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RESEARCH ARTICLE

Antioxidant and antimicrobial potentials of *Matricaria pubescens* from southeastern Morocco

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Abstract

This study explores the antioxidant and antimicrobial properties of *Matricaria pubescens* (Desf) Schultz, an aromatic plant traditionally used in Moroccan medicine. Collected from Errachidia, extracts were prepared using water, methanol and petroleum ether to assess polyphenol, flavonoid and tannin contents. Antioxidant capacity was evaluated via DPPH, ABTS and RPC assays, while antimicrobial activity was tested against six microbial strains using disc diffusion and microdilution methods. The methanolic extract exhibited the highest bioactivity, with inhibition zones of 16 mm (*Staphylococcus aureus*), 18 mm (*Bacillus subtilis subsp. spizizenii*), 10 mm (*Escherichia coli*), 12 mm (*Salmonella abony*), 14 mm (*Candida albicans*) and 12 mm (*Trichophyton rubrum*). *Bacillus subtilis* subsp. *spizizenii* and *Staphylococcus aureus* were the most sensitive (MIC: 1.87 and 3.75 mg/mL), while antifungal activity was observed against *Candida albicans* (7.5 mg/mL) and *Trichophyton rubrum* (15 mg/mL). These findings highlight the significant antimicrobial potential of *M. pubescens*, reinforcing its pharmaceutical value and encouraging further investigation into its bioactive compounds.

Keywords: antimicrobial activity; antioxidant potential; *Matricaria pubescens;* phenolic compounds; southeastern morocco

Introduction

Antibiotics have represented a groundbreaking advancement in the medical field and significantly contributed to reducing global mortality and morbidity rates. However, their increasing and often inappropriate use has facilitated the emergence of antimicrobial resistance in certain microbial strains. This phenomenon undermines the initial benefits of antibiotics and now constitutes a significant public health challenge (1, 2).

Due to the need for alternatives to synthetic antibiotics and the growing threat of antibiotic resistance, interest in medicinal plants has significantly increased to develop new agents capable of combating the emergence of resistant microorganisms (3). Medicinal plants are acknowledged for their vast bioactive compounds, mainly phenolic compounds, demonstrating significant antioxidant and antimicrobial properties (4). These compounds play a crucial role in the plants' defence mechanisms, safeguarding it against environmental stress, pathogens and herbivores (4). In the context of human health, phenolic compounds are appreciated for their potential to counteract diseases caused by oxidative stress and microbial infections (5). Therefore, evaluating these compounds' antioxidant and antimicrobial properties is crucial to harness their therapeutic potential and develop natural, plant-based alternatives to synthetic drugs (6).

Moroccos' diverse climatic conditions, resulting from its unique geographical location, support a rich flora of aromatic and medicinal plants (7,8). Several species have been identified with notable antimicrobial properties (9-12). *Matricaria pubescens* (Desf.) Schultz (*M. pubescens*), part of the Asteraceae family, stands out as a promising candidate for investigation. Used in Moroccan traditional medicine to treat various conditions, *M. pubescens* (Desf) Schultz has shown potential therapeutic effects, suggesting the presence of bioactive compounds with antioxidant and antimicrobial properties (13-18). Recent studies highlight the crucial role of phenolic compounds, secondary metabolites produced by plants, in combating resistant pathogens. These compounds exert antimicrobial activity by targeting microbial cell membranes, interfering with DNA/RNA or enzyme synthesis and disrupting cell communication and microbial resistance mechanisms. Furthermore, phenolic compounds can

enhance the efficacy of conventional antibiotics, offering a synergistic approach to overcoming drug resistance (19). While previous studies have emphasized the ethnobotanical significance of *M. pubescens*, its phytochemical profile and biological activities remain insufficiently explored.

This study aims to validate the traditional uses of *M. pubescens* by investigating its antioxidant capacity and antimicrobial activity while exploring its potential for new therapeutic applications.

Material and Methods

Plant materials

The aerial parts (Fig. 1) of *M. pubescens* were harvested in April 2023 from the province of Errachidia), located in Southeastern Morocco (Latitude: 31.9389767, Longitude: -3.6158102; Altitude: 938 m). An arid desert climate with hot and dry conditions characterizes the region. The plant sample was air-dried under shade at room temperature (20-25 °C) for 20 days.

