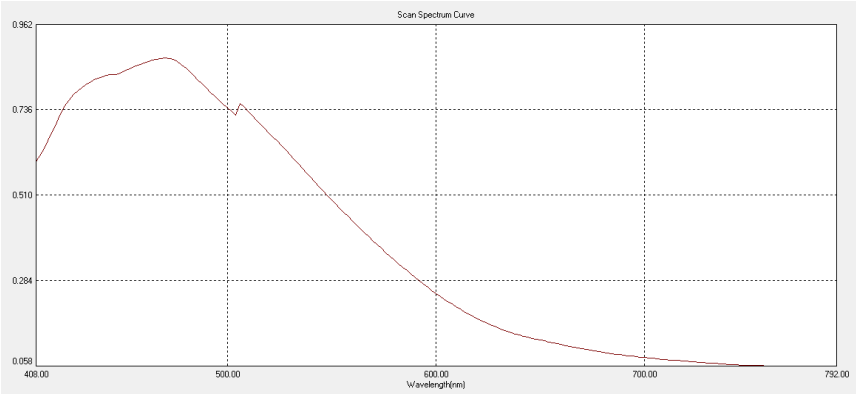

Fig No 1:- UV spectra of Lawsone.

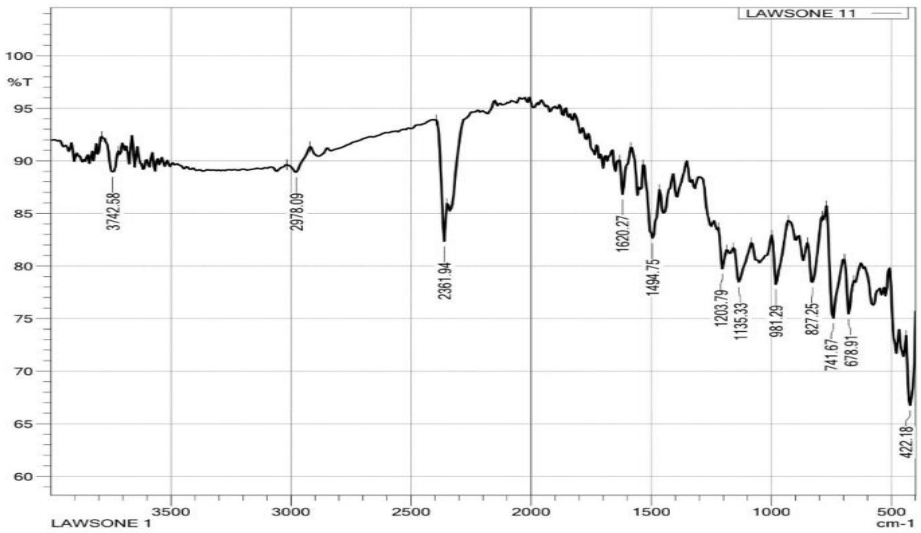

Fig No 2:- FTIR spectra of Lawsone.

