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BLACK GRAPE POMACE (*Vitis vinifera*) EXHIBITS POTENT ANTIFUNGAL AND IMMUNOMODULATORY ACTIVITY AGAINST *Candida albicans*: LC-MS CHARACTERIZATION AND IN VIVO VALIDATION IN A SILKWORM MODEL

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ABSTRACT
The search for viable and efficient substitutes is imperative due to Candida albicans' growing resistance to traditional antifungal treatments. This study explores the phytochemical content and multifunctional therapeutic potential of grape pomace, an underutilised agro-industrial by-product of *Vitis vinifera*. LC-MS and GC-MS analysis of two varieties, dark grape pomace (GP1) and light grape pomace (GP2), revealed different bioactive profiles, such as manumycin A and triamcinolone diacetate in GP1 and catechin and gallic acid in GP2. With a zone of inhibition of 22 mm and a minimum inhibitory concentration of 62.50 µg mL^{-1}, GP1 showed strong antifungal activity that was comparable to fluconazole's effectiveness. In addition to its antifungal properties, GP1 demonstrated dose-dependent anti-inflammatory activity through nitric oxide scavenging and IL-6 reduction, as well as improved wound healing in fibroblast tests. Crucially, better survival rates and decreased fungal load were validated by in vivo validation using the *Bombyx mori* model, underscoring its therapeutic value. This work demonstrates a robust relationship between biological activity and phytochemical composition, establishing grape pomace as a viable, affordable, and sustainable source of multipurpose bioactives. The results encourage the incorporation of agro-waste into pharmaceutical research and promote natural antifungal methods. Additionally, this work encourages circular economy techniques in drug discovery and healthcare applications and lays the groundwork for future translational research aimed at resistant fungal diseases.
Keywords: *Candida albicans*, Grape Pomace, LC-MS, GC-MS, Antifungal Activity, *Bombyx Mori*
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INTRODUCTION

Candida albicans infections have become a major global health concern in recent years, especially in immunocompromised people.[1] These infections can vary from minor mucocutaneous disorders to potentially fatal systemic illnesses. The efficiency of traditional antifungal medications has been significantly diminished by the rising incidence of antifungal resistance, biofilm formation, and pathogenicity adaptability of Candida albicans.[2] This has made the development of safer and more effective alternatives necessary.[3] The broad-spectrum antifungal, anti-inflammatory, and antibiofilm effects of natural products, particularly polyphenols and flavonoids derived from plants, have drawn a lot of interest.[4] The ability of phytochemicals, including catechins, resveratrol, and gallic acid, to disrupt fungal cell membranes, suppress virulence factors, and modify host immunological responses has been highlighted in recent research (2020–2024), suggesting their potential as next-generation antifungal agents.[5] Rich in phenolics, flavonoids, tannins, and stilbenes, grape pomace, a significant by-product of the wine and juice industries, represents a plentiful but underutilized agro-industrial waste.[6] The phytochemical makeup of grape pomace and its multifunctional biological activities, especially its antifungal efficiency, confirmed by in vivo validation, are not directly correlated, despite the increased interest in waste valorization. The majority of current research is restricted to in vitro assessments and lacks thorough molecular understanding and translational significance.[7] With an emphasis on their antifungal, anti-inflammatory, and wound-healing capabilities against Candida albicans, the current work

attempts to methodically examine the phytochemical profile and biological activities of grape pomace extracts. This study aims to close the current gap between phytochemical composition and medicinal performance by combining LC-MS-based chemical analysis with in vitro experiments and in vivo validation using the *Bombyx mori* animal.

Significantly, this study is in line with Sustainable Development Goal 9, which highlights resource efficiency, innovation, and sustainable industrial practices. In addition to supporting waste-to-wealth strategies, the conversion of grape pomace into bioactive medicinal agents aids in the creation of affordable and environmentally friendly pharmaceutical solutions.[8] Thus, our effort tackles the pressing issue of antifungal resistance while upholding the principles of the circular economy.

EXPERIMENTAL

Materials and Methods

Fresh grape pomace comprising skins, seeds, and residual juice from black grapes (Cabernet Sauvignon) and white grapes (Chenin Blanc) was collected from Grape City Winery, Sahakari Sanstha Ltd., Savlaj, during peak processing season. All chemicals and reagents used were of analytical grade.