Extract preparation

The extraction process was carried out using two methods:

a) Aqueous extraction: 500 mL of distilled water was brought to a boil with 5 g of plant material for 15 min, Following the procedure (12).

b) Organic solvent extraction: 50 g of powdered plant material was subjected to extraction in the dark, maintained at room temperature (25 ± 2 °C) for 48H with constant agitation using 500 mL of either 70 % methanol or 70 % petroleum ether. The mixture was filtered and the extract was concentrated to dryness under vacuum using a rotary evaporator (BUCHI, Switzerland; Rotavapor R-210 with Vacuum Pump V-700) at 40 °C. The resulting residue was stored at -20 °C for further analysis (20).

The yield of the three plant extracts was calculated using the Equation 1formula

$$Y (\%) = 100\ M2/M1, \qquad \text{(Eqn. 1)}$$

where Y is the extraction yield in %,

M_2 is the mass of the extract after solvent evaporation in mg,

M_1 is the mass of the plant sample in mg.

Phytochemical screening of *M. pubescens*

Total phenolic content (TPC)

The determination of total phenolic content was carried out using the Folin-Ciocalteu method (22). Briefly, 0.5 mL of the prepared extracts were mixed with 2.5 mL of Folin-Ciocalteu reagent (10 %) and 2 mL of sodium carbonate solution (4 %). The mixture was shaken and incubated at room temperature for 2 hr in the dark. Absorbance was measured at 750 nm. Total phenolics were expressed as gallic acid equivalents (GAE) in mg/g of crude extract.

Flavonoid content (FLA)

According to the aluminium chloride colourimetric protocol described by Ayyobi et al., the FLA content of the extracts was quantified spectrophotometrically (Zuzi 4255/50) at 510 nm (23). The total flavonoid content of the extracts was expressed in milligrams of quercetin equivalent (QE) per gram of dry weight.

Total condensed tannins (TCT)

The TCT were determined in the extracts of *M. pubescens* using the standard method (24). The condensed tannins were expressed as mg of catechin equivalents (CE) per g of DW.

Antioxidant and radical scavenging activities

Reducing power capacity (RPC)

The standard method was used to determine the RPC assay and butylated hydroxytoluene (BHT) was included as a positive control (25).

Free radical scavenging ability

The extracts' capacity to neutralize free radicals was assessed according to the standard procedure (26). BHTs' free radical scavenging activity was also evaluated and compared to the extracts.

ABTS radical assay

The free radical scavenging activity of the extracts was determined and the capacity of BHT was similarly investigated

Fig. 1. Aerial parts of *Matricaria pubescens* (Desf.) Schultz.

(22).

Antimicrobial activity

Microbial strains

In this study, the antimicrobial activity of *M. pubescens* extracts was evaluated against six microbial species, comprising four bacterial strains obtained from the culture collection of the Institute of Hygiene (Rabat): *Bacillus subtilis* subsp. Spizizenii (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Salmonella abony* (NCTC 6017) and *Escherichia coli (ATCC 8739)* and two fungal strains: *Candida albicans* (ATCC 10231) and *Trichophyton rubrum* (T.R), Isolated from a mucosal sample at the Errachidia medical analysis laboratory.

Disc diffusion method

The antimicrobial activity of *M. pubescens* extracts was determined using the disc diffusion technique, which was conducted on Mueller-Hinton agar (MHA) for bacterial strains and Sabouraud agar for fungal strains (27). Bacterial suspensions were prepared and adjusted to a density of 1-2 × 10^8 CFU/mL, while fungal suspensions were standardized to 1-5 × 10^6 CFU/mL. For bacterial tests, 100 µL of the prepared suspension was spread evenly onto the surface of MHA and for fungal tests, the suspensions were spread onto Sabouraud agar. Sterile Whatman paper discs were impregnated with 7 µL of the plant extract solution (30 mg/L, dissolved in 10 % DMSO) and positioned on the surface of the agar. Negative controls included discs impregnated with 10 µL of 10 % DMSO, while positive controls included imipenem (10 µg/disc), recognized for its broad spectrum of activity. Additionally, fluconazole (25 µg/disc) was used as a control for *C. albicans* and *T. rubrum*. The plates were maintained at 4 °C for 30 min to permit the extract to penetrate the agar. Following this, incubation in a bacteriological incubator (Model B 28 | Standard) at 37 °C was performed for bacterial strains (18-24 h) and 30 °C for fungal strains (48 h). Measurements of the inhibition zone diameters (inclusive of the discs) were recorded in mm.

