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Original article

Polyphenolic characterization and antifungal properties of *Urtica urens L.* extracts against *Candida* strains: A natural approach to fungal control

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Abstract

Urtica urens is classified within the Rosales subfamily and belongs to the Urticaceae family. Its well-documented health benefits are primarily attributed to its phenolic compounds. This study investigates the polyphenol, flavonoid, and condensed tannin content of *Urtica urens L.* (*U. urens L.*) extracts obtained using aqueous ethanol and water as solvents. The antifungal activity of ethanol and aqueous extracts derived from the aerial parts of *Urtica urens L.* was assessed in vitro against various *Candida* species (*C. parapsilosis, C. albicans, C. tropicalis, C. metapsilosis, C. orthopsilosis, C. glabrata*). Three methods were employed: the well diffusion method, disc diffusion method, and microdilution method. The findings revealed that the ethanol extract contained the highest levels of polyphenols. Both ethanol and aqueous extracts exhibited antifungal activity against the *Candida* strains tested. However, the aqueous extract demonstrated superior antifungal efficacy compared to the ethanol extract. The sensitivity of the strains to the extracts was concentration-dependent, with inhibition observed from a concentration of 1 g/mL. The ethanol extract displayed significant inhibitory activity (90.58%, 92.24%, and 91.33%, respectively) against *C. metapsilosis, C. glabrata,* and *C. orthopsilosis,* surpassing its activity against other strains. These findings suggested that *U. urens L.* extracts have potential as natural alternatives to chemical additives for controlling fungal diseases in plants.

Keywords: Antifungal activity; *Urtica urens* L; Extracts; Plant.

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1. Introduction

All living organisms rely on primary metabolism to produce essential molecules such as carbohydrates, amino acids, nucleic acids, proteins, and lipids, which serve as precursors for the synthesis of secondary metabolites through subsequent chemical reactions. In plants, these metabolites often play a crucial role in defensive mechanisms. To explore bioactive natural compounds, it is vital to employ simple biological assays to identify desired activities [1].

The preservative effects observed in many plants suggest the presence of antibacterial and antioxidant compounds [2-6]. Plants are rich in phenolic and nitrogenous compounds, including essential alkaloids and glycosides. Undoubtedly, they represent a significant source of biologically active, naturally occurring substances. Beyond their antioxidant and antimicrobial properties, polyphenols exhibit numerous additional health benefits [7].

Considering the therapeutic limitations of conventional chemical drugs, research into medicinal plants has increasingly focused on deriving herbal remedies from traditional medicines [8]. This approach is a crucial step in advancing industries beyond therapeutic applications, with cosmetics and food production, thereby supporting the broader utilization of natural resources [9].

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The Urticaceae family is a known source of polyphenols, and the health benefits of nettle are well-documented [10-13]. In Tunisia, the most abundant nettle species are *Urtica dioica* and *Urtica urens*. These plants grow spontaneously, and their aerial parts are commonly consumed as salad, particularly in North African and Mediterranean countries.

While some studies have investigated the antioxidant activity of *Urtica urens* [14], little to no information is available regarding its antifungal properties. The primary objective of this study is to assess the potential of *U. urens L.* as a novel source of natural phenolic compounds and to evaluate its antifungal activity. Additionally, this research aims to quantify the polyphenol, flavonoid, and condensed tannin content of *U. urens L.* extracts obtained using aqueous ethanol and water as solvents.

2. Material and methods

Plant Extraction

The aerial parts of *Urtica urens* were collected from Sfax region (Tunisia) and identified by Pr Chaieb from the Faculty of Sciences of Sfax (Tunisia). A voucher specimen (Pharm-PCT-2562) was deposited at INRAT. The plant material was carefully rinsed with distilled water, allowed to air-dry at room temperature for two days, and then ground

using a knife mill to obtain 100 g of *Urtica urens* powder. The resulting powder was stored in glass bottles at room temperature for subsequent use.

Ethanolic Extract: The powder was extracted with ethanol using the maceration method. Fifty grams of the powder were macerated for three days in 1 L of ethanol (70% volume fraction). The resulting solution was then filtered and concentrated.

Aqueous Extract: The aqueous extract of the dried aerial parts of *U. urens L.* (10 g) was prepared by infusing the powder in previously boiled distilled water (100 mL) for 20 minutes. After decantation and filtration, the filtrate was lyophilized [15].

Total phenolic content (TPC) determination: The TPC in the Urtica urens L. extracts was assessed calorimetrically using the Folin-Ciocalteu method [16]. The analysis was carried out in triplicate, and the TPC was reported as milligrams of gallic acid equivalents (GAEs) per 200 grams of each extract, based on a calibration curve created with a freshly prepared gallic acid solution. The equation $y=0.001x+0.014$ ($R^2=0.999$) was used to describe the absorbance-concentration relationship for gallic acid.

Total tannin content (TTC) determination: The TTC was determined from the AECS using the potassium iodide test [17]. The analysis was conducted in triplicate, and the results were expressed in milligrams of gallic acid equivalents per gram of dried extract (mg GAE/200 g DW), based on the calibration curve of gallic acid ($y = 0.121x + 0.011$, $R^2 = 0.9819$).