Phytochemical Screening

Qualitative phytochemical analysis was performed to identify alkaloids (Mayer's, Wagner's, Hager's, and Dragendorff's tests), flavonoids (Shinoda test), anthocyanins (sodium hydroxide test), anthraquinone glycosides (Borntrager's test), saponins (foam test), steroids and terpenoids (chloroform test), carbohydrates (Fehling's test), phenolic compounds (lead acetate test), and proteins (Biuret test).[9]

Chemical Analysis

pH Determination: After 30 minutes of stirring and filtering, the pH of a 10% (w/v) suspension of grape pomace in distilled water was measured using a calibrated pH meter.

Loss on Drying[10]

Powdered pomace weighing around 2 gm was dried at 105°C until it reached a consistent weight. Moisture content was calculated as:

$$\text{Moisture (\%)} = [(W_2 - W_3)/(W_2 - W_1)] \times 100.$$

Total Ash[11]

Samples were incinerated at 550°C for 4-6 hours in a muffle furnace.

$$\text{Total ash (\%)} = [(W_3 - W_1)/(W_2 - W_1)] \times 100.$$

Protein Content[12]

Determined using the Kjeldahl technique.

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25.$$

Carbohydrate Content

With glucose as a standard, the anthrone reagent method checked absorption at 620 nm[13].

Lipid Content[14]

Petroleum ether pulled compounds out over six to eight hours through a Soxhlet extractor.

$$\text{Lipid (\%)} = (\text{Weight of lipid/Weight of sample}) \times 100$$

Total Dietary Fiber

Determined by the enzymatic gravimetric technique after amyloglucosidase21, protease, and α-amylase treatments.[15]

Pectin Content

Extracted using 0.1 N HCl, precipitated as calcium pectate, and weighed.

Reducing Sugars

Fructose and glucose measured using the DNSA technique at 540 nm.[16]

Biochemical Analysis

Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent method was used to calculate TPC. After 30 minutes of incubation, absorbance was measured at 760 nm. Gallic acid equivalents (GAE)/g are used to express the results.[17]

Vitamin C

Titration happens through a dye called DCPIP, alongside an extract made with 3% metaphosphoric acid.

Anthocyanins

Determined by measuring absorbance at 520 and 700 nm using the pH differential method with buffers at pH 1.0 and pH 4.5. Cyanidin-3-glucoside equivalents are used to express the results.[18]

LC-MS Analysis

An Agilent 6550 iFunnel Q-TOF mass spectrometer with a Dual AJS ESI source running in positive ion mode and connected to an Agilent 1260/1290 Infinity UHPLC system was used for the LC-MS study. Methanol-water (80:20, v/v) was used to extract air-dried powdered samples, which were then filtered (0.22 µm) and 3 µL injected. Using water (0.1% formic acid) and acetonitrile (0.1% formic acid) with a gradient from 5% to 100% acetonitrile at a flow rate of 0.3 mL min^{-1}, separation was accomplished on a reverse-phase C18 column at 40 °C. The nebuliser pressure was 35 psi, the capillary voltage was 3500 V, the drying gas flow was 13 L min^{-1}, and the gas temperature was 250 °C. Mass spectra were obtained between 126 and 1200 m/z.

Chromatographic Separation and Structure Elucidation

TLC Analysis

Silica gel plates (0.4 mm thickness, 5×15 cm) activated at 110°C for one hour. Mobile phase: Toluene: Ethyl Acetate: Ammonia (3.3:6.7:0.01). Detection with iodine reagent.

FTIR Analysis

IR spectra recorded using ATR on FTIR Spectrophotometer (BRUKER) at Rajarambapu College of Pharmacy, Kasegaon.

GC-MS Analysis

Performed for qualitative and quantitative determination of volatile and semi-volatile organic compounds.