Minimum inhibitory concentrations (MIC) and fungicidal (MFC) determination

MIC and MFC were measured using a broth microdilution assay conducted in sterile flat-bottom 96-well microplates. A series of dilutions of the methanolic extract (MET-E) was prepared, ranging from 30 mg/mL to 0.234 mg/mL, using a 10 % DMSO solution (The methanolic extract was selected based on its performance in the disc diffusion test). Each well was filled with 50 µL of M-H Broth for bacterial strains or Potato Dextrose Broth (PDB) for yeast and fungal strains, followed by 50 µL of the extract solution. Next, the microbial suspension (30 µL) was added to each well. Positive controls consisted of Mueller-Hinton or Potato dextrose broth with Imipenem (10 µg/mL) for bacterial strains and Fluconazole (25 µg/mL) for *C. albicans* and *T. rubrum*. Negative controls contained broth with 10 % DMSO. The incubation conditions were set at 37 °C for 24 h for bacteria and 30 °C for 48 h for yeast and fungal cultures. Following the initial incubation, 5 µL of 0.01 % (w/v) resazurin solution was added to each well and the plates were incubated for an additional 4-5 h. The MIC and MFC were recorded as the lowest extract concentrations that prevented visible microbial growth compared to the negative control. All assays were performed in triplicate to ensure reproducibility (28).

Statistical data analysis

SPSS version 23.0 for Windows was employed for statistical analysis. The data were analyzed using analysis of variance, followed by Fishers' least significant difference (LSD) test at a 5 % significance level. All experiments were performed in triplicate.

Results and Discussion

Plant extract yield

Table 1 presents the yield percentages of the crude extracts. The highest yield was obtained from the aqueous decoction (22 %), followed by the methanolic extract at 7.8 %, while the lowest yield was observed for the petroleum ether extract at 4.2 %. The extraction yields were primarily influenced by the extraction method and the solvents utilized. Also, the variation in extraction yields is highly dependent on the polarity of the compounds within the plant. These results suggest that the plant contains a higher concentration of polar substances than non-polar ones. This observation is consistent with the work in which the result indicates that extraction yield and total polyphenol content depend on the extraction method and the nature of the solvent used (29). Similarly, solvent polarity has a significant impact on both the extraction efficiency and the bioactivity of the obtained extracts (30).

Table 1. Yield percentages of the crude extracts

Extract	Extract yield (%)
Water	22
Methanol (70 %)	7.8
Petroleum ether (70 %)	4.2

Total flavonoid, phenolic and condensed tannin contents

The data indicate clear variations in the total flavonoids, phenolics and condensed tannins contents across the different extracts, illustrating the significance of solvent polarity in determining the efficiency of extracting bioactive compounds (Table 2). The methanol extract displayed the highest flavonoid content (6.76 ± 0.012 mg QE/g of DW), highlighting the ability of methanol, a polar protic solvent, to extract these compounds effectively. The aqueous extract also contained a significant content of flavonoids (5.55 ± 0.13 mg QE/g of DW). In contrast, the petroleum ether extract showed the lowest content (3.41 ± 0.16mg QE/g of DW), consistent with the lower polarity of petroleum ether. Our findings highlight the significant impact of solvent choice on the efficiency of flavonoid extraction, with polar solvents such as methanol and ethanol demonstrating superior performance compared to less polar alternatives. These results agree with those presented for *Dicoma tomentosa* Cass. (Asteraceae), where extraction with 70 % ethanol yielded the highest total flavonoid content compared to water and dichloromethane extracts. The flavonoid content of *M. pubescens* aligns with the characteristic values reported for other plants within the same family, such as *Artemisia herba-alba* and *Anthemis arvensis* L.,

Table 2. Total phenolic, flavonoid and condensed tannin contents

Extract	Flavonoid content (mg QE/ g of DW)	Total tannins condenses (mg CE/g of DW)	Total phenolic content (mg GAE/g of DW)
Water	5.55 ± 0.13^{b}	2.33 ± 0.15^{c}	6.62 ± 0.09^{b}
Methanol	6.76 ± 0.12^{a}	2.60 ± 0.09^{b}	7.24 ± 0.14^{a}
Petroleum ether	3.41 ± 0.16^{c}	3.52 ± 0.10^{a}	4.17 ± 0.11^{c}

which range from 0.75 to 1.31 g QE/100 g of DW (32).

