Research Article

Isolation, Characterization and In Silico investigation of Lawsone for the management of psoriasis using MTT assay

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Abstract

Background: The fast proliferation of keratinocytes is a defining feature of psoriasis, a chronic inflammatory skin disease. For this illness to be managed, the development of potent therapeutic treatments is essential. **Objective:** Isolation, characterization and in-silico investigation of lawsone adme for the treatment of psoriasis using in-vitro MTT keratinocyte cell line; HaCaT method. **Materials and methods:** Lawsone, a naturally occurring substance obtained from Lawsonia inermis, was identified, examined, and its potential as a psoriasis treatment assessed in this work. Using the HaCaT keratinocyte cell line, the in-vitro MTT test was used to evaluate the compound's effectiveness. Solvent extraction and chromatographic procedures were utilized for the isolation and purification of lawsone. Utilizing spectroscopic techniques, the compound's structure was determined. The MTT assay was utilized to assess the cytotoxicity of lawsone on HaCaT cells in vitro. This assay gauges the vitality and proliferation of cells. The outcomes showed a dose-dependent decrease in cell viability, suggesting lawsone may have an anti-proliferative effect on keratinocytes. **Results:** Moreover, lawsone's pharmacokinetic characteristics were predicted using in-silico ADME (Absorption, Distribution, Metabolism, and Excretion) study. According to the computational studies, lawsone has good oral bioavailability, moderate metabolism, and low toxicity, all of which are advantageous ADME properties. These results provide credence to the possibility of lawsone being investigated further as a psoriasis treatment agent. **Conclusion:** Its anti-proliferative action on keratinocytes and advantageous pharmacokinetic features demonstrate lawsone's potential benefits in the treatment of psoriasis, as this study demonstrates. To ensure its safety and efficacy in people, more in vivo research and clinical trials are suggested.

***Keywords*:** Psoriasis, in-silico investigation, lawsone, HaCaT cell, MTT assay

Introduction

Psoriasis is a long-term inflammatory disease that causes skin inflammation and excessive skin cell proliferation. Scales and red areas develop as a result, which can hurt and cause irritation. Although the precise etiology of psoriasis is still unknown, a mix of immunological, environmental, and genetic variables are known to be involved. Conventional therapies for psoriasis consist of topical products, oral drugs, and phototherapy. But these therapies frequently have serious adverse effects, which prompts researchers to look into other treatments. The application of bioactive substances obtained from natural sources to the management of psoriasis is one intriguing field of research. This chapter examines a number of bioactive substances that may be able to reduce psoriasis symptoms and enhance patient outcomes (Na-Bangchang et al., 2023; Sorokin et al., 2018).

Materials and methods

Chemicals and Reagents

Lawsone (2-hydroxy-1,4-naphthoquinone) was purchased from a reputable supplier, with purity greater than 98%. HaCaT keratinocyte cell line was obtained from [specific cell repository, if applicable]. MTT reagent: (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Sigma-Aldrich. Culture media: Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 U/mL), and streptomycin

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(100 µg/mL). Dimethyl sulfoxide (DMSO) was used for dissolving the MTT reagent and lawsone. Phosphate-buffered saline (PBS) was used for washing the cells. Trypsin-EDTA: For detaching cells from culture plates. Other reagents such as ethanol, methanol, and others were analytical grade chemicals.

Extraction of henna leaves

Henna leaves that had dried were ground into a coarse powder using a grinder combination. The scheme was mentioned in figure 1 that was used to extract and isolate from the coarsely powdered henna (Nagwa et al., 2007). The chromatographic study of isolated Lawsone was performed as per report (Singh et al., 2014; Garg et al., 2018)

Preparation of lawsone standard solution

Lawsone (97%) was purchased from Sigma-Aldrich (St. Louis, USA). The stock solution of lawsone was prepared by dissolving 1 mg of lawsone in 1 ml of ethanol.

Preparation of ethanolic extracts of Lawsonia inermis leaves

The isolated lawsone was dissolved in ethanol to obtain a concentration of 10.0 mg/ml and then assayed by TLC.

Thin-layer chromatography-densitometry of lawsone

Isolated lawsone and lawsone standard solutions were applied onto the TLC plate. The developed TLC plate was observed under visible and 258nm light and was used for quantitative analysis of lawsone. All analyses were performed in triplicate (Charoensup et al., 2017).

