IN VITRO ANTIBACTERIAL, ANTIDERMATOPHYTE AND ANTICANDIDA POTENTIALS OF DIFFERENT ORGANIC EXTRACTS AND TOTAL ALCALOIDS OF INDIAN THORN APPLE (*DATURA METEL* L.) FROM TUNISIA

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Abstract. Leaf and seed extracts of *Datura metel*, a species known as a folkloric medicinal plant in Tunisia, were prepared with different solvents: PE, CH_2Cl_2, ACOEt and MeOH. Both the leaves and seed samples contained alkaloids, tannins, saponins, Iridoids, and flavonoids. Extracts were tested in vitro for antimicrobial activity against 9 fungi and 11 bacteria. Results have shown that different extracts have various levels of inhibition against all tested bacterial species (with MIC varied from 0.625 to 10 mg/mL). Differences in antibacterial activities between seed and leaf extracts were also observed. The highest inhibition potentials were shown for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* strains. Most fungi are quite resistant to PE and CH_2Cl_2 extracts at concentrations lower than 500 µg/mL, while the ACOEt and MeOH extracts demonstrated the greatest antifungal potential. The MIC against *Aspergillus fumigatus*, was found to be the lowest (62.5 µg/mL). The most promising results were shown against *Candida albicans*, which was totally inhibited by 250 µg/mL of both ACOEt and MeOH extracts. Findings from this study highlight not only the rich antibacterial potentials of *D. metel*, but also its very effective antidermatophytic and anticandidose activities, thus recommending the use of this plant against certain skin diseases.

Keywords: *antimicrobials, antifungal activity, organic extracts, phytochemical screening, organ variability*

Introduction

The emergence of newer multidrug resistant pathogens is threatening the clinical efficacy of existing antimicrobials. A lot of interest is given nowadays to new active substances with potent antimicrobial activity in all over the word. Polyphenols derived from plants and spices have been shown to have antimicrobial properties that could be used as a replacement for antibiotics (Luis et al., 2014; Khameneh et al., 2019). *Datura metel*, also known as Indian Thorn apple in Europe, is a member of the Solanaceae family, which includes over 100 genera and 3000 plant species. It is a worldwide shrub that can

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be cultivated for its chemical and ornamental properties. In traditional medicine, the plant is used widely. In Nigeria for example, *D. metel* is administered in case of cough, asthma convulsion and insanity. In other countries, it was used to treat skin diseases and burns, animal bites, cerebral as well as herpetic problems and diarrhoea. Mixed with other plants it is used as cataplasm against eczema (Priya et al., 2002; Dabur et al., 2004; Alam et al., 2021). Traditional Chinese medicine has used the plant for centuries to treat asthma, pain, convulsions, rheumatism, and antibronchitis (Xia et al., 2019). In Tunisia, *D. metel* is a spontaneous herb. Le Floch (1983) reported that the Arab physicians use this species for various uses: against insomnia, hair loss and injury oedema. The leaves are a source of scopolamine, used as nerve sedative, antiparkinsonian and in combination with morphine as a pre-anesthetic. However, according to many Pharmacopeia, it is extremely recommended that the plant should not be administered as ingestion form (Spadari et al., 2008).

Keeping in view the traditional beliefs, several research studies have investigated the antimicrobial activities of different aerial parts of *D. metel* and have documented promising antimicrobial effects (Sakthi et al., 2011; Rinez et al., 2013; Lim et al., 2020; Bawazeer and Rauf, 2021).

To our knowledge, none of the previous work however has considered tests on the seed extracts of the plant. In the same way, surprisingly, the antibacterial and antidermatophyte properties of Tunisian *D. metel* extracts have not been studied. As a result, this is the first attempt to determine the in vitro antimicrobial activity of different organic extracts from seeds, leaves, and total alkaloids from Tunisian *D. metel* roots against a variety of strains of bacteria, dermatophytes, hyphamycets, and one opportunist pathogenic yeast.

Material and methods

Plant material collection

D. metel was found in the Monastir region (35°46'N, 10°50'E) of Tunisia's central-east. Separately, the leaves, seeds, and roots were washed with tap water and dried in the shade at room temperature. They were powdered after drying and stored separately in a sterile and airtight container until further use. Pr. Fathia Skhiri, a Botanist at the Laboratoire de Génétique Biodiversité et Valorisation des Bio-ressources, Institut Supérieur de Biotechnologie de Monastir (ISBM), Université de Monastir, identified the plant material.