The results further demonstrated that the petroleum ether extract presented the highest concentration of condensed tannins (3.52 mg CE/g of DW), followed by the methanolic extract (2.60 mg CE/g of DW), while the aqueous extract presented the minimal content (2.33 mg CE/g of DW). Previous studies have demonstrated that organic solvents, such as acetone and petroleum ether, are more effective at extracting condensed tannins than aqueous solvents, owing to the higher affinity of high-molecular-weight tannins for less polar solvents (17, 33). Furthermore, research indicates that the lower yields obtained with aqueous extracts may be due to the slower diffusion of high-molecular-weight tannins in water than oligomeric tannins (34, 35).

The analysis of phenolic compounds revealed that the methanolic extract contained the highest concentration (7.24 mg GAE/g of DW), while the petroleum ether extract exhibited the lowest phenolic content. These findings underscore the pivotal role of secondary metabolites in plant defence mechanisms against abiotic and biotic stresses while highlighting their significant biological activities (36). Research has further established the remarkable phytochemical richness of *M. pubescens*, which includes sterols, steroids, tannins, saponins, flavonoids and triterpenicheterosides. These compounds are crucial to the plants' therapeutic efficacy, providing analgesic, anti-inflammatory and anti-edematous benefits (39). Moreover, The remarkable diversity of these phytochemicals offers scientific validation for the traditional use of *M. pubescens* in treating venous insufficiency, hemorrhoidal crises and conditions associated with capillary fragility (37).

The antimicrobial properties of *M. pubescens* extracts can be attributed to specific bioactive compounds, mainly flavonoids and phenolic acids, which have been widely recognized for their antibacterial and antifungal activities. Flavonoids, such as quercetin and kaempferol, exert their antimicrobial effects through multiple mechanisms, including inhibition of bacterial enzymes, disrupting microbial membrane integrity and interference with nucleic acid synthesis. These compounds can also chelate metal ions, depriving microorganisms of essential cofactors required for growth. Phenolic acids, such as gallic acid and caffeic acid, also contribute significantly to antimicrobial activity by increasing bacterial membrane permeability, leading to leakage of intracellular contents and eventual cell death. Additionally, they can act as enzyme inhibitors, targeting key microbial metabolic pathways. The high antimicrobial efficacy observed in the methanolic extract suggests that these bioactive compounds are best extracted in polar solvents, reinforcing their role in plant defence mechanisms (19).

Antioxidant activity

The antioxidant activity of *M. pubescens* extracts was assessed using three distinct assays: DPPH, ABTS and RPC. Table 3 illustrates the results obtained from the antioxidant activity evaluation. The capacity of plant extracts to scavenge the DPPH radical, represented by IC50, differed according to the solvent employed. A lower IC50 value reflects stronger antioxidant activity. This study revealed that the methanolic extract possessed the most effective DPPH scavenging activity, with an IC50 value of 4.28 mg/mL, whereas the aqueous decoction had an IC50 value of 4.51 mg/mL. The petroleum ether extract demonstrated the lowest antioxidant activity against DPPH, with an IC50 of 4.60 mg/mL.

