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HR-LCMS PROFILING AND CYTOTOXICITY STUDY OF CRUDE AQUEOUS EXTRACT OF *PHASEOLUS VULGARIS* LINN. LEAVES

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ABSTRACT

The objective of the current study is to evaluate the phytochemical profile and cytotoxic properties of the crude aqueous extract of *Phaseolus vulgaris* Linn. leaves. This plant is well-known for its nutritional and agricultural benefits, and its crude extract was obtained through the sequential extraction method. The extract was then subjected to High-Resolution Liquid Chromatography-Mass spectrometry (HR-LCMS) analysis to identify the phytoconstituents present. A total of 120 phytoconstituents were obtained, with 26 compounds being identified as major constituents in both positive and negative ESI modes. Sapropterin, Melibiose, Pirbuterol, Osmaronin, N-Isovalerylglycine methyl ester, Beta-D-Galactopyranosyl-(1->4) -beta-D-galactopyranosyl- (1->4) -D-galactose, 3-Methyladenine, 3-Beta, 6-Beta-Dihydroxynortropane, D-pipecolic acid, Lotaustralin, 8-Hydroxyadenine, L-threo-3-Phenylserine, L-isoleucyl-L-proline, Gentianadine, L-Arogenate, Prolyl-Arginine, Norcotinine, Ganolucidic acid E, Benzocaine, Succinoadenosine, D-Galactonate, Melibiose, Thiolactomycin, (Z)-5-[(5-Methyl-2-thienyl)methylene] -2(5H) Furanone, Rutin, and Kuwanon Z. The toxicity of crude extracts at various concentrations was

evaluated using the Sulforhodamine B assay on the HaCaT cell line. The results indicated that the IC50 value for the crude extract was 853.2 ± 0.11 g/ml. A dose-dependent cytotoxic effect of the crude aqueous extract was observed on the HaCaT cell line. Therefore, it is necessary to isolate, purify, and characterize bioactive compounds responsible for their bioactivity.

Keywords: HR-LCMS, HaCaT cell line, Leaves, *Phaseolus vulgaris* Linn

INTRODUCTION:

The outer layer of the body is referred to as the skin. It is responsible for protecting the body from harmful environmental substances. The frequency of skin cancer has increased during the last few decades. The primary cause of skin cancer is exposure to damaging ultraviolet (UV) radiation **[1]**. There are some causes of skin cancer, such as coal, tar, and viruses. There is no permanent cure for cancer, and the treatment of cancer includes radiation therapy, immunotherapy, and surgery. These treatments and medications are troublesome and costly. Alternatively, herbal medicine can show therapeutic effects with no side effects. Therefore, it is currently interesting for scientists to find a new therapeutic anticancer agent from herbal extract, as it has no side effects **[2]**.

Phaseolus vulgaris Linn. has many health benefits, and the seeds of the plant have high nutritional values **[3]**. The occurrence of this plant is more common, and the therapeutic importance of the leaves of the plant has not been documented yet. Therefore, the leaves of the plant were chosen for the study. The aqueous extract is more beneficial than the organic solvent extract due to its bioavailability, environmental safety, formulation studies, and cost effectiveness. In the field of phytochemistry, HR-LCMS is a widely utilized analytical method for identifying and quantifying complex blends of bioactive compounds in plant extracts. The integration of Mass Spectrometry (MS) with Liquid Chromatography (LC) facilitates highly sensitive and specific separation, identification, and structural elucidation of molecules. Liquid chromatography utilizes stationary and mobile phases to interact and separate the components of a mixture, while mass spectrometry determines the mass-to-charge ratios (m/z) of the separated components through ionization and measurement, providing extensive molecular information. High-resolution liquid chromatography-mass spectrometry (HR-LCMS) is indispensable for researching and developing novel herbal medicines. Identifying bioactive compounds in plants aids in creating potent herbal treatments and

understanding their pharmacological properties **[4]**.

The Sulforhodamine B (SRB) assay has gained considerable popularity for evaluating cytotoxicity and cell proliferation. This technique has proven particularly useful in research on the effects of herbal extracts on cells. By measuring the quantity of dye that binds to cellular proteins, the SRB assay provides a precise measurement of cell density, allowing for accurate assessments of cell growth or inhibition. Its suitability for high-throughput screening makes it ideal for testing multiple samples simultaneously, which is particularly advantageous for extensive research involving various herbal extracts or chemicals. The SRB assay has become widely adopted in research labs due to its relative simplicity and cost-effectiveness compared to other cytotoxicity assays **[5]**.

The objective of the current study is to evaluate the phytochemical profile through HR-LCMS screening of the crude aqueous extract of the leaves of the plant. Additionally, the study aims to assess the cytotoxicity of the extract using the SRB assay on the HaCaT cell line. The HaCaT cell line, derived from human skin and characterized by retaining many properties of normal keratinocytes, is a critical model for investigating skin cancer, as it was derived from spontaneously immortalized human keratinocytes **[6]**.