Plant extract preparation

Extracts from dried powder (100 g) were obtained by soaking it in increasing polarity organic solvents such as petroleum ether, dichloromethane, ethyl acetate, and methanol for one week. The resulting mixture was filtered through membrane filters with a diameter of 1 mm. The filtrates were vacuum evaporated in a rotary evaporator (BUCHI, Germany), and the dried extracts were stored at 4°C in the dark until further processing. Because the biosynthesis of alkaloids in *Datura* occurs primarily in the roots (Kohnen-Johannsen et al., 2019), total alkaloids were extracted and isolated from this organ using a liquid-liquid extraction based on the difference in solubility of the alkaloids in acidic and alkaline conditions. An electric grinder was used to finely crush 300 mg of roots. Sulfuric acid (3%) was added, followed by a 3-hour incubation at room temperature.

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Following filtration, the filtrate was basified with ammonia solution NH_4OH, which allowed alkaloids to transition from salt to organic form. A rotary evaporator is used to evaporate the collected extract under vacuum. The total alkaloids are represented by the dry residue.

Phytochemical screening of extracts

The leaves and seeds of *D. metel* were subjected to preliminary Qualitative phytochemical screening in order to detect major groups such as tannins, alkaloids, iridoids, saponins, and flavonoids by following the standard procedures described by Harbone (1998).

Tannins. After boiling 200 mg of plant material in 10 mL distilled water and adding a few drops of $FeCl_3$ to the filtrate, a blue-black precipitate indicated the presence of Tannins.

Alkaloids. 200 mg plant material was boiled and filtered in 10 mL methanol. 1% hydrochloric acid was added, followed by six drops of Dragendorff reagent, and the brownish-red precipitate was used to confirm the presence of alkaloids.

Iridoids. Trim & Hill color reagent was used to detect iridoid glycosides in the drug: 1 g of powered drug was placed in a test tube with 5 ml of 1 percent aqueous hydrochloric acid, and after 3–6 hours 0. 1 ml of the macerate is decanted into another tube containing 1 ml of the Trim–Hill reagent. After being heated, a dark color indicates the presence of iridoids.

Saponins (Frothing test). To 200 mg plant material, 5 mL distilled water was added. The filtrate was diluted to a volume of 5 mL with distilled water and vigorously shaken for 2 minutes. The presence of saponins is indicated by the formation of stable foam.

Flavonoids. 5 mL dilute ammonia solution was added to the aqueous filtrate, followed by concentrated H_2SO_4. The presence of flavonoids was indicated by a yellow coloration.

Antibacterial activity

Test microorganisms

For the antimicrobial tests, eight Gram-negative and three Gram-positive bacteria strains were used. The American Type Culture Collection (ATCC) provided the reference strains (USA). Stock cultures were stored at 80°C in the laboratory LR 99 ES 27, Monastir, Tunisia, as glycerol stock (20%). Before any antimicrobial testing, all strains were subcultured three times.

Method of disc diffusion

Different extracts of *D. metel* leaves, seeds, and total alkaloids were tested for antibacterial activity in Muller Hinton agar using the standard disc diffusion method. The bacterial inoculum was set at 0.5 Mcfarland (Bel Hadj Salah et al., 2006). Each extract (10 mg/ml) was inoculated onto the surface of the media using 6 mm sterilized Whatman No. 3 paper discs. As a positive control, a standard antibiotic disc (gentamycin, 30 µg /disc) was used, and a disc impregnated with ethanol 99 percent was used as a solvent control. The incubation of the plates is at 37°C for 24 hours. The diameter of the inhibition zone in mm, was used to calculate the antibacterial activity.

