Full Length Article

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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ARTICLE INFO

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.

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:

Percentage of scavenging activity $(\%) = (1 - A_t/A_o) \times 100$

where A_t is the absorbance of MA and A_0 is the absorbance of the control. The antioxidant activity was evaluated as IC_{50}, which is the MA concentration required to scavenge 50% of DPPH free radicals.

Hydrogen peroxide radical scavenging activity of MA

The H_2O_2 scavenging assay was performed to evaluate the ability of MA to scavenge hydrogen peroxide radicals. A 40 mM H_2O_2 solution in pH 7.4 phosphate buffer was prepared. Solutions of MA in distilled water (1 mL) at different concentrations (50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL) was added to 0.6 mL of hydrogen peroxide solution. The absorbance of each mixture at 230 nm was measured using a Shimadzu UV-1800 UV–VIS spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. A_t and A_0 for MA were then determined [20,21]. The IC_{50} value, which is the sample concentration at which scavenging activity is 50%, was also determined. The percentage of H_2O_2 scavenging activity of MA and ascorbic acid was calculated through the equation, Percentage of scavenging activity $(\%) = (1 - A_t/A_o) \times 100$.

Reducing power of MA and ascorbic acid

The reducing power of MA and ascorbic acid was resolved by using a modification of the methods described by Liu et al. [18] and Sun et al. [19]. MA solutions in DMSO at concentrations of 50–250 µg/mL were prepared. A 0.13-mL MA sample at each concentration was added to 0.125 mL of phosphate buffer (0.2 M, pH 6.6) and 0.125 mL of potassium ferricyanide (1%, w/v). This mixture was incubated for 20 min at 50 °C, and then 0.125 mL of trichloroacetic acid solution (10%, w/v) was added to complete the reaction. A ferric chloride solution (1.5 mL; 0.1%, w/v) was added to the mixture, and the absorbance at 700 nm was measured against the blank, DMSO. Ascorbic acid was used as the standard. The reducing ability of the mixture was calculated by observing the absorbance of the reaction mixture, that is, an increase in its absorbance indicates an increase in its reducing ability.

In vitro antimicrobial assay

Antibacterial and antifungal activity of MA

The antibacterial and antifungal activity of MA was evaluated through the agar-well diffusion method with modifications. Sterile nutrient agar was poured into sterile standard Petri plates (20 mL) and then allowed to congeal. The plates were inoculated with 100 µL of the microbial culture by spread-plate technique. By using a sterile borer, four wells were punched. For the antibacterial activity experiments, samples of 100 µg/mL MA solutions, the standards (ciprofloxacin and amoxicillin), and the solvent (DMSO) were loaded into respective wells, and then the Petri plates were incubated at 37 °C for 24 h. Incubation was done at 34 °C for 48 h for the antifungal activity experiments. The inhibition zone that appeared around each well, which represents the antibacterial potential of samples, was measured by using a zone reader scale [22].

Antimycobacterial activity of MA

The antimycobacterial potential of MA was determined by agar-well diffusion and disc diffusion methods.

Well diffusion method

A modification of the agar well diffusion method by Dahech et al. [22] was performed to evaluate the antimycobacterial activity. Middlebrook 7H11 agar with 0.1 mL of mycobacterial culture was spread and then incubated at 37 °C. Wells with 0.5 cm diameter were punched by using a sterile cork borer. A MA solution (50 µL, 50 mg/mL) and rifampicin were loaded into the respective wells, which were then incubated at 37 °C for specific periods (*M. smegmatis*: 1 day; *M. phlei*: 5 days; *M. tuberculosis*: 3 weeks). The diameter of the inhibition zone was observed after the incubation period and then measured by using a zone reader scale.