MATERIALS AND METHODS

Collection of plant material and authentication

The *Phaseolus vulgaris* Linn. was collected from Dapoli, a Taluka from Ratnagiri, State Maharashtra. The collected specimen was meticulously cleaned using water to eliminate any dust, soil, or other fine particles. Subsequently, the plant was dried under a shed to create a herbarium. Once fully dried, it was submitted to St. Xavier's College of Herbarium Section, and its species was confirmed to match the herbarium with voucher number D.P.2275.

Preparation of the extract:

The extraction method selected is the sequential method of extraction. The plant phytoconstituents have a small difference in their polarity range; therefore, the selection of solvent is crucial. The selected solvents are high-polar, mid-polar, and non-polar to separate the phytoconstituents **[7]**.

Procedure

Exactly 5 grams of dry leaf powder were taken into a 250-ml conical container, with 100 ml of petroleum ether poured into it. The conical flask is kept in a sonicator for about 10 minutes, and the filtrate is filtered through Whatman filter paper No. 1. The filtrate cake

was again washed with dichloromethane, ethyl acetate, methanol, and finally with water. The filtrate obtained from all the solvents is ignored. The final aqueous extract was collected and lyophilized on a lyophilizer. The yield obtained from the aqueous extract was recorded and used for further analysis.

HR-LCMS

The aqueous extract obtained by the sequential method of extraction is subjected to HR-LCMS profiling for analysis of the phytoconstituents present in it. Chemical fingerprints of the aqueous extract of the leaves of the plant were created using Agilent high-resolution liquid chromatography and mass spectrometry model G6550A with 0.01% mass resolution. The method used was to set the acquisition to the MS minimum range of 126 (m/z), with a maximum range of 1200 Dalton (m/z) and a scanning rate of one spectrum per second. The gas flow rate in gas chromatography was maintained at 13 psi/minute at 250 °C. Hip sampler with model G4226A was used with auxiliary speed 100 µl/minute, ejection speed 100 µl/minute, flush out factor 5µl, and 3µl injection volume used for HR-LCMS. The gradient solvent system used was solvent A (acetonitrile, 100%), solvent B (0.1% formic acid), and a Hemochrom C18 column to get chromatographic separation **[8]**.

***In vitro* SRB Assay:**

SRB assays can easily evaluate the cytotoxic potential of herbal extracts on various cell lines. This method of cytotoxicity testing is a colorimetric assay; therefore, it is cost-effective and environmentally friendly. This assay is very easy to perform and does not require expensive chemicals and instruments. Basically, sulforhodamine B is a dye that binds proteins present in the cell, thereby measuring the viability of the cells. The protein, upon solubilization, gives absorption around 490-530 nm. The absorption of solubilized dye is directly correlated to the quantity of cells present **[9, 10]**.

Cell Line

The crude aqueous extract of the leaves of *Phaseolus vulgaris* Linn. was tested on the HaCaT cell line provided by Aakar Biotechnologies Private Limited, Lucknow.

Origin of cell line

The HaCaT cell line is obtained from normal adult human skin keratinocytes. The cell line was used to test dermatological studies and skin cancer studies **[11]**. It is widely utilized for dermatological and skin cancer research. Unlike other cell lines, the HaCaT cell line is spontaneously immortalized and does not require virus manipulation or gene alteration. This distinct characteristic sets it apart from other cell lines. Additionally, HaCaT cells

exhibit sensitivity to UV light, making them ideal for studying the dermatological effects caused by UV radiation.

Procedure

The effectiveness of *Phaseolus vulgaris* Linn. leaf crude aqueous extract on the cytotoxicity of the HaCaT cell line was assessed using an SRB assay. Cells were cultured in 96-well plates at a density of 8000 cells/well in DMEM medium supplemented with 10% FBS and 1% antibiotic solution for 24 hours at 37°C with 5% CO_2. The following day, cells were exposed to different concentrations (0-5000 nM) of the extract, with untreated cells serving as controls. After incubation for 24 hours, the cells were treated with Tri Chloro Acetic Acid (TCA), followed by washing with DM water and air drying at room temperature. SRB solution was added to each well at a final concentration of 0.04% and left for 1 hour, after which the plate was washed with 1% (v/v) acetic acid to remove unbound dye. The wells were then incubated with Tris base solution (pH = 10.5) for 10 minutes on an orbital shaker to solubilize the protein-bound dye, which was measured in an Elisa plate reader at 510 nm. Images of the cells were captured under an inverted microscope using an AmSope digital camera **[12]**.

Statistical data analysis:

Excel 2016 from Microsoft Office was used to create the graphs. The linear regression formula in GraphPad Prism 5.0 was used to determine the IC50 value. A one-way ANOVA was used to determine statistical significance (p). Where $P<0.05$ was considered to be significant. Data are expressed as the means ± standard error of the mean (SEM; n = 4).