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Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC)

The Minimum Inhibitory Concentration (MIC) of *D. metel* organic extracts was determined using the serial dilution technique (Hajlaoui et al., 2009). In brief, 96-well plates were filled with 95 µl of nutrient broth, 5 µl of bacterial inoculum (10^6 cfu/ml), and 100 µl of plant extract (the initial concentration was 10 mg/ml). A total of 100 µl from their serial dilutions was transmitted into six consecutive wells. Column 12 was set aside for solvent control (ethanol), while column 1 was set aside for extract control. Columns 10 and 11 served as controls for bacterial culture and nutrient broth medium, respectively. Under the same conditions, the antibiotic gentamycin was manipulated. The test microplates were then incubated at 37°C for 24 hours, and the presence of turbidity and a pellet on the well bottom was used to assess bacterial growth. The minimum inhibitory concentration (MIC) was defined as the concentration that totally inhibited visible cell growth after a 24-hour incubation period at 37°C. 10 µl of each well medium with no visible growth was subcultured in MH plates to determine the minimum bactericidal concentration (MBC) results. The number of surviving pathogens was determined after 24 hours of incubation at 37°C. MBC was defined as the lowest concentration at which 99% of the bacteria were killed (Sahin et al., 2004). All experiments were done in triplicate.

Antifungal activity

Fungal strains

The fungal strains were acquired from the microbiology laboratory at the University of Franche-Comté in Besançon and the Pasteur Institute (France). Every three months, the strains were subcultured on Sabouraud Dextrose Agar (SDA) medium and incubated for seven days.

Agar incorporation method

The antifungal activity was measured using the method explained by Bel Hadj Salah et al. (2006). SDA medium containing 0.05% chloramphenicol was prepared. Appropriate amounts of leaf and seed extracts were mixed in the medium, kept cool to 45 to 50°C to obtain concentrations of 500, 250, 125, and 62 µg/ml. The medium was thoroughly mixed with the plant extracts. In sterile Petri dishes (33 mm), 3 ml of each concentration were poured. Following solidification, 5 mm diameter mycelial plugs were collected with a pre-sterilized cork borer from a 5 to 7 day old culture of test fungus and put in each Petri plate. On the SDA medium, a conidial suspension of *C. albicans* was placed. Incubation was 24 h at 37°C for *Candida* and *Aspergillus* and 7 days at 24°C for Scopulariopsis and dermatophytes. Each treatment was replicated thrice for every concentration and microorganism.

"Percentage growth inhibition of the fungal colonies (Eq1) was calculated by applying the following formula":

$$\text{Growth/inhibition (\%)} = (dC-dE)/dC \times 100 \text{ (Singh et al., 1993)} \qquad \text{(Eq.1)}$$

where dC: "*the diameters of the colonies in the control plate,* dE: *diameters of colonies in the treated plate"*.

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The minimal inhibitory concentration (MIC), for each species was also calculated. This is defined as the lowest concentration that suppresses observable growth of fungus throughout the incubation period.

Statistical analysis

Three replicates were analyzed using one-way analysis of variance (ANOVA) in the SPSS program Ver. 20. The mean of the replicates is used to calculate the standard deviation (SD). All tests were performed in triplicates (n = 3) and the error bars represent the SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ to compare the treatment, the differences were declared low, high and very high statistically significant, respectively.

Results

Phytochemical analysis

Organic extracts of *D. metel* were found to have a variety of chemical components. *Table 1* highlights the phytochemical content of leaves and seeds of *D. metel* extracts. All the extracts contained flavonoids, tannins, Iridoids, alkaloids and saponins.

Table 1. *Phytochemical Screening of Datura metel L leaves and seeds*

Phytochemicals	Seeds	leaves
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Iridoids	+	+
Tannins	+	+

Antibacterial activity

The diameters of the inhibition zones of various organic extracts were measured in mm. The antibacterial activities of leaf and seed extracts were then compared (*Fig. 1A, B, C and D*), as well as those of total alkaloids extract and the reference antibiotic Gentamicin (*Fig. 1E*). All of the organic extracts were efficacious against *Bacillus subtilis*, having inhibition zones ranged from 7 to 16 mm, whereas no inhibitory effects of the same extracts were observed against *Vibrio parahaemolyticus*. In most cases, methanolic extracts appeared to be more active over other extracts with zones of inhibition varying from 7 to 15 mm. Results also showed that the *D. metel* seed extracts possess in general significantly higher antibacterial activities over leaves extracts ($p < 0.01$, *Fig. 1*).

The total alkaloids extract, on the other hand, has lower activity than the control Gentamicin, as well as other leaf and seed extracts. It was efficient against *B. subtilis* (14 mm) (*Fig. 1E*).