Biological Characterization

***In vitro* Antifungal Activity**

Agar Well Diffusion Method

Candida albicans ATCC 14053 served as the test organism. Antibiotic Assay Medium No. 19 (pH 6.1±0.2) was inoculated with a culture suspension that was adjusted to 60–70% OD at 530 nm. Wells with a diameter of 6 mm were filled with 100 µL of test samples (10 mg/ml) and reference fluconazole (1 mg/ml). After plates were incubated at 30–35°C for 24–48 hours, zones of inhibition were evaluated 27–30 hours later.[19-21]

MIC Determination

The broth dilution method used serial dilutions ranging from 500 µg/ml to 31.25 µg/ml to determine MIC. Sabouraud's Dextrose agar was supplemented with 100 µL of cell culture (2×10^2 cells/ml) and 100 µL of different extract concentrations. Optical density was measured at 600 nm after a 48-hour incubation period. The minimum inhibitory concentration (MIC) is the lowest concentration that results in 50% inhibition.[22]

Anti-Inflammatory Assay

RAW 264.7 mouse macrophage cells seeded in 96-well plates (1×10^5 cells/well) and incubated 24 hours at 37°C, 5% CO_2. Cells were treated with LPS (1 µg/ml) + test sample for 24 hours. Controls included untreated cells, LPS-treated cells, and LPS + dexamethasone (10 µM). IL-6 measured by ELISA, nitric oxide by Griess assay (absorbance at 540 nm).[23]

$$\text{Inhibition (\%)} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100$$

Wound Healing Scratch Assay

Six-well plates were seeded with L929 fibroblast cells (2×10^3cells/ml). Following an overnight culture, DPBS was used to wash the cells, and a sterile 200 µL tip was used to make a scratch. 100 µL of the test sample and 5 µg/ml of Cipladine (positive control) were applied to the cells. Using an inverted microscope, cell migration and wound closure were monitored after 48 hours. SAGLO software was used to analyse wound closure.[24, 25]

***In vivo* Silkworm Model**

2 to 3 g of *Bombyx mori* caterpillars in their fifth stage were stored at 30°C. Only those who are strong are chosen, and their upper skin is lightly scraped. After applying a suspension of Candida albicans (1×10^2 CFU/ml, 10 µl) to the abraded area, it was incubated for 4-6 hours at 37°C with >70% humidity.[26] Six experimental groups (n=10 per group):

S1: Uninfected control (PBS/solvent)
S2: Infection control (PBS/solvent)
S3-S5: Infected + GP extract (50, 100, 150 mg/kg)
S6: Infected + fluconazole (5 mg/ml)

For two to three days, a 10 µl dosage was applied directly to the skin every 12 to 24 hours. Survival rates, tissue discolouration, and the quantity of fungus were all monitored. Darkening levels ranged from zero (no darkening) to three (severe cases). The specimens were carefully opened on the third day. A buffer solution was combined with the impacted areas. These mixes were diluted step-by-step. After that, samples were placed on fungus-specific agar plates. Plates were kept at 30°C in a heated room. Colonies were not visible for two full days. Examining growing areas revealed CFU/ml. A measure of the intensity of the infection was provided by the results.

Statistical Analysis

Data shown as mean ± SD. When looking at survival, researchers used Kaplan-Meier along with the log-rank test. For fungus levels and similar measures, analysis went through one-way ANOVA followed by Tukey's correction afterwards. A value under 0.05 marks a result that stands out. Each test was run three times.

RESULTS AND DISCUSSION

Phytochemical Screening

Several bioactive components, such as flavonoids, phenolics, alkaloids, and saponins, were found in both GP1 and GP2, according to the qualitative phytochemical profile (Table-1). Terpenoids, on the other hand, were only found in GP1, suggesting a relatively larger phytochemical variety. This compositional advantage is important since terpenoids and polyphenols are known to have antifungal and anti-inflammatory effects by disrupting membranes and modifying oxidative stress pathways. By showing that agricultural waste, like grape pomace, can operate as a reservoir of multipurpose bioactives, supporting its valorization in pharmaceutical applications, our findings add to the expanding body of knowledge on plant-derived antifungal agents.