RESULTS AND DISCUSSION

HR-LCMS profiling of the crude aqueous extract

HR-LCMS chromatograms of the crude aqueous extract of the leaves of the plant in a positive and negative mode, as shown in **Figure 1 and 2**.

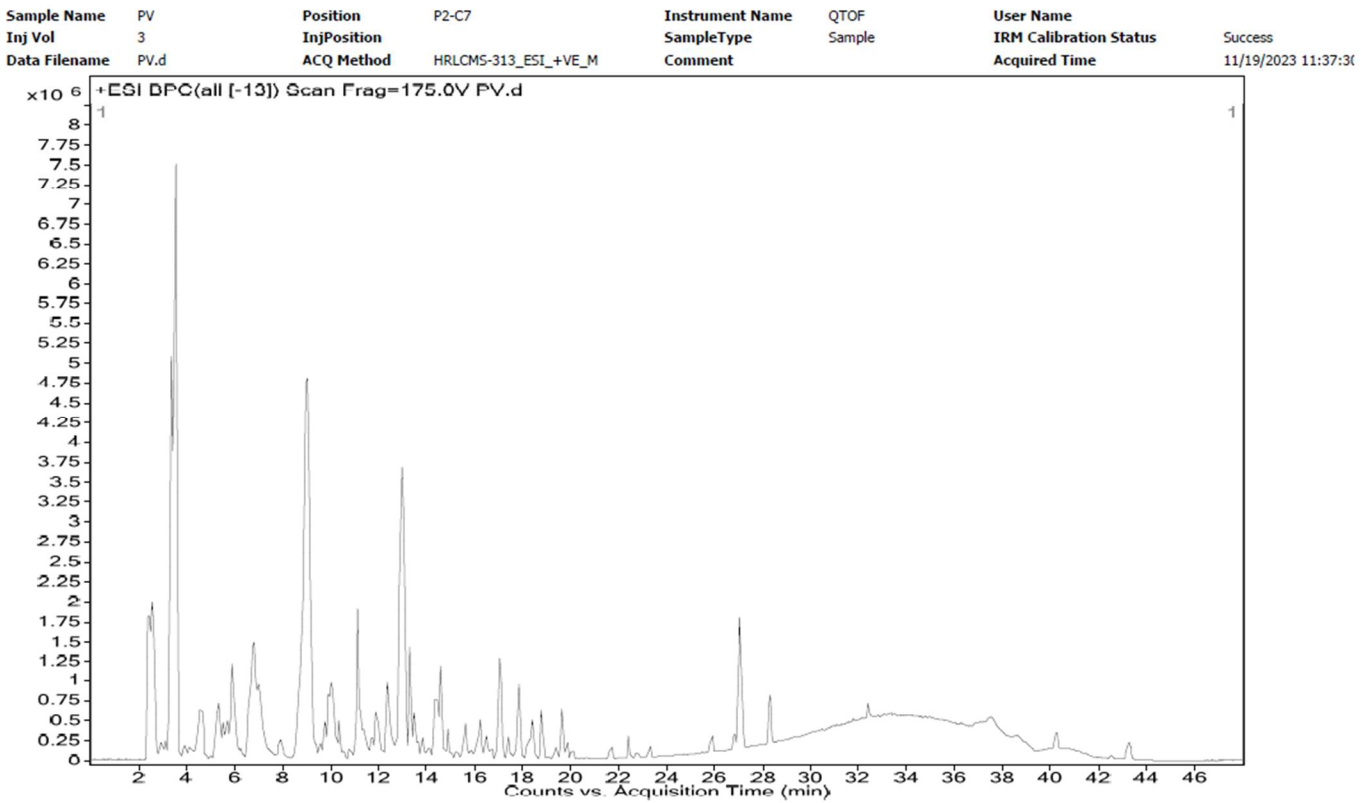


Figure 1: Positive mode of HR-LCMS chromatograms of crude extract of the leaves of *Phaseolus vulgaris* Linn