Table 3. Antioxidant activity of *M. pubescens* extracts

	IC_{50} values		
Extract	**DPPH (mg/mL)**	**RPC (mg/mL)**	**ABTS (mg/mL)**
Water	4.51 ± 0.04^{c}	18.06 ± 0.14^{c}	14.10± 0.12^{c}
Methanol	4.28 ± 0.02^{b}	14.57 ± 0.09^{b}	13.83± 0.05^{b}
Petroleum ether	4.60± 0.06^{d}	19.14 ± 0.04^{d}	18.03 ± 0.10^{d}
BHT	0.04 ± 0.001^{a}	2.41 ± 0.13^{a}	0.21 ± 0.002^{a}

BHT: Butylated Hydroxytoluene; IC50: Half Maximal Inhibitory Concentration;DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; RPC: Reversed-Phase Chromatography; ABTS: 2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid

Based on the findings of this study, all the tested *M. pubescens* extracts exhibited significant reducing power. In this regard, the methanol extract displayed the highest reducing capacity (14.57 mg /mL). Then, the aqueous extract showed a reducing capacity of 18.06 mg/mL. However, the petroleum ether extract, with a 19.14 mg/mL value, demonstrated the lowest ability to reduce Fe(III). According to the results obtained from the ABTS test, The extraction solvent significantly influenced the IC50 values measured. The methanol extract of *M. pubescens* displayed the highest ABTS radical scavenging potential, as indicated by an IC50 value of 13.83 mg/mL. However, an LC50 in the range of 14.10 mg/mL was observed for the ABTS scavenging capacity of the aqueous decoction, compared to the petroleum ether extract, which exhibited the least activity, with an IC50 value of 18.03 mg/mL.

The study of antioxidant activity in *M. pubescens* extracts indicated a variation in antioxidant power according to the solvent selected for extraction. Research suggests that the antioxidant properties of extracts are closely linked to the solvent employed during their preparation (40, 41). Additionally, this variation in antioxidant power can be

explained by the quantity and type of polyphenols, flavonoids and tannins present in the raw extracts obtained by each solvent (42). Furthermore, using solvents of varying polarities allows for extracting specific groups of antioxidants, affecting the overall antioxidant capacity (43). Consistent with our findings, research indicates a significant antioxidant capacity in *M. pubescens* extracts harvested from Algeria (16, 18).

Antibacterial and antifungal activity

Agar diffusion technique

The results of the diffusion method for evaluating the antimicrobial activity of *M. pubescens* extracts highlighted the methanol extracts' significant effectiveness against all tested microbial strains. In contrast, the petroleum ether extract showed no inhibitory effect against the strains examined (Table 4). For bacterial species, the methanol extract strongly inhibited the growth of *B. subtilis* subsp. *Spizizenii* and *S. aureus*, the inhibition zones were found to measure 18 ± 1.20 mm and 16 ± 0.09 mm in diameter, respectively. The most minor inhibition zone was observed for *E. coli* (10 ± 0.15 mm in diameter). The aqueous extract affected the growth of *B. subtilis* subsp. *Spizizenii* and *S. aureus*, with inhibition zones measuring 11± 0.30 and 9± 0.60 mm, respectively. However, no antibacterial activity was detected against *S. abonyi* and *E. coli*. Regarding antifungal activity, Significant inhibition was observed with the methanol extract, With a diameter of inhibition zones measuring 14 ± 0.4 mm for *C. albicans* and 12 ± 0.25 mm for *T. rubrum*. In contrast, the aqueous extract only affected *C. albicans*, with an inhibition zone of approximately 9 mm.

The values of MIC and MFC

The observed MIC values varied from 1.87 mg/mL to 30 mg/mL, as shown in Table 5 and Fig. 2. Specifically for bacterial species, *B. subtilis* subsp. *spizizenii* demonstrated the smallest MIC value of 1.87 mg/mL, the next lowest MIC value was observed for S. aureus, at 3.75 mg/mL. In contrast, the *S. abony* strain exhibited a 7.5 mg/mL MIC. However, *E. coli* exhibited a MIC of 15 mg/mL. In this context, it was observed that Gram-positive bacteria showed higher susceptibility compared to Gram-negative bacteria. This difference in susceptibility is primarily attributed to the structural variations between the cell walls of the two bacterial types (44). Regarding fungal microorganisms, the MFC values recorded for *C. albicans* and *T. rubrum* were 7.5 mg/mL and 15 mg/mL, respectively. Research indicates that *M. pubescens* extracts (infusion and ethanolic extracts) Presented antimicrobial activity against various microorganisms, including 9 bacterial species and *C. albicans* (37).