Disc diffusion method

The antimycobacterial activity of MA was analysed through the disc diffusion method. The mycobacterium inoculum was adjusted to the required concentration (approximately 1.2×10^8 CFU/mL). A 100 µL aliquot of inoculum was spread on the surface of an agar plate by using a sterile cotton-tipped swab to form an even layer. A paper disc (6 mm diameter) was impregnated with a dilute rifampicin solution (5 µg/disc) and then allowed to dry for 30 min. It was then placed on the surface of each plate by using a sterile pair of forceps. Similarly, MA was prepared and placed. The plates were incubated under aerobic conditions for specific periods (*M. smegmatis*: 1 day; *M. phlei*: 5 days; *M. tuberculosis*: 3 weeks) at 37 °C, and then the diameter of inhibition zone was measured by using a zone reader scale [23–25].

Determination of minimum inhibitory concentration and minimum microbicidal concentration

Each strain that produced an inhibition zone was tested for minimum inhibitory concentration (MIC) through serial dilution of MA. The MIC for antifungal activity was determined through the broth dilution method of Ye et al. [26]. Solutions of MA at different concentrations (50–150 µg/mL) were prepared. Nutrient broth (2 mL) was mixed with 0.5 mL of MA solution, and a loopful of each test microorganism was added to the test tube, which was then incubated for 28 h. The MIC, the lowest MA concentration at which the test microorganisms did not demonstrate any visible growth, was then determined. Clear solutions in the test tubes (i.e., those with no turbidity) were streaked on nutrient agar medium and then incubated for 28 h at 28 °C. The lowest MA concentration used with the medium having colonies fewer than five was taken as the minimum microbicidal concentration.

The MIC of MA for antitubercular activity was determined by using the well diffusion and disc diffusion methods. Strains producing inhibition zones were tested for MIC. MA solutions with the required concentrations (2–10 mg) were prepared. Five to six wells were punched into the agar medium. Each well was loaded with each MA solution and incubated for a specified period at 37 °C. In the disc diffusion

method, prepared discs were loaded. An inhibition zone of more than 6 mm diameter was taken as the MIC according to the standards of the Clinical and Laboratory Standard Institute [24].

In silico prediction using PASS

PASS (Prediction of Activity Spectra for Substances), a computer-based program, was used to screen the antimicrobial and antioxidant potential of MA. The software predicts biological activities of chemical structures, including those of phytochemicals, on the basis of structure–activity relationship with a known chemical entity. It predicts the desirable pharmacological effect, as well as molecular mechanisms of action and unwanted side effects such as mutagenicity, carcinogenicity, teratogenicity, and embryotoxicity [27]. It compares the structure with a training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activity. The activity is estimated in terms of Pa (probable activity) and Pi (probable inactivity). Structures with Pa greater than Pi were the only compounds considered for a particular pharmacological activity [28,29].

Statistical analysis

All data are presented as mean ± standard error of the mean. Results were evaluated by one-way analysis of variance followed by Tukey's test using Graphpad Prism (version 6, USA) and Graphpad Instat (version 3.0.1, USA) software. IC_{50} values for antioxidant activity were calculated by linear regression analysis of three measurements. The difference was considered to be statistically significant if $p < .05$.

Results

Antioxidant activity

Total antioxidant capacity
The total antioxidant activity of MA was determined through the phosphomolybdenum method. Reduction of Mo(IV) to Mo(V) and green phosphate/molybdenum(V) compounds by antioxidants present in the sample at low pH results in an absorption peak at 695 nm, which can be used to evaluate the antioxidant capacity. The dose-dependent increase (Fig. 1A) in absorbance values indicates that MA possesses substantial antioxidant activity, similar to the standard, ascorbic acid. IC_{50} values of MA and ascorbic acid were found to be 153.81 and 103.30 µg/mL, respectively (Table 1).