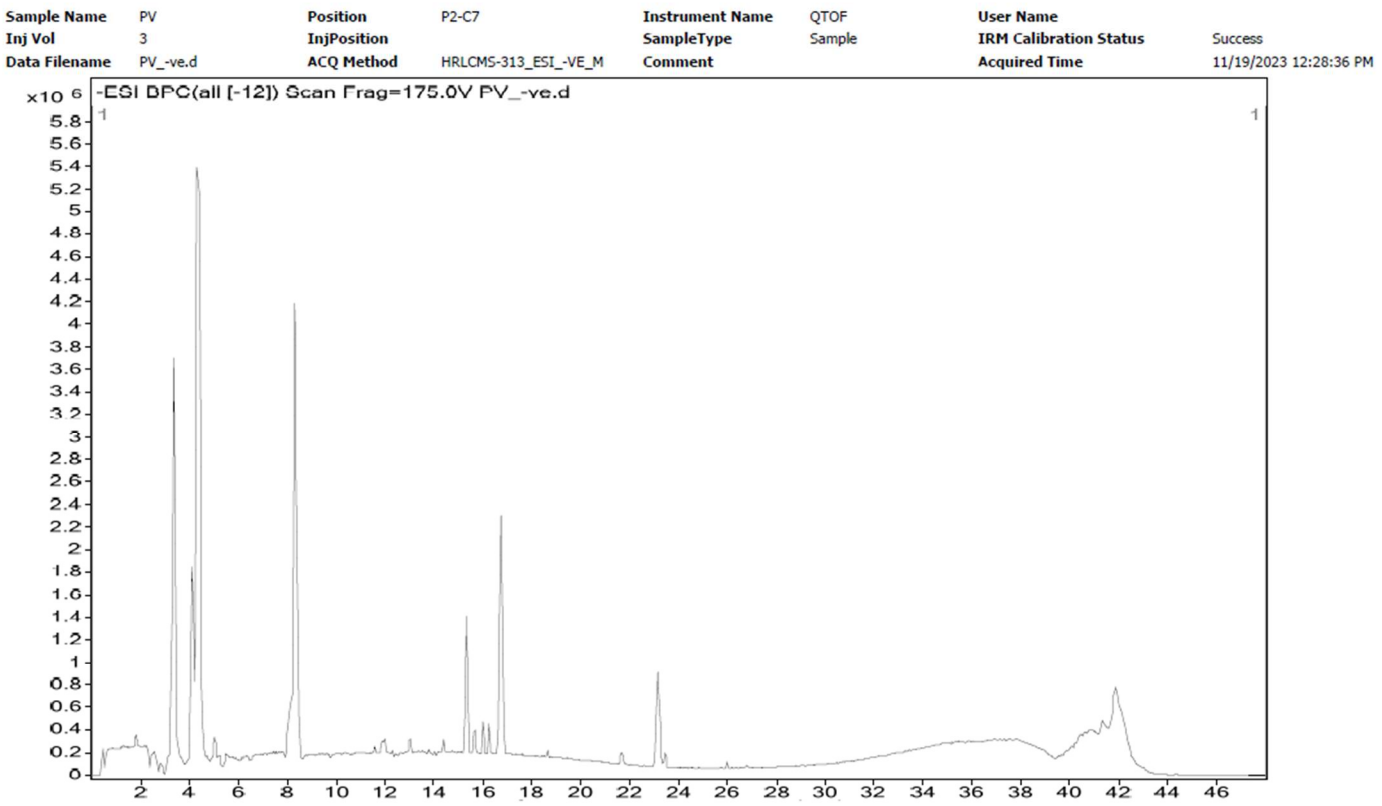


Figure 2: Negative mode of HR-LCMS chromatograms of crude extract of the leaves of *Phaseolus vulgaris* Linn

Table 1: HR-LCMS profiling of phytoconstituents found in positive mode of ESI

Sr.No.	Name of compound	Retention Time (Minutes)	Molecular Mass	Molecular formula	DB difference (ppm)	Biological Activity
1	Sapropterin	3.641	241.1165	$C_9H_{15}N_5O_3$	4.08	Used in the treatment of Phenylketonuria [9]
2	Melibiose	4.53	342.1145	$C_{12}H_{22}O_{11}$	4.98	Anticancer, Antiproliferative, Antifungal [10]
3	Pirbuterol	4.848	240.1453	$C_{12}H_{20}N_2O_3$	8.58	Antiasthma [11]
4	Osmaronin	5.213	259.1041	$C_{11}H_{17}NO_6$	5.79	Anticancer [12]
5	N-Isovalerylglycine methyl ester	5.275	173.1046	$C_8H_{15}NO_3$	3.55	Activity not reported
6	Beta-D-Galactopyranosyl-(1->4)-beta-D-galactopyranosyl- (1->4)-D-galactose	5.712	504.166	$C_{18}H_{32}O_{16}$	6.01	Activity not reported
7	3-Methyladenine	5.786	149.0692	$C_6H_7N_5$	6.39	Anticancer [13]
8	3beta,6beta- Dihydroxynortropane	5.857	143.0934	$C_7H_{13}NO_2$	8.32	Activity not reported
9	D-pipecolic acid	5.857	129.0782	$C_6H_{11}NO_2$	5.93	Antibacterial [14]
10	Lotaustralin	6.539	261.1202	$C_{11}H_{19}NO_6$	4.08	Antidepressant, cardio tonic, Anticancer [15]
11	8-Hydroxyadenine	6.686	151.0488	$C_5H_5N_5O$	4.12	Antiviral [16]
12	L-threo-3-Phenylserine	9.748	181.073	$C_9H_{11}NO_3$	4.84	Activity not reported
13	L-isoleucyl-L-proline	9.754	228.1449	$C_{11}H_{20}N_2O_3$	10.9	Anticancer, Normotensive [17]
14	Gentianadine	6.866	149.0466	$C_8H_7NO_2$	7.56	Wound healing [18]
15	L-Arogenate	9.796	227.0782	$C_{10}H_{13}NO_5$	5.01	Activity not reported
16	Prolyl-Arginine	9.957	271.1643	$C_{11}H_{21}N_5O_3$	0.53	Antibacterial [19]
17	Norcotinine	10.39	162.0782	$C_9H_{10}N_2O$	6.6	Activity not reported
18	Ganolucidic acid E	11.097	484.3159	$C_{30}H_{44}O_5$	6.07	Antioxidant and Antimicrobial agent [20]
19	Benzocaine	11.202	165.0782	$C_9H_{11}NO_2$	4.82	Local anaesthetic [21]
20	Succinoadenosine	11.49	383.1054	$C_{14}H_{17}N_5O_8$	6.01	Activity not reported