We used a second antibacterial technique, the serial dilution in 96 plates, to confirm the results obtained from the agar diffusion method. If we consider the problems of miscibility of organic extracts in an aqueous medium, it is said to be more efficient.

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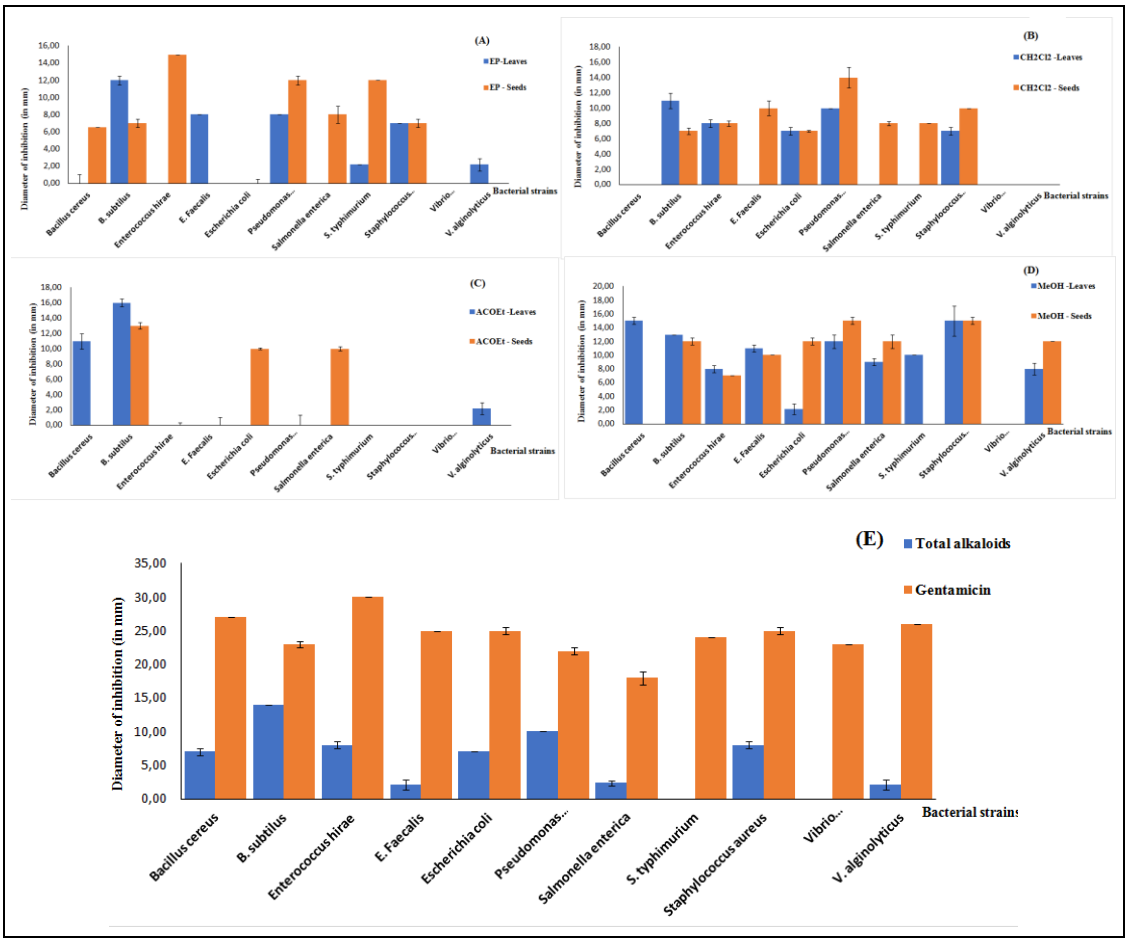