Additionally, research indicates that the essential oils of two *M. pubescens* varieties collected in Algeria displayed an inhibitory effect against the strains examined, including *E. coli*, *S. aureus* and *Pseudomonas aeruginosa*, With MIC values varying between 5.11 and 41.8 µg/mL (18). The study also revealed that the essential oils showed MFC values for *C. albicans* ranging from 2.67 to 21.82 µg/mL. Another survey by Makhloufi et al. (38) demonstrated that macerated methanolic and ethanolic extracts of *M. pubescens* (with a concentration of 1.86 mg/mL) completely inhibited the growth of five fungal species: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium purpurogenum, Fusarium oxysporum* f.sp. albedinis (FOA1 and FOA2).

The results of the antimicrobial test (agar diffusion technique, MIC and MFC) Indicated that the growth of the tested microorganisms is influenced by *M. pubescens* extracts, with varying efficacy depending on the solvent used. The notable antimicrobial activity observed in the methanolic extract is likely due to its elevated levels of flavonoids and phenolic compounds. In contrast, the lack of inhibitory effect in the petroleum ether extracts potentially results from the low concentrations of these secondary metabolites in this extract. Several studies have demonstrated that flavonoids and phenolic compounds extracted from various plants exhibit antimicrobial actions through different mechanisms, such as disrupting microbial membranes, altering membrane permeability and interfering with intracellular processes (44). These bioactive compounds can bind to the microbial cell envelope via hydrogen bonding, facilitated by their hydroxyl (-OH) groups, leading to membrane destabilization and leakage of cellular contents (44). Additionally, flavonoids and phenolic compounds can penetrate microbial cells, disrupt metabolic pathways, inhibit DNA and RNA synthesis and interfere with protein synthesis (45, 46).

Findings from this study confirm that the antioxidant and antimicrobial properties of *M. pubescens* extracts align with their traditional applications in managing digestive, genitourinary and respiratory conditions (47-49).

Table 4. Antimicrobial activity of *M. pubescens* (mm)

Strains / Extract	*S. aureus*	*B.subtilis* subsp. Spizizenii	*E. coli*	*S. abony*	*C.albecans*	*T. rubrum*
Water	9 ± 0.15^{c}	11± 0.30^{c}	6 ± 0.00^{c}	6 ± 0.00^{c}	9 ± 0.60^{c}	6 ± 0.00^{c}
Methanol	16 ± 0.09^{b}	18± 1.20^{b}	10 ± 0.15^{b}	12± 0.25^{b}	14± 0.4^{b}	12 ± 0.7^{b}
Petroleum ether	6 ± 0.00^{d}	6 ± 0.00^{d}	6 ± 0.00^{c}	6 ± 0.00^{c}	6 ± 0.00^{d}	6 ± 0.00^{d}
Imipenem	42 ± 0.50^{a}	40 ± 0.40^{a}	36 ± 0.50^{a}	38 ± 0.70^{a}	ND	ND
Fluconazole	ND	ND	ND	ND	28 ± 1.50^{a}	25± 0.90^{a}

ND: Not determined.

Table 5. Influence of MIC and MFC of methanolic extract of *M. pubescens* against pathogenic microorganisms

Antibacterial and antifungal activity of extract aqueous methanol of *Matricaria pubescens* (MIC and MFC values, mg/mL)					
S. aureus	*E. coli*	*S. abony*	*B. subtilis* subsp. Spizizenii	*C. albicans*	*T. rubrum*
3.75	15	7.5	1.87	7.5	15