Table 2: HR-LCMS profiling of phytoconstituents found in negative mode of ESI

Sr. No.	Name of compound	Retention Time (Minutes)	Molecular Mass	Molecular formula	DB difference (ppm)	Biological Activity
1.	D-Galactonate	3.349	196.0556	$C_6H_{12}O_7$	13.56	Activity not reported
2.	Melibiose	4.147	342.1118	$C_{12}H_{22}O_{11}$	12.97	Anticancer, Antiproliferative, Antifungal [10]
3.	Thiolactomycin	4.444	210.071	$C_{11}H_{14}O_2S$	2.03	Antimycobacterial [22]
4.	(Z)-5-[(5-Methyl-2-thienyl)methylene]-2(5H)-furanone	8.332	192.0234	$C_{10}H_8O_2S$	5.54	Activity not reported
5.	Rutin	15.336	610.1451	$C_{27}H_{30}O_{16}$	13.62	Antifungal, Antibacterial, Antifungal, Antimalarial, Antiretroviral, Antiviral, Hair, Sunscreen effects, Wound healing activity [23]
6.	Kuwanon Z	16.002	594.1507	$C_{34}H_{26}O_{10}$	3.23	Anti-allergic and Anti-inflammatory [24]

HR-LCMS profiling of the aqueous extract of the leaves gives 120 phytoconstituents. Based on the retention duration, 26 significant compounds were found among them, and the concentration of each phytoconstituent was expressed in decibels (DB) in ppm. The mass of each fragment is detected by a mass spectrometer to detect the possible structure of a compound. Electron Spray Ionization (ESI) is a type of mass ionizer with positive and negative modes used to predict the proton-acceptor and proton-donor capacities of phytoconstituents. HR-LCMS analysis of positive and negative ESI modes gives 20 and 7 important phytoconstituents, as shown in **Tables 1 and 2**. The phytoconstituents obtained from HR-LCMS are compared with the library and database supported for the same. The bioactivity of each phytoconstituent is obtained from a literature search.

SRB assay:

The cytotoxicity of crude aqueous extracts of different concentrations was tested by SRB assay on the HaCaT cell line, as shown in **Figure 3**. The percentage of viable HaCaT cells relative to the negative control is depicted in the bar graph with clarity, i.e., cells without treatment. The Y-axis represents the percentage viability of HaCaT cells, and the X-axis represents the concentration of crude aqueous extract of the leaves, ranging from 0 to 1000 µg/ml. It is observed from the graph that at 0 µg/ml, the viability of cells was kept at 100%. At low concentrations, from 1 µg/ml to 50 µg/ml, the viability of the cell was slightly changed. At 1 µg/ml, it is slightly reduced but still close to 100%; at 10 µg/ml, it becomes 90%; and at 50 µg/ml, the cell viability was 85%. In moderate concentrations from 100 µg/ml to 250 µg/ml, cell viability was around 75 to 80%. At a high concentration of 1000 µg/ml, the viability of the cell was reduced and found to be around 20%. Thus, the graph clearly shows that an increase in percentage leads to a dose-dependent decrease in cell viability. Preliminary screening of results can be done by observing **Figure 4**. A 96-microplate reader was used to determine the absorbance of the sample. The intense pink color (cells without treatment) on the left side of the microplate reader indicates the viability of the cells, while after loading the compound, the intense pink color decreases to faint pink. Therefore, it can be confidently declared that the leaves of *Phaseolus vulgaris* Linn. have a cytotoxic effect on the HaCaT cell line, and the effect of cytotoxicity goes on to increase with an increase in concentration. The dose-dependent IC50 value obtained for the crude aqueous extract on the HaCaT cell line was

853.2 ± 0.11 µg/ml. The IC50 value and potency of a drug have a strong relationship; a low IC50 value increases the potency of the drug. The percentage viability of cells at a given concentration shows a cytotoxic effect as compared to the negative control **[25]**.