Figure 1. *Comparison of the Diameters of inhibition (in mm) of D. metel L. leave and seed extracts (1mg/mL): (A) Petroleum Ether (PE); (B) Dichloromethane (CH_2Cl_2); (C) Ethyl acetate (ACOEt); (D)Methanol (MeOH);(E): total alkaloids (1mg/mL) and the reference antibiotic Gentamicin (30 µg /disc); bacterial strains are in the same order as in the Figure : Bacillus cereus(ATCC10987); Bacillus subtilus (ATCC6633); Enterococcus hirae (ATCC10541); Enterococcus faecalis (ATCC29212); Escherichia coli (ATCC25922); Pseudomonas aeruginosa (ATCC9027); Salmonella enterica (ATCC10708); Salmonella typhimurium (ATCC1408); Staphylococcus aureus (ATCC25923); Vibrio parahaemolyticus (ATCC17802) and Vibrio alginolyticus (ATTC17749). All tests were performed in triplicates (n = 3) and the vertical bars represent the standard deviations. All Treatments are highly significant at $p < 0.01$*

Fig. 2 represents the minimum inhibitory concentrations (MICs) of different *D. metel* extracts against the tested bacterial strains. *B. subtilis* (MIC from 1.25 to 2.5 mg/ml except the CH_2Cl_2 seed extract) and *P. aeruginosa* (MIC from 0.625 to 10 mg/ml) were the most vulnerable pathogens, whereas *V. parahaemolyticus, V. alginolyticus, Staphylococcus typhimurium, Escherichia coli* and *Enterococcus faecalis* were among the most resistant ones (MIC: 10 mg/ml for leave extracts). The lowest CMI/CMB (0.078/0.156) was observed with the EP seed extract against *Enterococcus hirae*. Differences in activities are very high significant ($p < 0.001$) (*Fig. 2 and Table 2*). These results correlate with those recorded with the disc diffusion method.

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Figure 2. *Comparison of MIC values (mg/mL) of D. metel L. leave and seed extracts (10mg/mL) Petroleum Ether (PE): A; Dichloromethane (CH_2Cl_2): B; Ethyl acetate (ACOEt): C; Methanol (MeOH): D; bacterial strains in the same order as in the Figure: Bacillus cereus (ATCC10987); Bacillus subtilus (ATCC6633); Enterococcus hirae (ATCC10541); Enterococcus faecalis (ATCC29212); Escherichia coli (ATCC25922); Pseudomonas aeruginosa (ATCC9027); Salmonella enterica (ATCC10708); Salmonella typhimurium (ATCC1408); Staphylococcus aureus (ATCC25923); Vibrio parahaemolyticus (ATCC17802) and Vibrio alginolyticus (ATTC17749). All tests were performed in triplicates (n = 3) and the vertical bars represent the standard deviations. All Treatments are very highly significant at p < 0.001 except Total alkaloid and Gentamicin are not Significant*

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Table 2. *Minimal bactericide concentrations values (MBC mg/mL) of D. metel organic extracts against bacterial pathogens*

Bacterial strains	Leave extracts				Seeds extracts				MBC	
	PE	CH_2Cl_2	ACOEt	MeOH	PE	CH_2Cl_2	ACOEt	MeOH	T. alkaloids	Gentamicin
B. cereus (ATCC10987)	*	*	1.25	1.25	*	*	*	2.5	*	5
B. subtilus (ATCC6633)	2.5	2.5	5	2.5	5	*	2.5	2.5	2.5	5
E. hirae (ATCC10541)	5	*	*	*	0.156	2.5	*	*	*	*
E. Faecalis (ATCC29212)	*	*	*	5	*	*	*	*	*	5
E.coli (ATCC25922)	*	*	*	*	*	*	5	2.5	*	5
P. aeruginosa (ATCC9027)	*	5	*	2.5	5	2.5	*	1.25	5	*
S. enterica (ATCC10708)	*	*	*	*	*	*	*	5	*	5
S. typhimurium (ATCC1408)	*	*	*	5	5	*	*	2.5	*	5
S. aureus (ATCC25923)	*	*	*	1.25	*	5	*	0.62	*	5
V.parahaemolyticus (TCC17802)	*	*	*	*	*	*	*	*	*	5
V.alginolyticus (ATTC17749)	*	*	*	*	*	*	*	*	*	5

*: MBC more than 10 mg/mL

The seed extracts are slightly more active over the leaves, and the methanolic extracts (0.625 to 10 mg/ml) seem to be more active over the organic extracts. All treatments are highly significant at $p < 0.001$ except Total alkaloid and Gentamicin which are not Significant. For many of the bacterial strains tested, *Datura* leaves and seeds extracts seem to have less MICs than the reference antibiotic Gentamicin (*Fig. 2*). The minimum bactericidal concentrations MBCs of *D. metel* organic extracts were noted to be close to the MIC values (*Table 2*).