Table-1: Phytochemical Constituents of Grape Pomace Extracts

Phytochemical Class	GP1	GP2
Alkaloids	+	+
Flavonoids	+	+
Anthocyanins	+	+
Anthraquinone Glycosides	+	+
Saponins	+	+
Terpenoids	+	–
Steroids	–	–
Carbohydrates	+	+
Phenolic Compounds	+	+
Proteins	+	+

(+) Present, (–) Absent

Chemical Analysis

There are clear compositional differences between GP1 and GP2 in the physicochemical characteristics of grape pomace extracts (Table-2). Compared to GP2 (4.11 ± 0.02), GP1 had a much lower pH (3.59 ± 0.02), indicating increased acidity, which is known to improve the stability of polyphenolic chemicals, especially anthocyanins. Both samples' moisture contents stayed below 10%, indicating strong storage stability and decreased vulnerability to microbial deterioration. GP1 had higher protein content (11.28 ± 0.04%) and total ash (4.88 ± 0.03%) than GP2, which could be explained by a higher percentage of seed and skin material, which are known to be rich in structural proteins and mineral-bound phenolics. On the other hand, GP2's increased pulp and seed oil composition was reflected in its higher lipid content (9.23 ± 0.10%) and carbohydrate content (58.77 ± 0.25%). Better functional qualities, such as gelling and stabilising capability, are suggested by GP1's higher pectin concentration (6.27 ± 0.08%), which may also lead to increased wound healing activity. Overall, the physicochemical profile shows that GP1 has a composition that is more advantageous for the stability of bioactive compounds and the effectiveness of treatment.

Table-2: Chemical Parameters of Dark (GP1) and Light (GP2) Grape Pomace

Parameter	GP1 (Dark Grape Pomace)	GP2 (Light Grape Pomace)
pH	3.59 ± 0.02	4.11 ± 0.02
Moisture Content (%)	6.18 ± 0.06	7.03 ± 0.08
Total Ash (%)	4.88 ± 0.03	4.21 ± 0.04
Protein Content (%)	11.28 ± 0.04	9.89 ± 0.04
Carbohydrate Content (%)	55.67 ± 0.40	58.77 ± 0.25
Lipid Content (%)	8.13 ± 0.10	9.23 ± 0.10
Total Dietary Fiber (%)	48.40 ± 0.15	52.70 ± 0.17
Pectin Content (%)	6.27 ± 0.08	5.75 ± 0.05
Reducing Sugars (%)	9.80 ± 0.13	9.03 ± 0.10

Values are expressed as mean ± SD (n = 3)

Biochemical Analysis

GP1 is substantially more concentrated in bioactive chemicals than GP2, according to biochemical examination (Table-3). GP1's total phenolic content (62.93 ± 0.37 mg GAE/g) was almost 29% higher than GP2's (48.90 ± 0.16 mg GAE/g), suggesting a higher concentration of antifungal and antioxidant components. GP1 also had a higher vitamin C content (12.27 ± 0.15 mg/100 g), which would help explain its improved immunomodulatory and antioxidant qualities. One important type of bioactive flavonoids, anthocyanins, was found only in GP1 (4.88 ± 0.04 mg/g) and not in GP2. This distinction is explained by the black grape pomace's colored skins. GP1's enhanced antifungal, anti-inflammatory, and wound-healing properties are highly connected with its higher quantities of phenolics and anthocyanins. These substances are known to disrupt membranes, inhibit fungal enzymes, and alter inflammatory mediators, including nitric oxide and IL-6. By explicitly connecting phytochemical richness with antifungal and immunomodulatory activity, this study closes a crucial gap between chemical profiling and biological validation, strengthening the body of current evidence. The idea that phytochemical richness directly affects antifungal and immunomodulatory efficiency is supported by these results, which provide a solid compositional basis for the increased biological activity of GP1.

Table-3: Biochemical Constituents of GP1 and GP2 Extracts

Parameter	GP1 (Dark Grape Pomace)	GP2 (Light Grape Pomace)
Total Phenolic Content (mg GAE/g)	62.93 ± 0.37	48.90 ± 0.16
Vitamin C (mg/100 g)	12.27 ± 0.15	10.97 ± 0.15
Anthocyanins (mg/g)	4.88 ± 0.04	---

Values are expressed as mean ± SD (n = 3)

LC-MS Analysis

Different metabolic patterns were shown in Fig.-1 of the LC-MS profiling, with GP1 displaying broader signals (1.5–10 min) corresponding to polar and semi-polar compounds like phenolics, alkaloids, and glycosides, and GP2 displaying early peaks (1.3–1.6 min) primarily from organic acids and low-molecular-weight phenolics. Subsequent eluting substances included hydroxycinnamic acids, flavan-3-ols,

and derivatives of benzoic acid, which are compatible with documented constituents of grape pomace such as gallic, caffeic, vanillic acids, catechin, and ellagic acid.