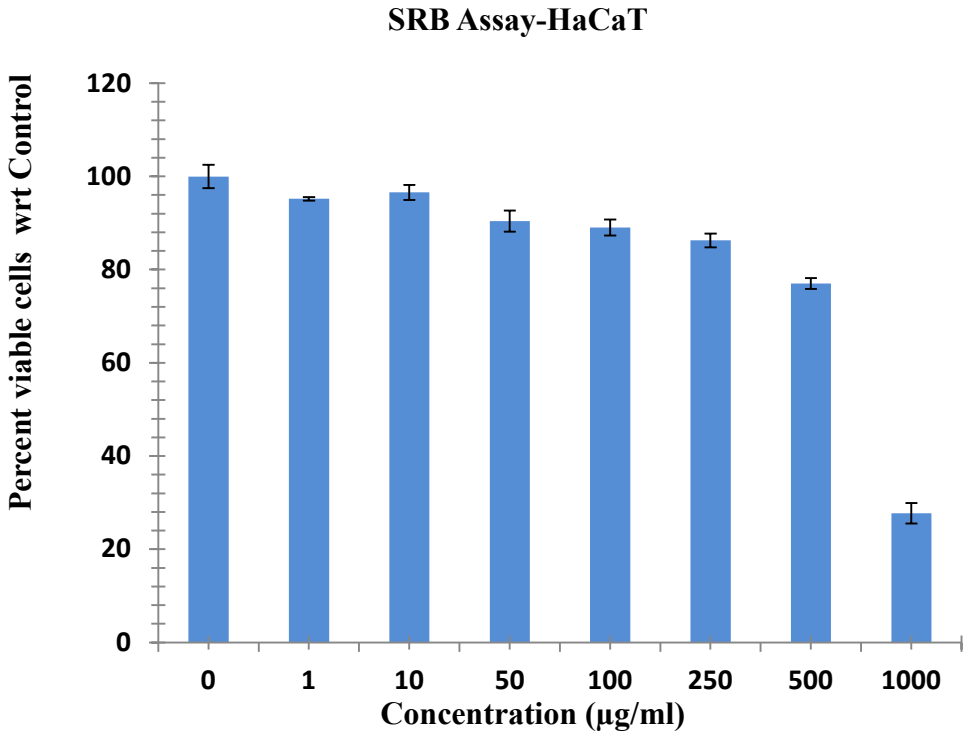


Figure 3: The bar graph of cytotoxicity study of crude aqueous extract of the leaves of the plant by *in vitro* SRB assay