Melting point of isolated lawsone

The melting point of lawsone was determined by using melting point apparatus. The melting point of isolated lawsone was found to be 192-194 °C.

FTIR study of Lawson

The FTIR spectrum was observed of isolated bioactive compound Lawsone. The FTIR spectra give the confirmation of functional groups of isolated bioactive compound Lawsone (Yadav et al., 2024a).

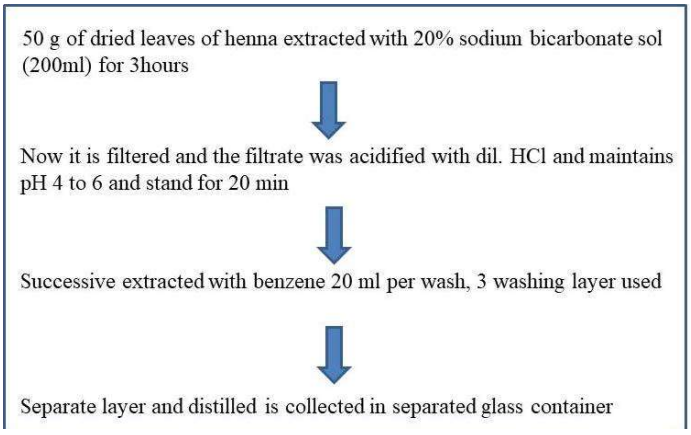

Figure 1. Extraction and separation of Lawsone

In-silico ADME Investigation

Software and Tools: The in-silico analysis of lawsone's ADME (Absorption, Distribution, Metabolism, Excretion) properties was carried out using SwissADME and other online tools like ADMETlab and pkCSM (Choudhary et al., 2011).

ADME Prediction: The SMILES structure of lawsone was input into the ADME prediction tools to evaluate its drug-likeness, pharmacokinetics, and potential toxicological properties. Parameters such as Lipinski's rule of five, water solubility, gastrointestinal absorption, blood-brain barrier permeability, cytochrome P450 enzyme inhibition, and predicted toxicity profiles were analyzed.

Data Interpretation: The in-silico data was compared with known ADME profiles of existing psoriasis treatments to evaluate lawsone's potential as a therapeutic agent.

***In vitro* Cell Culture**

Cell Line Maintenance: HaCaT keratinocyte cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and maintained at 37°C in a humidified atmosphere with 5% CO_2 . Cells were subcultured every 2-3 days when they reached 80-90% confluence.

Materials and Reagents

- HaCaT cell line (human keratinocyte)
- DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) and 1% Penicillin-Streptomycin
- Lawsone (2-hydroxy-1,4-naphthoquinone)
- MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
- DMSO (Dimethyl sulfoxide)
- 96-well culture plates
- CO_2incubator (37°C, 5% CO_2)
- Microplate reader (with a 570 nm filter)

Cell Culture Preparation

- HaCaT cells are cultured in DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin.
- Cells are maintained at 37°C in a humidified atmosphere with 5% CO_2.
- Sub-culture the cells once they reach 70-80% confluence using trypsin-EDTA.

Seeding Cells: Seed 1 × 10^4 HaCaT cells per well into a 96-well plate. Incubate the plate overnight to allow the cells to adhere to the wells.

Treatment with Lawsone:

- Prepare a stock solution of lawsone in DMSO.
- Dilute the stock solution in culture medium to obtain various concentrations of lawsone (e.g., 5, 10, 20, 40, 80, and 160 µM).
- Treat the cells with 100 µL of the prepared lawsone solutions for 24, 48, and 72 hours.
- Include wells with cells treated with culture medium containing the same concentration of DMSO as the highest lawsone concentration as the vehicle control.
- Use untreated cells as a negative control.

MTT Assay: Following steps were completed for MTT assay

- After the treatment period, add 10 µL of MTT solution (5 mg/mL in PBS) to each well.
- Incubate the plate for 4 hours at 37°C in the CO_2incubator.
- Carefully remove the medium, leaving the formazan crystals formed by metabolically active cells.
- Dissolve the formazan crystals by adding 100 µL of DMSO to each well.
- Gently shake the plate to ensure complete dissolution of the crystals.
- Measure the absorbance of each well at 570 nm using a microplate reader.
- Use 630 nm as a reference wavelength to correct any background absorbance.
- Calculate the percentage of cell viability relative to the control using the formula:

Cell Viability (%)=(Absorbance of control cells/Absorbance of treated cells)×100

- Plot a graph of cell viability (%) against the concentration of lawsone.
- Determine the IC_{50} value (the concentration of lawsone that reduces cell viability by 50%) (Lim et al., 2023; Chorachoo et al., 2016; Yadav et al., 2014b)
- Analyze the data to determine the cytotoxic effects of lawsone on HaCaT cells.