Activity in DPPH radical scavenging
An assay for activity in DPPH radical scavenging was used to determine the ability of MA to scavenge reactive species by donating hydrogen (Fig. 1B). The odd electron in DPPH has an ultraviolet–visible absorption maximum at 517 nm. This electron becomes paired off in presence of a hydrogen donor, a free-radical scavenging antioxidant. The decrease in absorbance is thus considered a measure of the antioxidant activity. Percentages of the scavenging activity of MA at 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL were

31.408 ± 0.12%, 36.765 ± 0.16%, 40.021 ± 0.06%, 43.873 ± 0.21%, and 46.254 ± 0.12%, respectively, indicating a concentration-dependent increase in the radical scavenging ability similar to that of ascorbic acid. The scavenging ability of MA, measured in terms of the IC_{50} (the concentration resulting in 50% inhibition; Table 1), was found to be 116.20 µg/mL.

Activity in H_2O_2 radical scavenging
The ability of MA to scavenge hydrogen peroxide, depicted in Fig. 1C, was compared with that of the reference standard, ascorbic acid. MA demonstrated activity in scavenging hydrogen peroxide radicals in a dose-dependent manner. Percentages of inhibition of hydroxyl radical scavenging by MA are 37.67 ± 0.01%, 57.97 ± 0.01%, 63.83 ± 0.01%, 79.82 ± 0.01% and 80.37 ± 0.01% at MA concentrations of 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL, and 250 mg/mL, respectively. These results show that an increase in MA concentration leads to a corresponding increase in the scavenging rate. Similarly, dose-dependent scavenging was observed with ascorbic acid (Fig. 1C). IC_{50} values for MA and ascorbic acid are 85.03 and 61.06 µg/mL, respectively (Table 1).

Assay of the Fe^{3+} reducing power
We determined the ability of MA and ascorbic acid to reduce a ferric ion/ferricyanide complex to the ferrous form via electron donation. The reducing power of MA was 0.606 ± 0.001, 0.655 ± 0.001, 0.087 ± 0.001, 1.268 ± 0.008, and 1.581 ± 0.001 at concentrations of 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL, and 250 mg/mL, respectively; whereas at the same concentrations, that of ascorbic acid was 0.809 ± 0.005, 0.701 ± 0.008, 1.401 ± 0.042, 1.846 ± 0.022 and 2.170 ± 0.103, respectively. Therefore, MA showed dose-dependent reducing power similar to that of ascorbic acid (Fig. 1D).

Antimicrobial activity

Antibacterial and antifungal activity
The antimicrobial potential of MA was estimated by using four bacterial strains and six fungal strains. Results for the antibacterial activity experiment (Table 2) suggest that MA is completely inactive against all of the tested bacterial strains. MA produced no zone of inhibition, while standard ciprofloxacin and amoxicillin showed potent antibacterial activity against all of the strains (Table 2).

The antifungal activity of MA and clotrimazole are indicated in Table 3. MA exhibited potent activity against A. niger (8.16 ± 0.44), similar to that of clotrimazole (10.33 ± 0.33), whereas A. flavus (4.33 ± 0.33) and U. maydis (3.66 ± 0.33) moderately reduced growth. MA was found to be ineffective against S. cerevisiae, C. albicans, and A. fumigates.