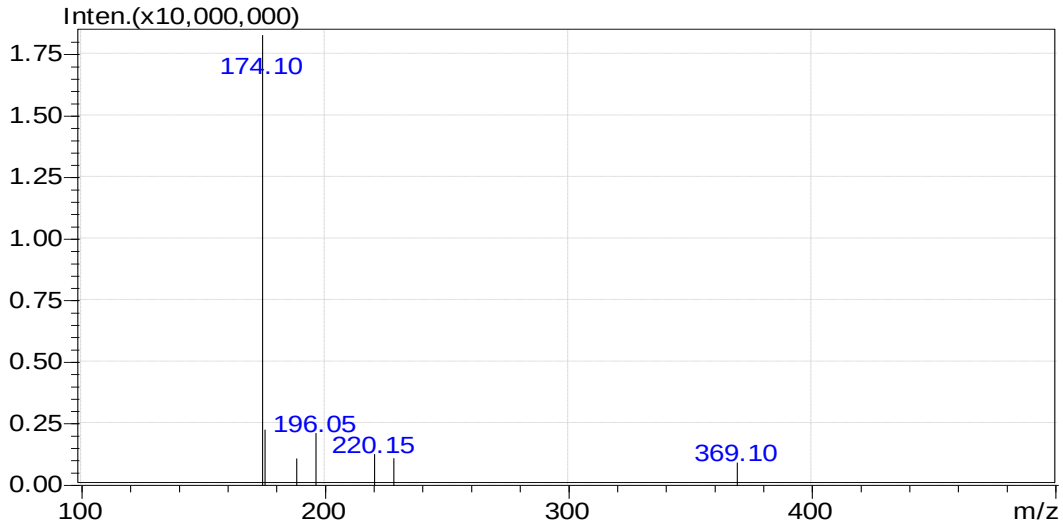

Fig No 3:- LC-MS spectra of Lawsone.

CONCLUSIONS

The extraction, isolation of lawsone was carried out successfully and synthesis, purification, characterisation of lawsone synthesized as described which resemble the identification standard values reported. Lawsone hair dye gel is the hair colorant gel prepared and evaluated and was found to be effective as hair colorant L3 formula as shown Table No 1 shows the best results. It has seen that exposure to 2 hours of sunlight daily caused hair color to fade after 16 days whereas it remained unchanged for 20 days when kept at room temperature protected from sunlight.

ACKNOWLEDGEMENT

We are very thankful to your principal sir Dr. Ashish Jain for providing college facility to do research work.

REFERENCES

1. Chaudhary G, Goyal S, Priyanka Poonia et al '*Lawsoniainermis Linnaeus: A Phytopharmacological Review',* International Journal of Pharmaceutical Sciences and Drug Research, 2010; 2(2): 91-98.
2. Mikhaeil BR, Badria FA, Maatooq GT, Amer MM Antioxidant and immunomodulatory constituents of henna leaves, Z Naturforsch C J Biosci, 2004; 59(7-8): 468-76.
3. Utkarsha Shivsharan, Yash Kothari et al "*Antimicrobial Activity and Isolation of Lawsone from Lawsoniainermis Using Column Chromatography*", Research Journal of Pharmacognosy and Phytochemistry, 2020; 12(4): 219-221.
4. Catino. S. C. and Farris. R. E., 'Concise encyclopedia of chemical technology', M. Grayson Ed. New York: John Wiley & Sons, 1985.
5. Fieser. L. F and Martin E. L, 'A Publication of Reliable methods for preparation of organic compounds', VIII, 1925; 300.
6. Utkarsha Shivsharan, Yash Kothari et al "Antimicrobial Activity and Isolation of Lawsone from Lawsoniainermis Using Column Chromatography", Research Journal of Pharmacognosy and Phytochemistry, 2020; 12(4): 219-221.
7. Syed Muhammad Ghufran Saeed, Syed Asad Sayeed et al "*a New Method For The Isolation And Purification of Lawsone From LawsoniaInermis And Its Ros Inhibitory Activity*", Pakistan Journal of Botany, 2013; 1432.
8. Singh M, Kaur M, Dangi CBS, Singh H, '*Photochemical and TLC profile of lawsoniainermis (heena)'*, International Journal of Pharmaceutical Research Scholars, 2014; 3(1): 2077- 7873.
9. Christopher T. Kruger; Dirk W. Lachenmeier, Evamaria Kratz; and Gerd Mildau, *Rapid Colorimetric Analysis of para-Phenylenediamine in Henna-based, Non-permanent Tattoo Color Mixtures;*Cosmetics & Toiletries magazine, 2011; 126(7): 496-501.
10. S. Elavarasan,M. Gopalakrishnan, Synthesis, structural analysis, theoretical studies of some lawsone derivatives; *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, 2014; 133: 1-6.
11. Fatima-Zahra Alem , Samira Ananthabothla Gita , et.al, Lawsone quantification in *Lawsonia inermis L.* by HPLC-MS: How does the temperature and pluviometry affect lawsone concentration? Industrial Crops and Products, 2020; 158: 112960.
12. Ramakrishna S &Gopikrishna U. V (2022) 'Formulation and Evaluation of Herbal Hair Gel'. Sch Int J Tradit Complement Med, 5(2): 28-32.