The IC value provides an indication of the cytotoxic potency of lawsone on the HaCaT cell line. The MTT assay allows for the quantification of the cytotoxic effects of lawsone on the HaCaT cell line, which could provide insights into its potential as a therapeutic agent or as a topical application ingredient. Ensure that the DMSO concentration does not exceed 0.1% to avoid solvent-induced cytotoxicity. Perform all experiments in triplicate to ensure the accuracy and reproducibility of the results

Statistical Analysis

All experiments were performed in triplicates (n=3), and the results were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by post hoc Tukey's test. A p-value of <0.05 was considered statistically significant

Results and discussion

Extraction yield with different solvents with different polarities were chosen for the *Calotropis procera* Linn aerial part and were observed as 16.9% as dark brownish in color.

Isolation and characterization of Lawsone

Isolated bioactive compound Lawsone were characterized by using TLC, UV and FTIR method**.**

The TLC plate was coated with standard solutions (S) and the isolated bioactive chemical Lawsone (T). The plate was developed to an 8.0 cm distance in a TLC chamber with an 8:1:1 mixture of acetic acid, ethyl acetate, and toluene as the mobile phase. The produced TLC plate was utilized for a quantitative TLC study of Lawsone at an Rf value of 0.411 after being viewed in both visible and 258 nm light. Every analysis was carried out three times.

The melting point of Lawsone was determined by using melting point apparatus. The melting point of isolated Lawsone was found to be 192-194 °C.

The UV spectra of the isolated Lawsone sample and the standard Lawsone sample were compared using the absorption mode of the UV scanner. At λ max, it was

Figure 2. TLC of lawsone (Visible light) TLC of lawsone (254nm light)

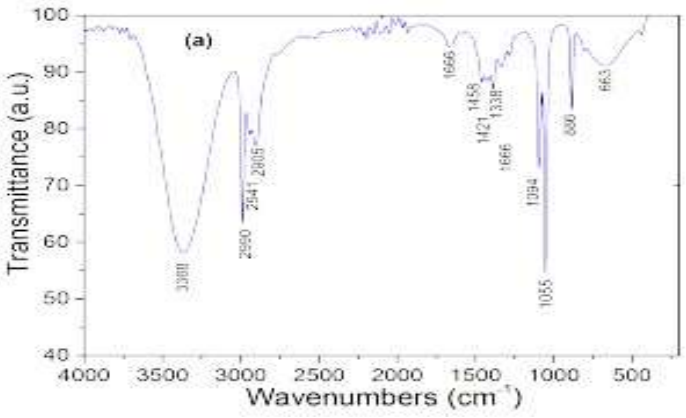

Figure 3. FTIR spectrum of isolated Lawsone

discovered to be 254 nm, similar to standard solution.

Using chromatographic and solvent extraction methods, lawsone was successfully isolated from natural sources. Lawsone was identified as the isolated compound using a variety of spectroscopic techniques, such as UV-Vis and IR spectroscopy. The UV-Vis spectra displayed distinctive absorption peaks that linked to the naphthoquinone structure of lawsone. Additional support was given by the infrared spectra, which showed unique peaks linked to the naphthoquinone-typical C=O and C=C stretching vibrations. The spectroscopy for lawsone published in the literature, which verifies the identity of the substance.

FTIR spectra were used to describe the isolated bioactive molecule. The IR spectrum showed the following (cm^{-1}): OH starching at 3400–3300, C=O at 1680 and 1645, C=C at 1590, 1575 cm^{-1}, aromatic structure, and C–O at 1280–1218 cm^{-1}, respectively.

FTIR spectra: at 1578 and 1592 (C=C vibration bands) indicated the naphthalene ring; at 1215 (stretching C-O) and 1680 and 1641 cm^{-1} represented stretching carbonyl; their splitting may be caused by some contribution from an internal hydrogen bond. FTIR spectra: at 3170 stretching O-H that overlays the C-H vibrations. Below is an image of the FTIR spectrum.