Antifungal activity

The effects of different tested extracts on fungal growth are presented in *Fig. 3* and *Fig. 4*.

The comparison of the growth inhibition percentages (%) of different organic Leave & Seed extracts from *D. metel L.*, at a concentration of 500 µg/ml are represented on *Fig. 4*. The percent inhibition values vary from 0 to 100%.

The MeOH and ACOEt extracts showed the strongest inhibition effects with percent inhibition values ranging from 56.5 to 100% and from 31 to 100% respectively with a very high significance ($p < 0.001$). The most inhibited fungi were *A. fumigatus* (90.9%), *A. niger* (82.7%), *S. brevicaulis* (83.3%), *T. mentagrophytes* (86.6%) and *C. albicans* (100%) with the methanolic leave and seed extracts. They were more effective against the majority of fungal strains (*Fig. 4*). MIC values ranged from 62.5 to 250 g/ml as shown on *Table 3*. No revealing difference was observed between the leaves and seed extracts, only with ACOEt extracts (*Table 3*).

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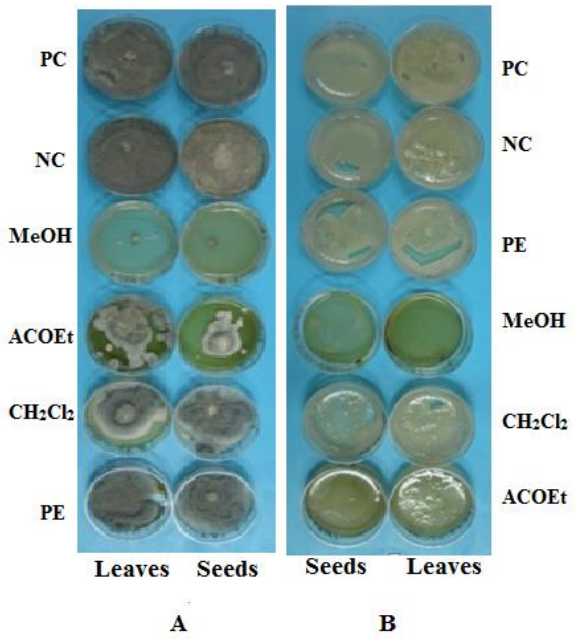

Figure 3. *The antifungal activity of D.metel Leaves and seeds extracts 500µg/ml; (A) Aspergillus fumigatus; (B) Candida albicans; (PC) positive control; (NC) negative control: alcohol 99%; (PE) petroleum ether; (CH_2Cl_2) dichloromethane; (ACOEt) ethyl acetate; (MeOH) methanol*

