



A New Eco-Friendly Innovation Enhances Food Security and Prevents Fungal Pests in Post-harvest Crops

Prosopis juliflora water-soluble leaf ethanolic (PJ-WS-LE) extract as a preventive coating for post-harvest fruits



Prosopis juliflora leaves

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Introduction

Food security is one of the major global challenges and a cornerstone of Qatar National Vision 2030. Although the agricultural industry is a growing sector in Qatar, about 90% of the fruits consumed in the country are imported. Many challenges hinder the cultivation of local fresh produce, including high summer temperatures and water scarcity. Postharvest diseases impact both locally produced fruits and vegetables, as well as imported ones, especially during storage. These diseases are primarily caused by fungal and bacterial pathogens, which can result in significant economic losses for both farmers and consumers.

Chemical pesticides and fungicides with their environmental pollution footprints are not a recommended option to extend fresh produce shelf life. Many countries around the world are setting a maximum residue limit (MRL) of chemicals on the skin of their fruits and vegetables. When pesticides are applied, only 0.1% of the applied amounts reach the target pests; the remaining (99.9%) are drifted to affect non-target populations and/or remain in the environment to cause biodiversity loss, water pollution, and soil contamination. Therefore, pesticides are reaching humans directly on their fresh produce and indirectly through the environment to cause various health problems. A successful replacement for chemical pesticides would be a product that effectively controls pre-existing spoilage causes, leaves residues that prevent subsequent infections, and inhibits the sporulation of existing spores, thereby reducing economic losses.

Prosopis juliflora (Mimosaceae), the focus of this study, is a short tree, native to Mexico, South America, and the Caribbean. It is an increasingly spreading invasive species in Asia, Austria, and other places around the world. Among the globally distributed plants, *P. juliflora* is considered one of the world's 100 most invasive species. *P. juliflora* is an invasive species in the state of Qatar, introduced in the 1950s as a soil stabilizer and for greening purposes. Mechanical irradiation of *P. juliflora* is costly and without effective control when it comes to the spread of seedlings of the plant. Nowadays, the idea of control through mass utilization is recommended. Using the leaves of this plant to prepare an extract that can serve as a bio-controller or natural anti-spoiling agents would help in utilizing the plant and would solve a major food security problem. This research would provide a solution to the worldwide agricultural problem.

Description of the Proposed Technology

The ethanolic extract from *Prosopis juliflora* leaves is prepared by incubating leaf powder in 70%

ethanol at 45°C with shaking at 50 cycles per minute for 48 hours. The supernatant contains all active phytochemicals, which are collected as a powdery or gummy material after the solvent has evaporated. The ethanolic crude extract is typically re-suspended in sterile distilled water to create stock solutions. Only the water-soluble portion is usually sterilized using a 0.2µm syringe filter for use in further in vitro and in vivo investigations.

Proof of Concept

In vitro analysis of PJ-WS-LE extract showed solid results on the efficacy of PJ-WS-LE extract against a wide range of pathogenic microorganisms. Antimicrobial tests showed total inhibition of *Botrytis cinerea*, *Alternaria alternata*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* with minimum inhibitory concentrations (MICs) ranging between 0.125 and 1 mg/ml. The in vitro results were supported by fruit bioassay results during which PJ-WS-LE extract was explored as an alternative biological controller. Artificially inoculated tomato and mango samples were completely protected from the disease caused by fungi that were inhibited by the extract in the laboratory assays, including *B. cineraria* and *A. alternata*. Figure 1 shows the effectiveness of the extract in protecting mangoes from *A. alternata* (preventive treatment) and in curing *A. alternata* in mangoes (curative treatment). PJ-WS-LE extract embedded in edible coating has also been demonstrated as an effective coating material to maintain mangoes quality at low temperature for up to five weeks of storage. On the other hand, spraying cucumber samples with 8mg/ml of PJ-WS-LE extract extended cucumber shelf-life at 22°C by 77% (Figure 2) and maintained samples' acceptable quality for three weeks of storage at 8°C. PJ-WS-LE extract alone extended strawberries' shelf-life at 4°C by 2.32 folds and maintained the best storage quality parameters when embedded in 1% chitosan, which includes sensory characteristics, maintenance of firmness and total soluble solids levels, lower surrounding microbial count, lower percent weight loss, and maintaining total antioxidant levels. In citrus fruits, *Penicillium italicum*, one of the main citrus fruits spoiling agents, was 100% cured in lemons and prevented in oranges when fruits are treated with PJ-WS-LE extract. *Penicillium digitatum* infection was also controlled by the extract (Figure 3). PJ-WS-LE extract also showed success as a coating material to maintain lemons and oranges storage parameters at 4°C. The effectiveness of PJ-WS-LE extract against postharvest phytopathogens opens doors for the application of this natural product as a replacement for chemical pesticides and to manage the invasive plant via utilization.