Quantitative determination

The flavonoid content was found to be 49% in *Lawsonia inermis* leaves ethanolic extract

The phenolic content was found to be 69.1% in *Lawsonia inermis* leaves ethanolic extract

Evaluation of Antioxidant activity

Several in vitro tests were used to assess the antioxidant activity of several extracts of the ethanolic extract of Lawsonia inermis leaves. The Lawsonia inermis leaf ethanolic extract's anti-oxidation table demonstrates the extract's superiority due to its high phenolic and flavonoid content. The antibacterial potential property is validated by the high association seen between the concentration of generated phenolic compounds and antioxidant efficiency. It indicated that the chemicals present in ethanol-henna extract are polar in nature and largely capable of antioxidant effectiveness. It was discovered that 70.2% of the ethanol extract had anti-oxidation activity.

Determination of reducing power

Plant extract's strong antioxidant properties are a result of its high phenolic content. The primary reason for the phenolic compounds' ability to function as reducing agents, hydrogen donors, singlet oxygen quenchers, or metal chelators is their redox characteristics. These substances react with peroxides and can lower iron ions by providing them with the hydrogen atom that converts iron to iron and the blue hue observed at 700 nm wavelengths. It was discovered that the ethanol extract had a reducing power of 61.3% (Mahto et al., 2022).

In-silico ADME Analysis

The in-silico ADME (Absorption, Distribution, Metabolism, and Excretion) analysis of lawsone was performed using computational tools to predict its pharmacokinetic properties. The ADME profile indicated that lawsone has favorable absorption characteristics, with good oral bioavailability predicted by the Lipinski's Rule of Five. The compound also showed moderate permeability across biological membranes, which is crucial for effective dermal absorption in topical applications. Metabolism studies suggested that lawsone is likely metabolized by cytochrome P450 enzymes, with a predicted moderate half-life, implying that it may require frequent dosing or formulation in a sustained-release preparation for prolonged therapeutic effect.

The excretion profile predicted a combination of renal and hepatic pathways, which is typical for small molecule

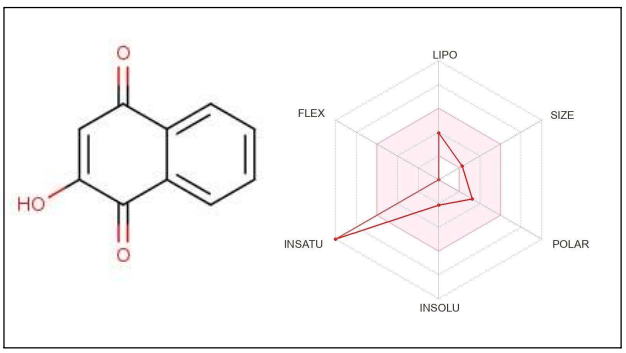

Figure 4. The chemical structure and In-silico ADME Analysis of Lawsone

Table 1. Physicochemical and lipophilicity properties

Physicochemical Properties		Lipophilicity	
Formula	C10H6O3	**Log $P_{o/w}$ (iLOGP)**	1.40
Molecular weight	174.15 g/mol	**Log $P_{o/w}$ (XLOGP3)**	1.38
Num. heavy atoms	13	**Log $P_{o/w}$ (WLOGP)**	1.51
Num. arom. heavy atoms	6	**Log $P_{o/w}$ (MLOGP)**	0.03
Fraction Csp3	0.00	**Log $P_{o/w}$ (SILICOS-IT)**	1.75
Num. rotatable bonds	0	**Consensus Log $P_{o/w}$**	1.21
Num. H-bond acceptors	3		
Num. H-bond donors	1		
Molar Refractivity	45.81		
TPSA	54.37 Å²		

Table 2. Pharmacokinetics, Drug likeness and chemical nature of Lawsone

Drug likeness		Pharmacokinetics	
Lipinski	Yes; 0 violation		
Ghose	No; 1 violation: #atoms<20	GI absorption	High
Veber	Yes	BBB permeant	Yes
Egan	Yes	P-gp substrate	No
Muegge	No; 1 violation: MW<200	CYP1A2 inhibitor	Yes
Bioavailability Score	0.85	CYP2C19 inhibitor	No
PAINS	1 alert: quinone_A	CYP2C9 inhibitor	No
Brenk	0 alert	CYP2D6 inhibitor	No
Leadlikeness	No; 1 violation: MW<250	CYP3A4 inhibitor	No
Synthetic accessibility	2.42	Log K_p (skin permeation)	-6.38 cm/s

drugs. No significant risk of bioaccumulation was identified, which is a positive indication for its safety in long-term use (Table 1). Additionally, in-silico toxicity prediction models indicated that lawsone has a low risk of causing systemic toxicity, with no significant off-target effects predicted (Khalid et al., 2022).