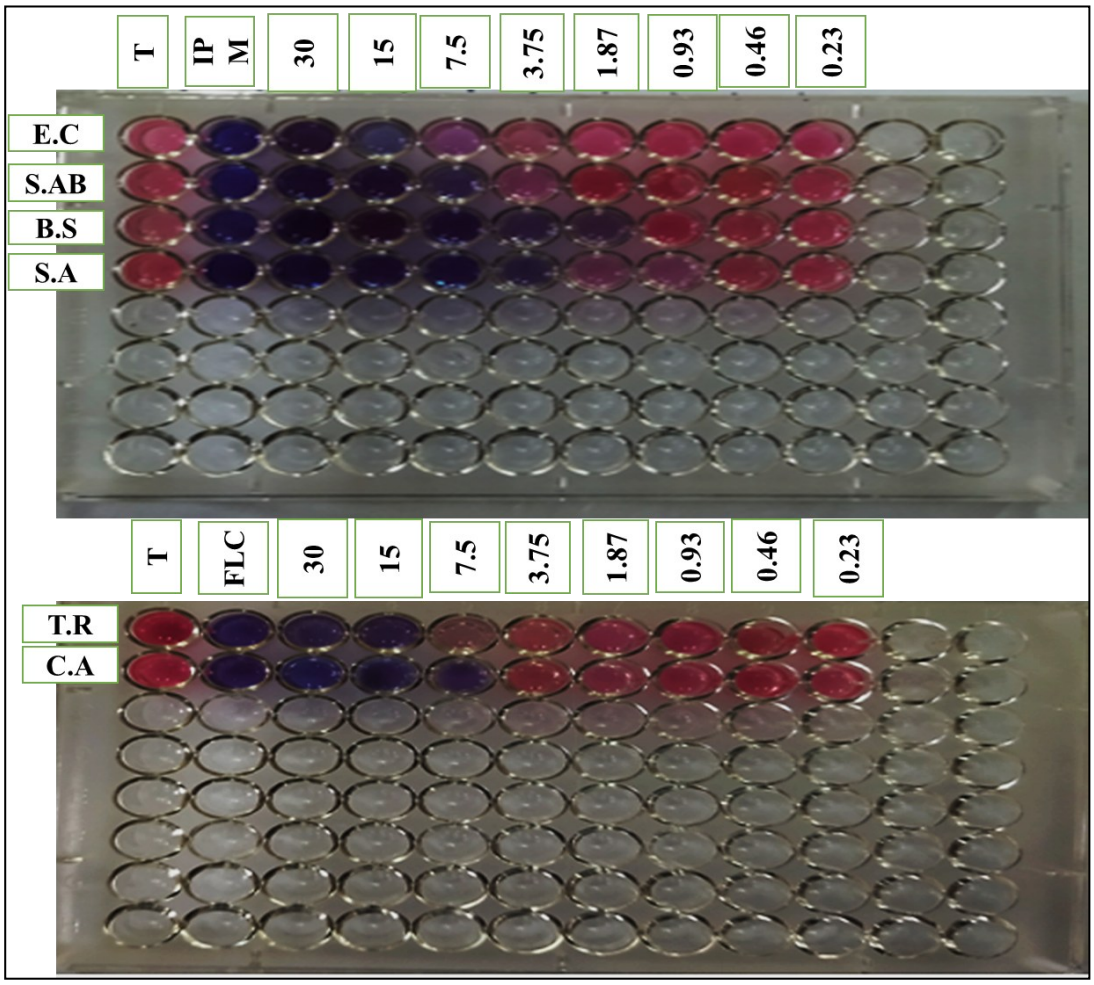

Fig. 2. Determination of the MIC and MFC of methanolic extract of *M. pubescens* against pathogenic microorganisms using the microdilution method. T:ve control; IMP: Imipenem, FLC: Fluconazole, E.C: *E. coli,* S.AB: *S.abony,* B.S: *B. subtilis* subsp. Spizizenii, S.A: *S. aureus*, C.A: *C. albicans*, T.R: *T. rubrum.*

Conclusion

Medicinal plants have emerged as valuable resources in addressing the growing challenge of microbial resistance, offering natural alternatives to conventional antibiotics. *M. pubescens* has demonstrated significant antimicrobial properties in this context, complemented by its potent antioxidant potential. These activities are attributed to the rich diversity of bioactive secondary metabolites, notably phenolic compounds, flavonoids and tannins, which exhibit antibacterial and antifungal effects. This studys' findings lend scientific evidence to the traditional applications of *M. pubescens* in combating infectious diseases. Upcoming research should concentrate on the precise identification and the mechanisms underlying their biological activities to further explore their therapeutic potential.

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Authors' contributions

AE and IJ did the conceptualization, investigation, methodology, software, visualization and writing - original draft. KS and MB did the methodology, writing-review & editing. ME, AH and NL did Supervision, Validation, review & editing.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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