From left: Dr. Atifa Ismail, Master's student Bushra Al-Janahi, Prof. Mohammed Abu-Dieyeh, Dr. Iman Saleh, and Master's student Amal Hadi.



Figure 1: Disease incidence in mango samples inoculated with *A. alternata*. All samples are 10 days old after treatment at 25 °C. **(a)** Preventive effect of PJ-WS-LE extract. **(b)** Curative effect of PJ-WS-LE extract. **(c)** Control samples inoculated with *A. alternata* and not treated with PJ-WS-LE extracts.



Figure 2: Cucumber samples of the shelf-life evaluation experiment. **(a)** The 30 fresh samples coated with 8 mg/ml PJ-WS-LE extract at 0-day post-treatment. **(b)** The remaining 20 control (non-treated) samples at 6 days post-treatment. **(c)** 20 of the PJ-WS-LE-treated samples at 6 days post-treatment.

The novel extraction method and the selection for a water-soluble end-product make future applications easier. Derived from an invasive tree that is widely available, the extract offers a low-cost solution for countries most in need of effective spoilage control for fresh produce. Time stability and heat stability were evaluated, and the extract maintained its antimicrobial effectiveness up to six months of the trial, and remains active upon exposure to high temperature up to 70°C. These results are very important for the future commercialization of the extract.

Novelty

As a naturally based antifungal agent, PJ-WS-LE extract is different than the currently used pesticides in the agricultural domain. Firstly, it is a novel solution, making it more effective as spoilage agents have not yet developed resistance to it. Additionally, it does not pose a threat to the environment or human health. Compared to other biological controllers described in the literature, the novelty of the PJ-WS-LE extract lies in the unique characteristics of the end product. It is water soluble, stable with time, and heat stable, which means that it can be easily applied in the form of a spray or embedded in any stabilizing packaging. It is also suitable for the hot weather of the Qatari environment. The extraction method is simple and not expensive. The tree is an invasive species, widely available in many countries, and it can be cultivated easily in places where it doesn't usually grow.

Development Roadmap and Requirements

PJ-WS-LE extract is ready to be taken to the next stage as an antifungal agricultural solution in the current development status for large-scale in-field trials. It can also be used as an antifungal storage coating material for large-scale storage preservation trials. The extract has a published patent application number 18/772,403 under the title "PROSOPIS JULIFLORA WATER-SOLUBLE LEAF ETHANOLIC (PJ-WS-LE) EXTRACT AS A PREVENTIVE COATING FOR POST-HARVEST FRUITS". Under the scope of the ARG01-0531-230424 project, further analysis would be conducted, which includes:

1. Fractionation of the crude extract using preparative HPLC to identify the active fraction.
2. Chemical identification of the active phytochemicals in the active fraction. The final structure of the active phytochemical(s) can be determined using Nuclear Magnetic Resonance (NMR).
3. Active fraction cytotoxicity evaluation will also add value to the end-product and would facilitate future commercialization.

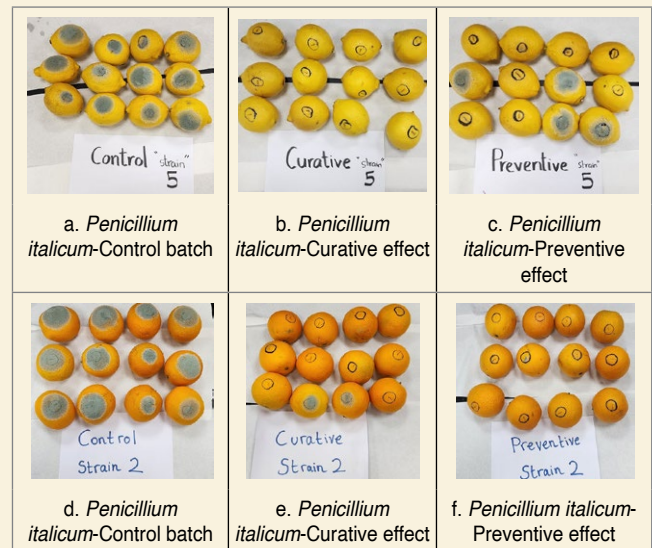


Figure 3: Preventive and curative effect of PJ-WS-LE extract on *Penicillium italicum* artificially inoculated into lemon and orange samples.

Acknowledgments

The researchers would like to express their sincere gratitude to Qatar University, represented by the College of Arts and Sciences and the Department of Biological and Environmental Sciences, for providing all the necessary resources for the success of this research. Special thanks and appreciation are also extended to the Qatar Research, Development, and Innovation Council for their financial support of the research projects ARG01-0531-230424 and UREP29-146-4-004.



The researcher while isolating fungi from tomatoes.