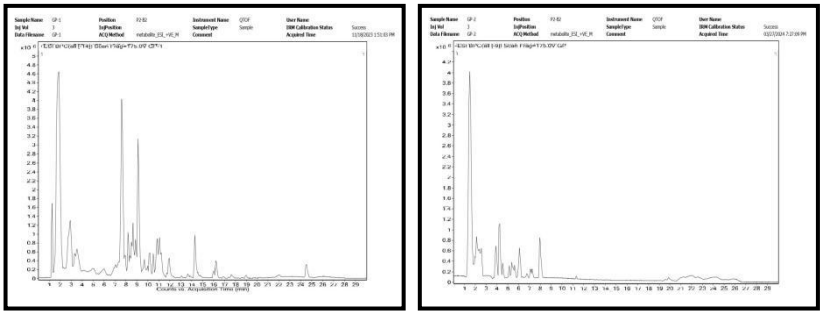

Fig.-1: LC-MS Chromatographic Profiles of GP1 and GP2 Extracts Showing Differential Distribution of Phytoconstituents

Isolation and Characterisation

TLC Analysis

Four fractions isolated with Rf values: GP1-F1 (0.86, Triamcinolone Diacetate), GP1-F2 (0.70, Manumycin A), GP2-F1 (0.89, Catechin), GP2-F2 (0.58, Gallic Acid).

FTIR Analysis

Spectral analysis confirmed the presence of hydroxyl groups (3448 cm^{-1}), carbonyl groups (1796, 1694 cm^{-1}), aromatic rings (1589 cm^{-1}), and ether linkages (1051 cm^{-1}), supporting polyphenolic structures.

GC-MS Analysis

Figure-2 depicts the GP1 revealed 36 compounds with major constituents including Triamcinolone Diacetate (35.60%), Manumycin A (28.30%), Ellagic Acid (10.479%), and DL-Glucitol (3.10%). GP2 showed 22 compounds with Catechin (39.05%), Gallic Acid (19.03%), Proanthocyanidins (5.712%), and Hexadecane (4.44%) as major components.

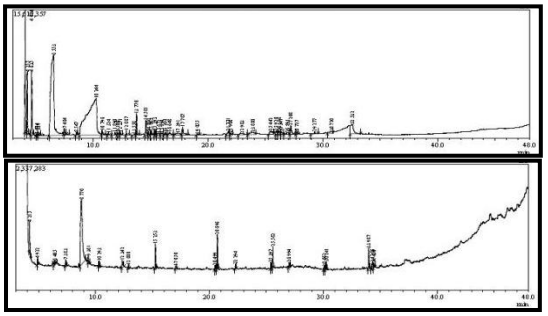

Fig.-2: GC-MS Analysis of GP1 and GP2 Extracts Illustrating the Qualitative and Quantitative Distribution of Volatile and Semi-Volatile Compounds

***In vitro* Antifungal Activity**

Agar Well Diffusion

GP1's effect on *Candida albicans* was doubled by a single dose, increasing from 9 to 22 mm when the concentration changed from 5 to 10 mg/ml. Fluconazole only required a tenth of that dose to attain an additional 33 mm. GP1's effectiveness stemmed from both its potency and its mode of action, which included disrupting fungal membranes, preventing the generation of essential sterols, and even disrupting the pathogen's ability to cause damage.

MIC Determination

GP1 demonstrated a growth limit of 62.50 µg/ml at half the concentration. As seen in Fig.-3, inhibition started at a little less than one-third and dropped to 31.25 µg/ml. Suppression increased as levels did, peaking at over 62.82% at the highest test point. Fluconazole was more effective, blocking between 76.59 and 94.89%. In contrast, its effective dose was also reduced by half. Teamwork among its components, rather than brute force, is what sets GP1 apart. Quercetin and resveratrol work together. Anthocyanins are

joined by catechins. Grip is increased by tannins. When combined, they produce observable fungal resistance. Given the extract's natural origin, the observed antifungal effectiveness is noteworthy despite being somewhat less potent than fluconazole. Polyphenols, flavonoids, and tannins work in concert to disrupt fungal cell membranes and prevent ergosterol manufacture, which is responsible for the activity. In light of growing antifungal resistance, our results encourage the development of plant-based antifungal medicines as substitutes or supplements to traditional treatments.