MIC and minimum microbicidal concentration
MA exhibited no activity against all tested bacterial strains in the agar well diffusion method. However, the antimicrobial activity of MA varied with the microbial species. MA produced an inhibition zone around A. flavus, U. maydis and A. niger. Thus, the MIC and minimum fungicidal concentration (MFC) of MA (Table 3) confirm its antifungal activity in the broth dilution method. MICs of MA for the tested fungi range from
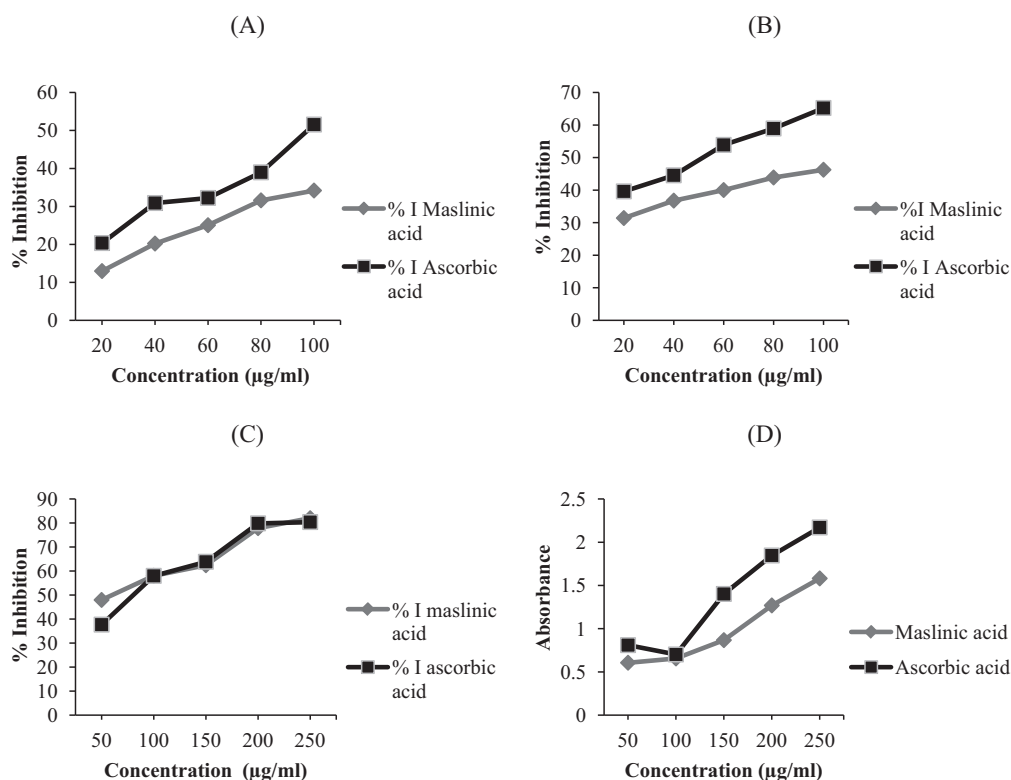
Fig. 1 – Antioxidant activities of maslinic acid evaluated by using in vitro antioxidant models: (A) total antioxidant capacity; (B) DPPH radical scavenging activity; (C) H_2O_2 radical scavenging activity; and (D) Fe^{3+} reducing power assay. Each value represents mean ± standard error of the mean ($n = 3$). Note: DPPH = 2,2-diphenyl-1-picrylhydrazyl.

Table 1 – IC$_{50}$ values of MA and ascorbic acid.[a]

Sr. No.	Antioxidant assay/method	IC$_{50}$ (µg/mL)	
		MA	Ascorbic acid
1	Total antioxidant capacity	153.81	103.30
2	DPPH radical scavenging activity	116.20	52.62
3	H_2O_2 radical scavenging activity	61.06	85.03

Note: DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC$_{50}$ = half maximal inhibitory concentration; MA = maslinic acid.
a IC$_{50}$ values were calculated by linear regression analysis of three measurements.

Table 2 – Activity of MA, DMSO, and standard antibiotics against bacterial strains tested through the agar well diffusion method.

Bacterial species	Diameter of inhibition zone (mm)			
	MA	DMSO	Standard antibiotic	
			Amoxicillin	Ciprofloxacin
Bacillus megaterium	—	—	8	15
Bacillus subtilis	—	—	11	14
Escherichia coli	—	—	9	11
Pseudomonas aeruginosa	—	—	10	18

Note: DMSO = dimethyl sulphoxide; MA = maslinic acid.

Table 3 – MIC and MFC of MA and the activity of MA, DMSO, and standard antibiotics against fungal strains tested through the agar well diffusion method.

| Fungal species | Diameter of inhibition zone (mm) | | | MA | |
	MA	DMSO	Clotrimazole	MIC (µg/mL)	MFC (µg/mL)
Aspergillus flavus	4.33 ± 0.33	—	7.33 ± 0.88	90 ± 5.77	103.33 ± 3.33
Ustilago maydis	3.66 ± 0.33	—	6.33 ± 0.33	86.66 ± 3.33	103.33 ± 3.33
Aspergillus niger	8.16 ± 0.44	—	10.33 ± 0.33	96.66 ± 3.33	106.66 ± 3.33
Aspergillus fumigates	—	—	—	—	—
Candida albicans	—	—	—	—	—
Saccharomyces cerevisiae	—	—	—	—	—

Note: DMSO = dimethyl sulphoxide; MA = maslinic acid; MFC = minimum fungicidal concentration; MIC = minimum inhibitory concentration.