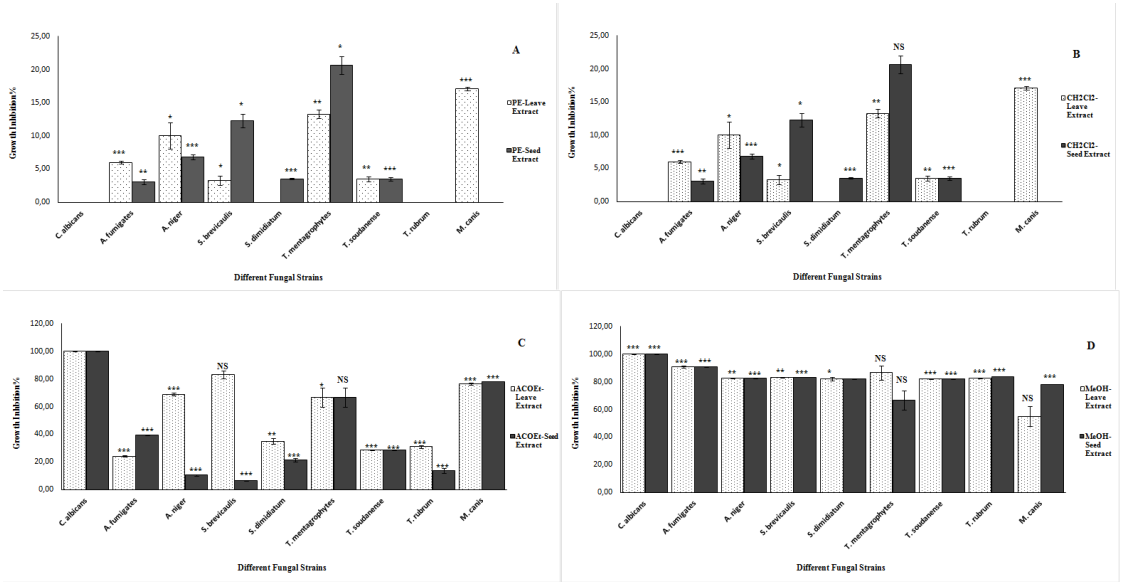

Figure 4. *Comparaison of the Growth Inhibition percentage (%) of. different organic Leave & Seed extracts from D. metel L. (500µg/ml): Petroleum Ether (PE): A; Dichloromethane (CH_2Cl_2): B; Ethyl acetate (ACOEt): C; Methanol (MeOH): D; fungal strains strains in the same order as in the Figure: Candida albicans; Aspergillus fumigates; Aspergillus niger; Scopulariopsis brevicaulis; Scytalidium dimidiatum; Trichophyton mentagrophytes; Trichophyton soudanense; Trichophyton rubrum and Microsporum canis. Inhibitory power was interpreted as follows: 0–25%, no or little inhibition; 26–50%, average inhibition; 51–100%, strong inhibition. All tests were performed in triplicates (n = 3) and the error bars represent the SD. Asterisks denotes significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*

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Table 3. *The MIC (µg/mL) of Datura metel organic extracts against fungal strains*

Fungal strains	MIC (µg/mL)							
	Leaves extracts				Seeds extracts			
	PE	CH_2Cl_2	ACOEt	MeOH	PE	CH_2Cl_2	ACOEt	MeOH
Candida albicans (B)	*	*	250	250	*	*	250	250
Aspergillus fumigates (B)	*	*	*	62.5	*	*	*	62.5
Aspergillus niger (B)	*	*	*	250	*	*	*	250
Scopulariopsis brevicaulis (B)	*	*	250	250	*	*	*	250
Scytalidium dimidiatum (IP)	*	*	*	250	*	*	*	250
Trichophyton mentagrophytes (B)	*	*	*	250	*	*	*	*
Trichophyton soudanense (B)	*	*	*	250	*	*	*	250
Trichophyton rubrum (B)	*	*	*	250	*	*	*	250
Microsporum canis (IP)	*	*	250	*	*	*	125	250

*: minimum inhibitory concentration (MIC): more than 500 µg /mL; (B): Microbiological Laboratory, Besancon; (IP): Institut Pasteur, Paris; Petroleum Ether (PE); Dichloromethane (CH_2Cl_2); Ethyl acetate (ACOEt)

Discussion

Due to their wealth of active components, plant extracts are widely assessed for their biological properties. To our knowledge, this is one of the few studies that have investigated the antibacterial, antidermatophyte, and anticandida properties of Tunisian *D. metel* organic extracts from seeds, leaves, and total alkaloids from roots.

The qualitative preliminary phytochemical analysis of *D. metel* extracts from seeds and leaves revealed the presence of saponins, alkaloids, tannins, Iridoids and flavonoids. Our findings in this context are in line to several other reports (Nandakumar et al., 2017; Partap et al., 2019; Alam et al., 2021; Sharma et al., 2021). On the other hand, tannins were not identified in the extracts of *D. metel* in some other literature but are present in seed and leave extracts considered in this study (Irudayaraj et al., 2010; Muthusamy et al., 2014).

Phytochemical substances are involved in a wide range of biological activities, including antimicrobial properties. Numerous among them, have shown significant antibacterial action (Cowan, 1999; Mahizan et al., 2019). In our study, *D. metel* extracts inhibited all of the microorganisms examined, except for the genus *Vibrio*. This discovery supports the use of this herb in traditional medicine to cure cough, diarrhea, and certain skin disorders (Dabur et al., 2004; Al-Snafi, 2017; Alam et al., 2021). Furthermore, our findings are consistent with earlier studies that found antibacterial properties of *D. metel* extracts from Oman, Pakistan and India, but with variable potency (Al-Jafari and Hossain, 2015; Krishnan et al., 2017; Bawazeer and Rauf, 2021).