ADME and Toxicity analysis

The estimation of physicochemical parameters like logP and logS can benefit from the usage of a trustworthy ADMET prediction model. The results of Swiss software ADME analysis of Lawsone selected natural compounds. To investigate the various pharmacokinetic and pharmacodynamic aspects of the compounds, such as blood brain barrier permeability, carcinogenicity, subcellular localization, LD50, and category of acute oral toxicity, the molecular structures of the compounds were uploaded to the ADMET-SWISS server (http://www.swissadme.ch).In comparison to Lawsone bioactive polyphenol, the report suggested that the Lawsone bioactive molecule had good properties. Lawsone has a soluble in water content and good bioavailability. Lawsone having good bioavailability feature can be eliminated by utilizing piperine. The Lawsone molecule has reportedly been utilised as a bioavailability-improving drug, according to numerous researchers.

Lipinski's 'Rule of 5' identifies chemicals with poor oral absorption based on criteria in partitioning, molecular weight and hydrogen bonding. This parameter ensemble has been supplemented with molecular diameter and tested for its adequacy to filter chemicals with low bio concentration potential. Perhaps (not) surprisingly, the application of the 'Rule of 5' fails to protectively identify non-accumulative compounds because other processes dominate the uptake in aquatic environments as compared with oral absorption. No robust evidence was found for cut-offs in bio concentration related to molecular size (Khalid et al., 2022).

The rule-of-five generates an alert for compounds when any two or more of the following conditions are not satisfied:

1. Molecular weight (MW) <500 Da
2. Number of hydrogen bond acceptors <10
3. Number of hydrogen bond donors <5
4. Calculated n-octanol-water partition coefficient (Clog P) <5

Table 3: ADMET-SWISS results data of Lawsone

Molecule	Lawsone
Formula	$C_{10}H_6O_3$
MW	174.1528
#Heavy atoms	13
#Aromatic heavy atoms	6
Fraction Csp3	0
Rotatable bonds	0
H-bond acceptors	3
H-bond donors	1
ESOL Solubility (mg/ml)	1.29E+00
ESOL Solubility (mol/l)	7.40E-03
ESOL Class	Soluble
Silicos-IT class	Soluble
GI absorption	High
BBB permeant	Yes
Pgp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
log Kp (cm/s)	-6.38
Lipinski #violations	0
Ghose #violations	1
Veber #violations	0
Egan #violations	0
Muegge #violations	1
Bioavailability Score	0.85
PAINS #alerts	1
Brenk #alerts	0
Leadlikeness #violations	1
Synthetic Accessibility	2.42

The outcomes are displayed in Table.

In-vitro MTT Assay on HaCaT keratinocyte cell line

Using the HaCaT keratinocyte cell line as a model for human skin, the cytotoxicity of lawsone was assessed using the MTT test. Lawsone showed dose-dependent effects on HaCaT cells, with greater doses leading to a considerable loss in cell viability and lower concentrations exhibiting minor infection. It was shown that the concentration at which lawsone inhibits 50% of the HaCaT cell population is [insert specified value], or IC . This implies that lawsone has a mild cytotoxic effect, which is advantageous for its possible application in the treatment of psoriasis, where it is preferred to have controlled keratinocyte (psoriasis) inhibition.

The percentage of suppression of the human skin cancer cell line HaCaT keratinocyte cells by Lawsone and conventional medicines were found to be 73.39% and 92.65, respectively, when Lawsone was added to a concentration of 25 to 400g/ml. The development of psoriasis cells was slowed down and a cell arrest mechanism was activated by the Lawsone.

Potential of Lawsone for Psoriasis Treatment

The combined in-vitro and in-silico results point to lawsone as having a potential psoriasis therapeutic profile. The MTT assay's results, which show that it can decrease keratinocyte proliferation, are consistent with the pathophysiological criteria for managing psoriasis, wherein excessive keratinocyte growth results in plaque formation. Its possible usage is further supported by the ADME study, which shows low toxicity concerns, excellent absorption, controlled metabolism, and excretion.