86.66 µg/mL to 90 µg/mL, and its MFCs range from 103.33 µg/mL to 106.66 µg/mL.

Antimycobacterial activity

MA was analysed for its activity against *M. phlei*, *M. smegmatis*, and *M. tuberculosis* through the disc diffusion and well diffusion methods. Calculation results were expressed as MICs in µg/mL (Table 4). In the disc diffusion method, MA exhibited inhibitory effect against *M. smegmatis*, *M. phlei*, and *M. tuberculosis* (Fig. 2A–C), producing inhibition zones with diameters of 4.33 ± 0.33, 8 ± 1.15, and 5.66 ± 0.33 mm, respectively. MICs of MA for *M. phlei* and *M. tuberculosis* are 4.96 ± 0.08 µg/mL and 2.50 ± 0.05 µg/mL, respectively.

Similarly, MA produced inhibition zones around *M. smegmatis*, *M. phlei*, and *M. tuberculosis* (Fig. 2D–F) in the well diffusion method. MA showed activity against *M. smegmatis*, *M. phlei*, and *M. tuberculosis*, giving inhibition zones with diameters of 8 ± 1.15, 7 ± 0.57, and 6.3333 ± 0.33 mm, respectively. MA had an MIC (Table 5) of 2.56 ± 0.03 µg/mL for *M. phlei* and 2.53 ± 0.03 µg/mL for *M. tuberculosis*.

PASS calculations for antioxidant, antibacterial, and antifungal activities

The biological activity spectra of MA was determined by using an online version of PASS software. The results obtained (Table 6) were interpreted and used in a flexible manner. MA showed the highest Pa for antifungal activity (0.589), and its Pa values for antioxidant and antibacterial activity are 0.479 and 0.363, respectively.

Discussion

The rapid development of mutants and resistant pathogenic strains is a major reason for the failure of synthetic antibiotics [30]. The constant exposure of plants to diverse environmental changes and stress conditions causes the plants to synthesise metabolites that protect them from these unfavourable situations [2]. Free radicals generated during normal metabolism of cells contribute to immunity from pathogenic infections. Generation of these radicals is primarily regulated by the antioxidant system, which acts through diverse mechanisms such as inhibition of chain initiation, binding of transition-metal ion catalysts, and scavenging of radicals [8,31]. Certain conditions associated with stress, such as infections, contribute to the formation of free radicals, thus disturbing its balance. This imbalance, in turn, disrupts cellular components such the cell membrane, mitochondria, nuclear components, as well as macromolecules. It also leads to aging, vital-organ damage, neurodegenerative complications, diabetes mellitus, and other diseases [32,33]. Bacterial and fungal infections perturb the balance of oxidation and reduction processes, exacerbating oxidative stress [34,35]. Such oxidative effects hasten disease progression, which can be delayed by proper use of antioxidants [9].

Olean-type pentacyclic triterpenoids are transcriptional regulators of antioxidants and detoxifying enzymes. Oleanolic acid has ability to scavenge reactive oxygen free radicals by direct chemical reactions. The mechanism for this potent antioxidant property is the increase in Nuclear factor-erythroid 2-related factor (Nrf2)-mediated expression of antioxidant enzymes such as catalase and thioredoxin

Table 4 – Antitubercular activity and MIC of MA in the disc diffusion method.

| *Mycobacterium* species | Diameter of inhibition zone (mm) | | MA |
	MA	Rifampicin	MIC (µg/mL)
Mycobacterium smegmatis	8 ± 1.15	24 ± 0.66	—
Mycobacterium phlei	4.33 ± 0.33	23 ± 0.57	4.96 ± 0.08
Mycobacterium tuberculosis	5.66 ± 0.33	26 ± 0.57	2.50 ± 0.05