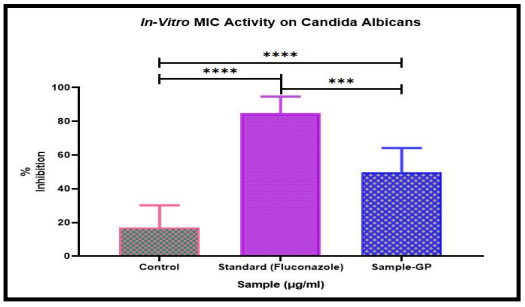

Fig.-3: Dose-Dependent Antifungal Activity of Grape Pomace Extracts against *Candida albicans,* Showing Percentage Growth Inhibition at Varying Concentrations

Anti-Inflammatory Activity

IL-6 Inhibition

One way to look at it is that, like diclofenac, bigger doses worked better. GP reduced IL-6 to 120 pg/ml when evaluated at 100 µg/ml, precisely mirroring diclofenac. This type of decline suggests potent anti-inflammatory actions, mostly through modifying the NF-κB pathway. With each increase in concentration, the pattern was not random; rather, it followed a distinct trend.

Nitric Oxide Scavenging

As indicated in Fig.-4, GP exhibited concentration-dependent NO radical scavenging: 45.36% at 10 µg/ml, 56.02% at 40 µg/ml, and 66.68% at 100 µg/ml, compared to ascorbic acid (62.66-84.00%). Results confirm significant antioxidant capacity through hydrogen atom donation and free radical stabilisation. The immunomodulatory potential of GP1 is highlighted by the inhibition of pro-inflammatory mediators, which implies modification of important signalling pathways, including NF-κB. When treating infections where inflammation worsens tissue damage, this combination of antifungal and anti-inflammatory action is especially beneficial, improving therapeutic results.

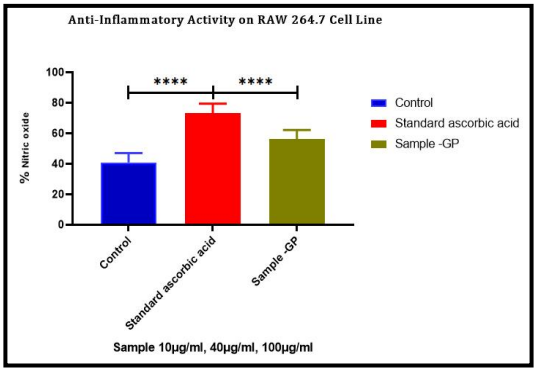

Fig.-4: In vitro anti-inflammatory activity of grape pomace extracts in RAW 264.7 macrophage cells

Wound Healing Activity

Higher concentrations of GP extract caused cells to migrate more quickly, as demonstrated by the results in Fig.-5. At 10 µg/ml, coverage was 54.38%; at 40 µg/ml, it was 59.84%; and at 100 µg/ml, it was 65.16%. These outcomes are comparable to the 80.35-87.53% range observed with Cipladine. Polyphenols, which aid in the repositioning of skin cells, the construction of structural proteins, the formation of new blood vessels, and the calming of harmful chemicals, seem to be the cause of this effect.

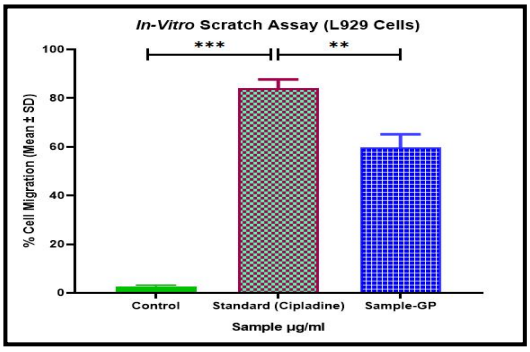

Fig.-5: Effect of Grape Pomace Extracts on Wound Healing in L929 Fibroblast Cells Assessed by Scratch Assay

In vivo Silkworm Model Validation

Survival Analysis

After three days without treatment, survival fell to zero. GP extract-treated groups remained stable; half of them survived with S3, more with S4, and the majority with S5 (Fig.-6). As the dosage grew, protection increased. At 80%, fluconazole performed somewhat better. The substance directly combats fungus and modifies the immune system. Evidence clearly demonstrates its efficacy within living things.