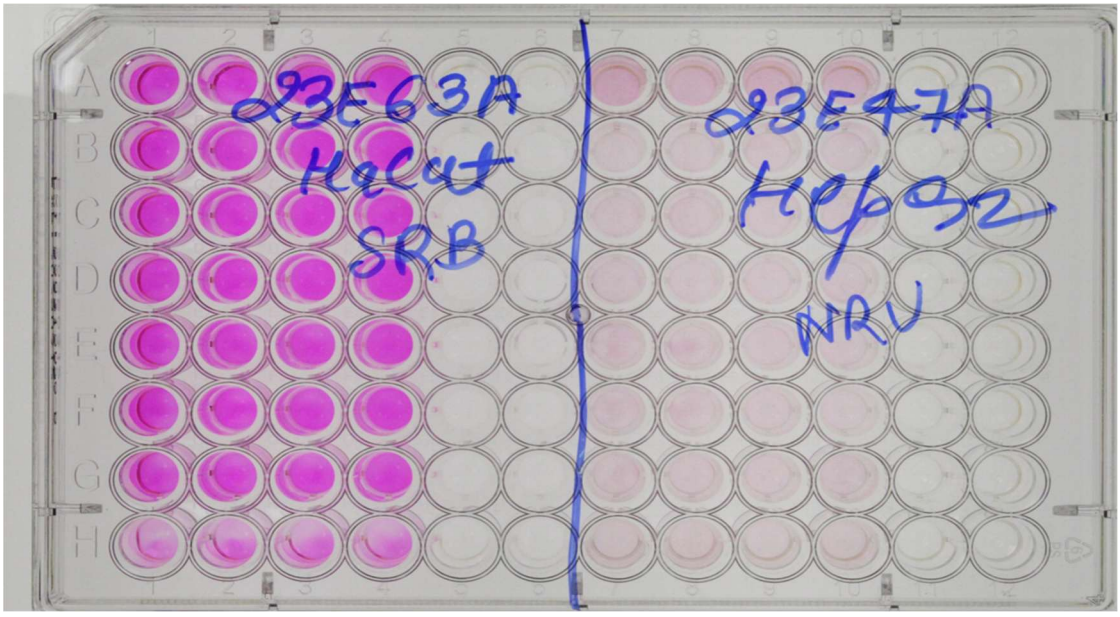
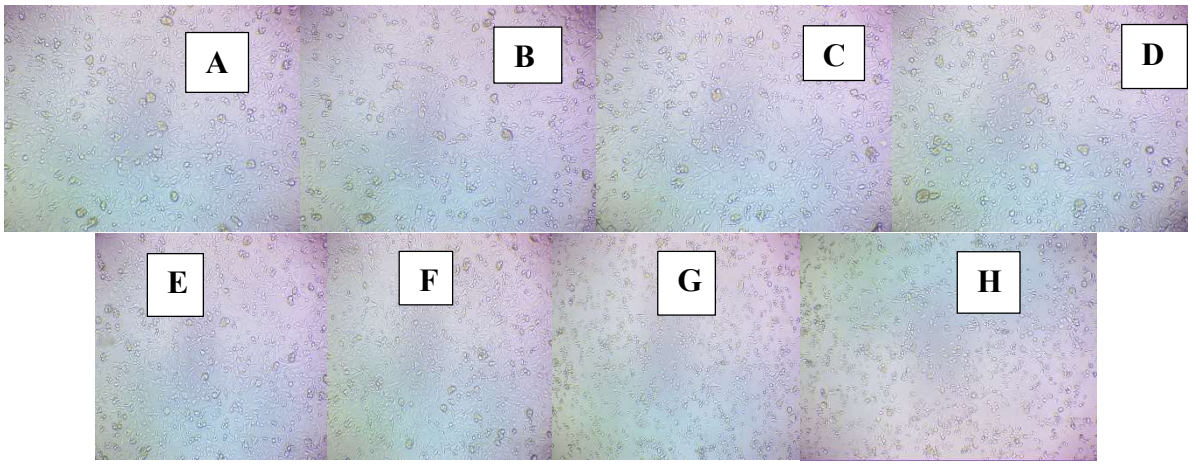


Figure 4: 96 well microplate reader used to test aqueous extract for HaCaT cell line



A) Negative Control B) 1 µg/ml C) 10 µg/ml D) 50 µg/ml E) 100 µg/ml F) 250 µg/ml G) 500 µg/ml H) 1000 µg/ml.
Figure 5: Stained images of SRB cytotoxicity assay against HaCaT cell line

The images obtained by an inverted microscope (Olympus eK2) using a camera (AmSope digital camera 10 MP Aptima CMOS) are shown in **Figure 5**. The images are captured for concentrations ranging from 1 µg/ml to 1000 µg/ml (images from B to H), including the negative control (image A). The image at 1000 µg/ml shows a significant change in the cytotoxicity against the HaCaT cell line and confirms the dose-dependent cytotoxicity of the crude aqueous extract of the leaves of *Phaseolus vulgaris* Linn.

CONCLUSION

For a comprehensive understanding of herbal plants and their extracts, it is very important to integrate HR-LCMS proofing along with the SRB assay. The SRB assay is used to study the biological effect of phytoconstituents on cell viability, while HR-LCMS gives in-depth knowledge of plant secondary metabolites, their possible structures, and ensures quality control. When combined, these techniques significantly expand our understanding of the effectiveness and security of herbal remedies. Assessing the toxicity of aqueous extracts on HaCaT cells is a crucial first step in dermatological research for developing safe and effective treatments for skin cancer. To create cancer treatments that are highly effective and safe for healthy skin, extensive research is essential to ensure that these extracts do not harm healthy skin cells.

The HR-LCMS and SRB assays of the aqueous extract of the leaves of *Phaseolus vulgaris* Linn. give important pharmacological bioactive compounds. Therefore, isolation, purification, and characterization of these bioactive phytoconstituents must take place.

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REFERENCES:

[1] Silpa SR, V C. a Review on Skin Cancer. Int Res J Pharm 2013;4(8):83–8.

[2] Bharadwaj R, Haloi J, Medhi S. Topical delivery of methanolic root extract of *Annona reticulata* against skin cancer. South African J Bot [Internet] 2019;124:484–93. Available from: https://doi.org/10.1016/j.sajb.2019.06.006

[3] Devi M, Dhanalakshmi S, Thillai Govindarajan GE, Tanisha BA, Sonalika T, Ruth JE, *et al*. A review on phaseolus vulgaris linn. Pharmacogn J 2020;12(5):1160–4.

[4] Ck S, Ps U. GC-MS and HR-LCMS fingerprinting of various parts of *Oroxylum indicum* (L .) Vent . A comparative phytochemical study based on plant part substitution approach. J Pharmacogn Phytochem 2020;9(6):1817–24.

[5] Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 2006;1(3):1112–6.