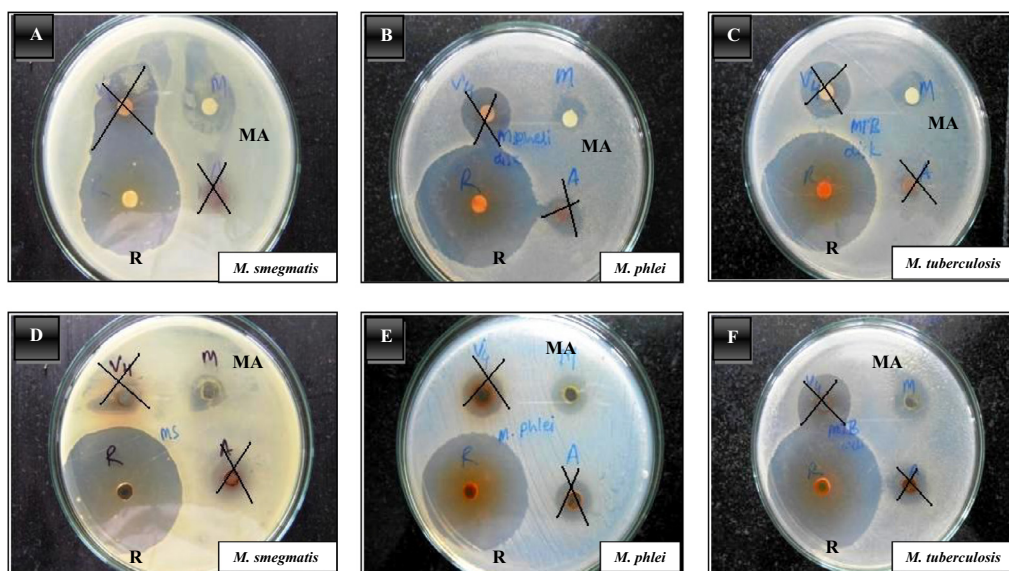*Note:* MA = maslinic acid; MIC = minimum inhibitory concentration.

Fig. 2 – Activity of maslinic acid (MA) and rifampicin (R) against *Mycobacterium smegmatis*, *Mycobacterium phlei*, and *Mycobacterium tuberculosis* using the (A–C) disc diffusion method and (D–F) well diffusion method.

Table 5 – Antitubercular activity and MIC of MA in the well diffusion method.

Mycobacterium species	Diameter of inhibition zone (mm)		MA
	MA	Rifampicin	MIC (µg/mL)
Mycobacterium smegmatis	8 ± 1.15	26 ± 0.57	—
Mycobacterium phlei	7 ± 0.57	25 ± 0.57	2.56 ± 0.03
Mycobacterium tuberculosis	6.33 ± 0.33	25 ± 0.57	2.53 ± 0.03
Note: MA = maslinic acid; MIC = minimum inhibitory concentration.			

Table 6 – Results of PASS calculations for antioxidant, antibacterial and antifungal activity of MA.

Activity	PASS predictions of MA	
	Pa	Pi
Antioxidant	0.479	0.008
Antibacterial	0.363	0.037
Antifungal	0.589	0.020
Chitinase inhibitor	0.831	0.002
Membrane integrity antagonist	0.934	0.001
Note: MA = maslinic acid; Pa = probable activity; PASS = Prediction of Activity Spectra for Substances; Pi = probable inactivity.		