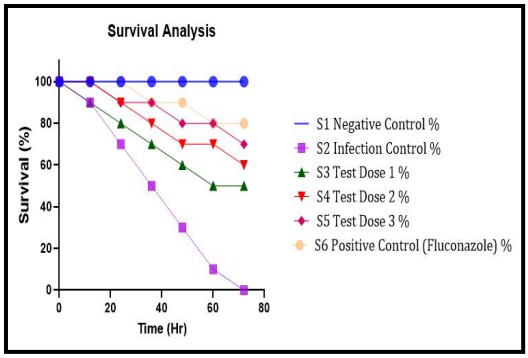

Fig.-6: Kaplan-Meier Survival Curves of *Bombyx mori* Larvae Infected with *Candida albicans* and Treated with Grape Pomace Extracts

Melanization Scoring

Disease levels decreased more as the dosage increased. While untreated plants scored 3.0, almost matching fluconazole at 0.6, S3 reached 1.8, S4 fell to 1.4, and S5 fell to 0.9. Less tissue browning indicates that the fungus's spread has slowed.

Fungal Burden

GP treatment progressively reduced fungal load: 780 CFU (S3), 910 CFU (S4), 1070 CFU (S5) compared to infection control (551 CFU), approaching fluconazole (1230 CFU) exhibited in Fig.-7. Results demonstrate effective fungal growth inhibition *in vivo*. With higher survival rates and a lower fungal burden in infected larvae, the in vivo investigation validated GP1's therapeutic efficacy. The findings validate the bioactivity's potential for practical applications by showing how well it translates into a live system. The silkworm model contributes to translational research approaches by offering an economical and morally sound substitute for early-stage in vivo validation. By encouraging the conversion of agro-industrial waste into high-value medicinal goods, the study's conclusions firmly support Sustainable Development Goal 9. An example of resource efficiency and sustainable innovation is the transformation of grape pomace into bioactive antifungal and therapeutic compounds. In addition to addressing the problem of antifungal resistance, this strategy reduces environmental waste and produces financially feasible healthcare solutions by supporting circular economy principles.

CONCLUSION

This study shows that black grape pomace (GP1) from *Vitis vinifera* has strong antifungal, anti-inflammatory, and wound-healing effects against *Candida albicans*. LC-MS and GC-MS profiling have

established a robust correlation between GP1's increased biological activity and its higher phenolic and anthocyanin content. With a minimum inhibitory concentration of 62.50 µg/mL, GP1 demonstrated significant antifungal activity in addition to effectively suppressing inflammatory mediators (nitric oxide and IL-6) and promoting fibroblast-mediated wound healing.

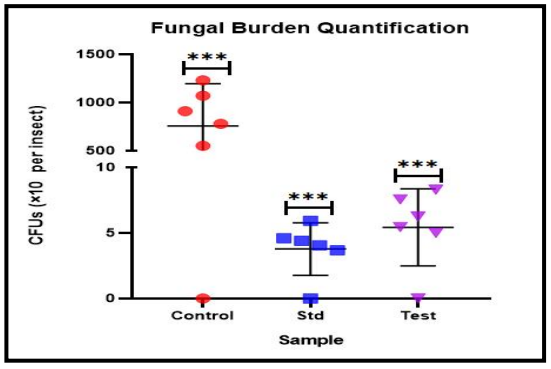

Fig.-7: Quantitative analysis of fungal burden in *Bombyx mori* larvae following treatment with grape pomace extracts

Crucially, its therapeutic value was established by in vivo validation using the *Bombyx mori* model, which verified increased survival rates and decreased fungal burden. These results demonstrate grape pomace as a viable, affordable, and sustainable source of multipurpose bioactive chemicals with potential uses in wound care and antifungal therapy. In order to confirm its safety and effectiveness in human models, it is advised that future research concentrate on the isolation of active ingredients, thorough mechanistic analyses, and clinical translation. Additionally, by encouraging the valorisation of agro-industrial waste into medicinal goods with added value, this work supports Sustainable Development Goal 9. In drug research, the conversion of grape pomace into medicinal medicines promotes circular economy, resource efficiency, and sustainable innovation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-9: Industry, Innovation, and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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