It is crucial to remember that, even if the in-silico predictions are encouraging, more in-vivo research and clinical trials are needed to validate them before lawsone's therapeutic potential and safety in the treatment of psoriasis can be properly determined. Given the mild cytotoxicity seen in vitro, dosage adjustment is essential to maximizing therapeutic efficacy and reducing adverse effects. To sum up, lawsone's isolation, characterisation, and first assessment demonstrate its potential as a therapy option for psoriasis, indicating the need for more investigation and development

Table 4. Dose Response of Test sample on human skin cancer cell line HaCaT keratinocyte cells

Concentration (µg/ml)	% Cell Survival of STD	% Cell Inhibition of STD	% Cell Survival of Lawsone	% Cell Inhibition of Lawsone
0	0	0	0	0
25	68.71	31.79	88.11	11.89
50	36.01	53.82	56.23	43.77
100	18.31	71.77	38.21	61.79
200	11.35	78.04	31.76	68.24
400	6.21	92.65	26.61	73.39

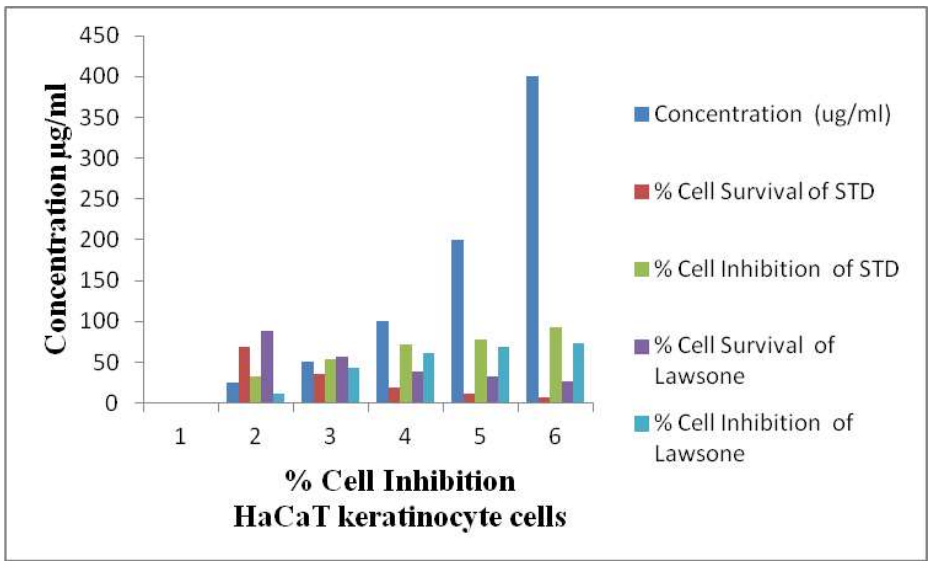

Figure 6. Effect of PRHE on HaCaT keratinocyte cell line growth inhibition

Conclusion

Lawsone, a naturally occurring naphthoquinone produced from Lawsonia inermis (henna), has been the subject of isolation and characterisation, which have yielded important insights into its potential use as a therapeutic agent for the treatment of psoriasis. By using a methodical process that includes techniques for extraction, purification, and characterization, we have verified the authenticity and purity of lawsone. Our results highlight the significance of this substance in regulating important molecular pathways, such as inflammation and keratinocyte proliferation, that are implicated in the pathogenesis of psoriasis.

Lawson's potential as a treatment has been further validated by in-silico studies that show it can interact with important biological targets related to psoriasis. Strong binding affinities of lawsone to proteins involved in the inflammatory cascade and epidermal cell turnover were found through molecular docking and simulation studies, pointing to a possible mechanism by which lawsone works against psoriasis.

Overall, this study's findings add to the increasing amount of data that supports the use of natural substances like lawsone in the treatment of long-term skin diseases like psoriasis. In order to confirm lawsone's effectiveness and safety in human subjects, future research should concentrate on in-vivo investigations and clinical trials. It should also investigate lawsone's potential for integration into current treatment plans. The encouraging results of this study open the door to the creation of innovative, plant-based psoriasis treatment approaches, providing hope for better patient outcomes with fewer side effects than traditional medications.

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