peroxidase, as well as the enhanced biosynthesis of the antioxidant glutathione [12]. MA is an olean-type pentacyclic triterpenoid that has been studied for its antioxidant potential, in particular, activity in DPPH radical and hydrogen peroxide radical scavenging, total antioxidant activity, and Fe^{3+} reducing power. The free radicals DPPH and hydrogen peroxide possess absorbance maxima at particular wavelengths. Scavenging or complexation of these radicals by radical scavengers decreases concentrations of radicals, which induce oxidative stress [19]. MA showed dose-dependent, moderate neutralisation of DPPH radicals. H_2O_2 is involved in the formation of reactive oxygen species such as superoxide anion radicals and hydroxyl radicals [18]. MA was found to scavenge hydrogen peroxide free radicals as effectively ascorbic acid does. The total antioxidant assay, which reflects the antioxidant capacity of the nonenzymatic defence system [18], showed high values for the reaction mixture, thus implying the strong total antioxidant activity of MA. MA shows antioxidant activity by donating an electron, forming a ferrous complex in a dose-dependent manner. This effect is due to the reducing tendency of the compounds due to its capacity to break the free radical chain reaction by donating

hydrogen [36]. Although the antioxidant activity of MA varied in the antioxidant assay, it was close to that of ascorbic acid, indicating its strong antioxidant potential.

Since ancient times, secondary metabolites of plants have been known for their medicinal properties [23]. Many comprehensive reports indicate the antibacterial, antifungal, antioxidant, antiviral and antihelminthic properties of plants [24,25,37]. Pentacyclic triterpenoids, in particular, have an extensive range of biological activity [10]. In search of an effective antimicrobial agent, we focused on MA in the present study. MA was not effective against the tested pathogenic bacterial strains, but it produced a clear inhibition zone around all of the tested *Mycobacterium* strains. Triterpenoids such as ursolic acid and oleanolic acid possess strong antimycobacterial activity, damaging the bacterial complex cell envelope (antilipidic activity) and the immunoregulatory mechanism mediated by nitric oxide and TNFα (tumor necrosis factor) synthesis [38]. Such mechanisms seem to partly explain the strong antimycobacterial activity of MA. Whereas MA exhibited moderate antifungal activity, its effect on *A. niger* was prominent, as showing by its clear inhibition zone. *A. niger* is much more resistant to various biocides than are pathogenic strains of Gram-positive and -negative bacteria [39]. Therefore, MA may be the best treatment for the effective management of *Aspergillus*-associated infections.

Calculations using PASS software have been used extensively to confirm and correlate biological activities of chemicals [28,29]. Our results are confirmed by a structural study of MA using the software, which works on the principle of structure-based comparison of MA with existing antimycobacterial compounds. The Pa value of MA for antifungal activity is higher than those for antibacterial and antioxidant activity. As the fungal cell wall contains compounds such as mannan, chitin, and glucans, which are unique to fungi [40], the calculation results suggest that MA is a chitinase inhibitor (Pa, 0.831) and that it is an antifungal agent. Membrane integrity, an indicator of the viability of microorganisms [41], was also apparently disrupted by MA (Pa, 0.934). Thus, MA may be an antimicrobial compound. Thus, the chitinase inhibition and disruption of membrane integrity could contribute to the antimicrobial effects of MA.

To date, very few studies comprehensively describe the antimicrobial profile of triterpenoids from medicinal plants. Our study is the first to explore the in vitro antioxidant and antimicrobial properties of triterpenoids using software. The study showed variable effects of MA on bacterial and fungal strains, as well as potent inhibitory effects against mycobacterial strains. Therefore, MA is a potential candidate compound for the treatment of tuberculosis and fungal infection.

Conclusion

Our study showed that MA is a biologically active triterpenoid that exhibits mild antioxidant and antifungal activity. The antioxidant property can ameliorate oxidative damage induced by oxidative stress. MA exhibited substantial antifungal activity, especially against *A. niger*. The significant antimycobacterial activity of MA may be used to prevent complications associated with tuberculosis. These findings indicate that MA could be an antimicrobial agent and a promising source of natural antioxidants. However, further studies are needed to uncover the therapeutic importance of MA as an antioxidant, antifungal, and antimycobacterial agent for the treatment of infectious diseases.

Conflicts of interest

The authors disclose that there are no conflicts of interest.

Acknowledgments

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