[6] Colombo I, Sangiovanni E, Maggio R, Mattozzi C, Zava S, Corbett Y, *et al*. HaCaT Cells as a Reliable in Vitro Differentiation Model to Dissect the Inflammatory/Repair Response of Human Keratinocytes. Mediators Inflamm 2017;2017.

[7] Laware SL. Original Research Article Sequential Extraction of Plant Metabolites. IntJCurrMicrobiolAppSci [Internet] 2015;4(2):33–8. Available from: http://www.ijcmas.com

[8] Subhashrao Pawar D, Nasreen S, Digambar Subhashrao Pawar C. HR-LCMS of phytoconstituents and antifungal activity of medicinal plants. ~ 173 ~ J Med Plants Stud 2018;6(1):173–6.

[9] Lachmann R. Sapropterin hydrochloride: Enzyme enhancement therapy for phenylketonuria. Ther Adv Endocrinol Metab 2011;2(3):127–33.

[10] Lin P, Tzi BN. Preparation and biological properties of a melibiose binding lectin from *Bauhinia*

variegata seeds. J Agric Food Chem 2008;56(22):10481–6.

[11] Mali RG, Dhake AS. A review on herbal antiasthmatics. Orient Pharm Exp Med 2011;11(2):77–90.

[12] Shariful Islam M, Mia M, Aminul Islam Apu M, Halder J, Farzanoor Rahman M, Islam M, *et al*. A comprehensive review on region based traditional Ayurvedic practitioner's plants secondary metabolites and their phytochemical activities in Bangladesh. ~ 202 ~ J Pharmacogn Phytochem 2015;3(6):202–16.

[13] Fronza G, Gold B. The biological effects of N3-methyladenine. J Cell Biochem 2004;91(2):250–7.

[14] Vogel-Adghough D, Stahl E, Návarová H, Zeier J. Pipecolic acid enhances resistance to bacterial infection and primes salicylic acid and nicotine accumulation in tobacco. Plant Signal Behav 2013;8(11):1–9.

[15] Jørgensen K, Morant AV, Morant M, Jensen NB, Olsen CE, Kannangara R, *et al*. Biosynthesis of the cyanogenic glucosides linamarin and lotaustralin in cassava: Isolation, biochemical characterization, and expression pattern of cyp71e7, the oxime-metabolizing cytochrome P450 enzyme. Plant Physiol 2011;155(1):282–92.

[16] Hirota K, Kazaoka K, Niimoto I, Kumihara H, Sajiki H, Isobe Y, *et al*. Discovery of 8-hydroxyadenines as a novel type of interferon inducer. J Med Chem 2002;45(25):5419–22.

[17] Bernard BK, Nakamura Y, Bando I, Mennear JH. Studies of the toxicological potential of tripeptides (L-valyl-L-prolyl-L- proline and L-isoleucyl-L-prolyl-L-proline): II. Introduction. Int J Toxicol 2005;24(SUPPL. 4):5–11.

[18] Pasdaran A, Naychov Z, Batovska D, Kerr P, Favre A, Dimitrov V, *et al*. Some European Gentiana Species Are Used Traditionally to Cure Wounds: Bioactivity and Conservation Issues. Diversity 2023;15(3):1–20.

[19] Sepahi M, Jalal R, Mashreghi M. Antibacterial activity of poly-l-arginine under different conditions. Iran J Microbiol 2017;9(2):103–11.

[20] Ahmad MF, A. Alsayegh A, Ahmad FA, Akhtar MS, Alavudeen SS, Bantun F, *et al*. Ganoderma lucidum: Insight into antimicrobial and

antioxidant properties with development of secondary metabolites. Heliyon 2024;10(3).

[21] Taha I, Keshk EM, Khalil AGM, Fekri A. Benzocaine as a precursor of promising derivatives: synthesis, reactions, and biological activity [Internet]. Versita; 2021. Available from: https://doi.org/10.1007/s11696-021-01808-3

[22] Slayden RA, Lee RE, Armour JW, Cooper AM, Orme IM, Brennan PJ, *et al*. Antimycobacterial action of thiolactomycin: An inhibitor of fatty acid and mycolic acid synthesis. Antimicrob Agents Chemother 1996;40(12):2813–9.

[23] Ganeshpurkar A, Saluja AK. The Pharmacological Potential of Rutin. Saudi Pharm J [Internet] 2017;25(2):149–64. Available from: http://dx.doi.org/10.1016/j.jsps.2016.04.025

[24] Jin SE, Ha H, Shin HK, Seo CS. Anti-allergic and anti-inflammatory effects of Kuwanon G and Morusin on MC/9 mast cells and HaCaT keratinocytes. Molecules 2019;24(2).

[25] Vajrabhaya L ongthong, Korsuwannawong S. Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays. J Anal Sci Technol 2018;9(1).