Moreover, the comparison of the bacteriostatic and bactericid action of extracts with different polarities in the present study is as follow: MeOH > Gentamicin > ACOEt >

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CH2Cl2 > total alkaloids > EP. Several earlier studies have documented the antibacterial potentials from alkaloid component (Al-Jafari and Hossain, 2015; Pervaiz et al., 2019; Sharma et al., 2021). In our study, total alkaloids extracted from the roots of *D. metel* were active against only two bacteria among a total of eleven strains tested. This difference in activity among different extracts could be explained by the synergistic action between phytochemicals found in every crude extract. We call synergism, the fact that the therapeutic effect of two or more compounds is higher than if each one is used alone. Thus, toxicity can be decreased since fewer concentrations can be used from both compounds (Chung, 2011).

In the same context, *E. coli*, was not only inhibited but completely killed by 2.5 mg/ml of seed methanolic extract from Tunisian *D. metel*, while it was not inhibited by "5`, 7 `, dimethyl 6 ` hydroxyl 3 amine β-yne sitosterol", isolated from the leaves of Nigerian *D. metel* (Donatus et al., 2009). In another study, the methanolic extract of *D. metel* was effective against *E. coli* and *P. aeruginosa* (Bachheti, 2018). Furthermore, for the same solvent, seed extracts were significantly more active than leaf extracts. Previous antibacterial research has also deduced differences in activity between extracts and organs of *D. metel* (Al-Snafi, 2017; Sharma et al., 2021). According to Carrubba et al. (2021), the variability in activity for the same species may be related to the existence of bioactive secondary metabolites, which are known to cause damage to cell membranes and vary from organ to another, as well as climatic circumstances, according to the place of collection (geographical origin) and physiological growth period of the plant (Rouis et al., 2013).

Continuing with the antimicrobial activities, *D. metel* leaf and seed extracts were tested for antifungal activity. Our findings indicated a high significant inhibition of many harmful fungi (*T. mentagrophytes, T. soudanense, T. rubrum, C. albicans and M. canis*). As shown in our experiments, the methanol extract of *D. metel* was the most efficacious, with the highest percentage of inhibition seen against *S. brevicaulis* radial growth (83.3%). Similarly, Bawazeer and Rauf (2021) discovered that the chloroform and methanol fractions of *D. metel* exhibited a promising antifungal activity against *Aspergillus flavus, Fusarium solani,* and *Microsporum canis,* but *Candida albicans*, on the other hand, was resistant to all extracts tested.

Furthermore, our data support the use of *D. metel* in traditional medicine to treat dermatitis of the hair, nails, and skin. Although *C. albicans* frequently demonstrated resistance to natural extracts in our previous researches (Bel Hadj Salah et al., 2006; Ben Othman, 2017), the current findings revealed an interesting and a high significant anti-Candida activity for ACOEt and MeOH extracts of *D. metel* (MIC 250 µg/ml). The presence of flavonoids, saponins and alkaloids might explain Thornapple's intriguing antifungal effectiveness. According to Trdá et al. (2019), saponins have been reported to have antifungal potential (Tagousop et al., 2018). Moreover, flavonoids were revealed to have inhibition effects against the germination of plant fungi (Al Aboody et al., 2020).

Tropane alkaloids scopolamine and atropine characterise *D. Metel* in many reports and indole alkaloids were identified in seeds of *D. metel* (Yang et al., 2010; Kohnen-Johannsen et al., 2019; Pervaiz et al., 2019; Alam et al., 2021; Sharma et al., 2021).

Moreover, Atropine has a strong antiviral impact against HSV-1 DNA viruses PI-3 as well as HIV-1 RNA viruses and COVID-19 (Devkar et al., 2021).

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Conclusion

Overall, the present study suggests that extracts from Tunisian *D metel* have efficient and a high significant antimicrobial potential, supporting its folkloric use. This research also points to the promising value of *D. metel* as anticandida and antidermatophyte source. Further analysis, dealing with bioactive molecules from the organic extracts and their eventual cytotoxicity, are required to confirm the possible therapeutic and/or industrial valorization of this plant.

Conflict of interests. The authors state that they do not have any conflict of interests.

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