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In silico PASS analysis and determination of antimycobacterial, antifungal, and antioxidant efficacies of maslinic acid in an extract rich in pentacyclic triterpenoids

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ABSTRACT

Objective/background: Microbial infections such as tuberculosis is a major cause of mortality worldwide. Plant-derived phytochemicals have a long history of providing much-needed novel therapeutics. Triterpenoids are among the prominent phytochemicals that possess numerous biological activities. Among them is maslinic acid (MA), a biologically active olean-type pentacyclic triterpenoid. In search of a novel antimicrobial agent, we aimed to evaluate the antimicrobial potential of MA.

Methods: Antibacterial and antifungal activity was evaluated through the agar well diffusion method. Antitubercular activity was analysed through the agar well diffusion and disc diffusion methods, respectively. Antioxidant capacity was determined through assays for total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl radical scavenging, hydrogen peroxide radical scavenging, and Fe^{3+} reducing power. The program Prediction of Activity Spectra for Substances was used to calculate the possible biological activity of MA.

Results: MA showed dose-dependent antioxidant activity similar to that of ascorbic acid. It had no inhibitory effect on bacterial strains, but it had moderate activity against the fungi *Aspergillus flavus* and *Ustilago maydis*, with *Aspergillus niger* being the most sensitive to MA. MA also exhibited strong antimycobacterial activity. Probable antioxidant, antibacterial, and antifungal activity of MA based on software calculations are 0.479, 0.363 and 0.589 respectively.

Conclusion: This work provides scientific evidence of the antioxidant, antifungal, and antimycobacterial activities of MA, showing its potential application in the development of natural antioxidants and antimicrobial agents for the agro-food and pharmaceutical industries.

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Introduction

The increasing availability of life-saving drugs such as antibiotics has led to advances in the developed medical world. However, extensive use of these synthetic drugs has led to life-threatening side effects and development of resistant strains of deadly pathogenic microorganisms [1,2]. Development of novel antimicrobial drugs that overcome these problems is therefore a major challenge for pharmaceutical industries. Plant-derived antimicrobials and antioxidants have a long history of providing much-needed safe and novel therapeutics [3–5]. Plants constantly interact with rapidly changing and potentially damaging external environmental factors such as microbial attack and oxidative stress. This interaction involves alternative defence strategies, including synthesis of a wide variety of chemical metabolites that counter these stressors. According to the World Health Organization, medicinal plants are the best source of various biologically active drugs [2]. To date, only 10–15% of plant species have been explored for their therapeutic potential for various ailments [6].

Free radicals generated during various metabolic processes affect multiple functions such as defence against infections and cancers [7,8]. Enzymatic and nonenzymatic defence systems within the body neutralise these free radicals by balancing their oxidation and reduction reactions. Under certain conditions such as infections, which induce oxidative stress, free radicals overwhelm this balance and exacerbate the adverse effects of such diseases [9]. The literature indicates that use of phytochemicals with antioxidant and antimicrobial potential are associated with a low risk of mortality and sepsis [3].

Triterpenes, secondary metabolites of isopentenyl pyrophosphate oligomers, constitute a large group of phytoconstituents. More than 20,000 natural triterpenoids are known, some of which exist in free form and some in combination with other constituents [10]. Most of them are present in plants such as seaweed, and some are part of the wax-like coatings of numerous fruits and medicinal herbs [11]. Maslinic acid (MA) is an olean-type pentacyclic triterpenoid that is structurally similar to oleanolic acid, a pentacyclic triterpenoid. It possesses activity against type-2 diabetes [12], malaria [13], and cancer [14]. Oleanolic acid is a potentially hepatoprotective, anti-inflammatory, antioxidant, anticancer, antitubercular agent [15–17]. Therefore, it is of interest to evaluate the biological activities of MA against common human pathogens.

Materials and methods

Collection of MA

A MA-rich alcoholic fraction of leaves of the plant *Eriobotrya japonica* was obtained from 3 W Botanical Extract (batch no LLPE-111227, China).

Reagents and chemicals

Analytical-grade dimethylsulphoxide (DMSO), methanol, sodium hydroxide, hydrogen peroxide, and potassium dihydrogen phosphate were obtained from Rankem (Mumbai, India). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), nutrient agar, potato dextrose agar, and yeast potato dextrose agar were purchased from Himedia (Mumbai, India). Ascorbic acid was obtained from Oxford Laboratory (Mumbai, India).

Test microorganisms

The MA-rich fraction was tested against four bacterial strains, namely, *Pseudomonas aeruginosa* CC 488, *Bacillus subtilis* B 28, *Bacillus megaterium*, and *Escherichia coli* MTCC 170. Fungal strains selected for antifungal experiments were *Aspergillus flavus* MTTC 873, *Ustilago maydis* NCIM 983, *Aspergillus niger* MTCC A, *Aspergillus fumigates* MTCC 2551, *Saccharomyces cerevisiae* MCIM 170, and *Candida albicans* MTCC 3018. All strains were obtained from the School of Life Sciences at Swami Ramanand Teerth Marathwada University (Nanded, India). Three species of *Mycobacterium*, namely, *Mycobacterium tuberculosis*, *Mycobacterium phlei*, and *Mycobacterium smegmatis*, were procured from the Microbial Type Culture Collection and the gene bank at the Institutes of Microbial Technology (Chandigarh, India).

Antioxidant activity

Total antioxidant capacity

The total antioxidant capacity of MA was calculated through the total antioxidant capacity assay. MA and ascorbic acid solutions in DMSO at concentrations ranging from 20 μg/mL to 100 μg/mL were prepared. The reagent solution (3 mL of a 0.6 M sulphuric acid/28 mM sodium phosphate/4 mM ammonium molybdate mixture) was mixed with 0.30 mL of the working standards. The reaction mixture was incubated for 90 min at 95 °C and then cooled to room temperature. Absorbance at 695 nm was measured against the blank, DMSO, and ascorbic acid was used as the standard. All tests were performed in triplicate. The total antioxidant capacity was expressed in terms of equivalents of ascorbic acid. The antioxidant capacity of MA was expressed in terms of the absorbance: a high absorbance represents high antioxidant activity [18,19].

DPPH radical scavenging activity

The radical scavenging potential of MA was evaluated in terms of its activity in DPPH radical scavenging according to the method of Sun et al. [19]. Selected concentrations (20–100 μg/mL) of working standards of MA and ascorbic acid, as well as a 0.1 mM solution of DPPH in methanol, were prepared. A 1 mL aliquot of the DPPH solution was added to 2 mL of the MA solution at different concentrations (20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, and 100 mg/mL). Each mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The control solution contained all reagents except MA, and the blank was DMSO. The absorbance of each mixture was measured spectrophotometrically at 517 nm (UV-1800 UV–VIS spectrophotometer, Shimadzu). The percentage of the activity of MA in DPPH radical scavenging was calculated through the formula:

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