Determination of total flavonoid content (TFC): The TFC was determined using the colorimetric assay developed by Zhishen et al. [18]. The analysis was conducted in triplicate, and the TFC in the extract was expressed as catechin equivalents (CEs) per gram of extract, based on a calibration curve of freshly prepared catechin solution. The equation $y = 0.0049x$ ($R^2 = 0.998$) represents the absorbance-concentration relationship for catechin.

Total flavonol content (TFl) determination: In the plant extract was estimated using the method outlined by Yermakov et al. [19]. Each determination was carried out in triplicate. The flavonol content was expressed as milligrams of rutin equivalents (REs) per gram of extract, based on a standard curve created with varying concentrations of rutin. The equation $y = 0.002x + 0.009$ ($R^2 = 0.998$) defines the absorbance-concentration relationship for rutin.

Determination of ortho-diphenol content (ODC): The ODC in the *Urtica urens L*. extracts was determined using the method of Mateos et al. [20]. The ODC was expressed in milligrams of caffeic acid equivalents (CAEs) per gram of extract, based on a calibration curve of freshly prepared caffeic acid solution. The equation $y = 0.021x - 0.017$ ($R^2 = 0.999$) represents the absorbance-concentration relationship for caffeic acid.

Estimation of vitamin D: Vitamin D levels were measured using commercial reagent kits (Ref: 20151 and 20091) obtained from Biomaghreb (Ariana, Tunis, Tunisia), utilizing colorimetric methods.

Estimation of vitamin E: Vitamin E was extracted using the method outlined by Katsanidis and Addis [21].

Estimation of vitamin C: Vitamin C (ascorbic acid) levels were measured using the method described by Jacques-Silva et al. [22].

Antifungal activity

Fungal Strains Collection and Identification: The antifungal activity of the various extracts of *Urtica urens L.* was evaluated against different species of the genus *Candida*, including *Candida albicans* (n = 33), *Candida glabrata* (n = 1), *Candida parapsilosis* (n = 31), *Candida tropicalis* (n = 1), *Candida metapsilosis* (n = 6), and *Candida orthopsilosis* (n = 4). These strains were sourced from the Parasitology-Medical Mycology Laboratory, Habib Bourguiba University Hospital in Sfax and the Faculty of Medicine of Sfax. The selection of these species was primarily based on their frequent involvement in various candidal infections and their associated problems with resistance to conventional antifungal treatments.

In vitro antifungal activity: To evaluate the antifungal activity of the leaf extracts, we employed the agar diffusion method as described by Bauer and Kirby [23], along with the microdilution method to determine the minimum inhibitory concentration (MIC).

Disc Method: The protocol followed was that of Choi et al. [24]. Whatman paper discs (7 mm in diameter) were soaked for a few seconds in the different extract solutions, which were dissolved in distilled water for the aqueous extract and in ethanol for the ethanolic extract. The discs were then placed under sterile conditions on agar plates that had been inoculated with a yeast suspension of 0.5 McFarland optical density. An appropriate reference antibiotic disc (Amphotericin B or fluconazole) was applied to each Petri dish as a positive control. Discs soaked in sterile distilled water were used as a negative control. The plates were incubated for 24 to 48 hours at 37°C. Antifungal activity was assessed by measuring the diameter of the growth inhibition zone in millimeters (including the 7 mm disc diameter) [25].

Well method: This method involved seeding the fungal suspension (0.5 McFarland density) on the surface of Sabouraud agar medium. Five small wells (6 mm in diameter) were created using sterile yellow plastic cones. Each well was inoculated with 40 µL of extract, distilled water (negative control), or fluconazole/amphotericin B (positive controls). The plates were incubated for 24 to 48 hours at 37°C. The antifungal activity was assessed by measuring the diameter of the growth inhibition zone in millimeters (including the 6 mm well diameter).

Microdilution method and determination of MIC: The microdilution technique was applied to assess the MIC of the plant extracts using a 96-well Elisa plate, as described by Elof [26]. The MIC is defined as the lowest concentration of extract that inhibits yeast growth by more than 90%, when compared to the positive control [26]. The percentage inhibition was calculated using the formula: % inhibition=((OD negative control–OD extracted)/OD negative control)×100, where OD negative control = Optical density of the negative control and OD extracted = Optical density of the wells containing the extract.

Statistical analysis

Statistical analysis was conducted using SPSS version 27. All results are presented as the mean ± standard error of the mean (SEM).

2. Results

Total phenolic, flavonoid, tannin, flavonol, and ortho-diphenol contents

The contents of polyphenols, tannins, flavonoids, flavonols, and ortho-diphenols were evaluated in different Urtica urens L. extracts, with the results summarized in Table 1. The total phenolic content was measured using the Folin-Ciocalteu reagent and expressed in terms of gallic acid equivalents (GAEs). The findings from the total phenolic assay highlight the important role that phenolic compounds play in scavenging and/or reducing free radicals.

The total phenolic content of the various Urtica urens L. extracts was found to be solvent-dependent. Among the different solvent extracts, the ethanol (EtOH) extract exhibited the highest phenolic content (6.81 ± 1.72 mg GAEs/200 g extract), followed by the water extract (5.34 ± 0.21 mg GAEs/200 g extract).

Flavonoid content was reported as mg catechin equivalents (CEs) per 200 g of extract. The EtOH extract of Urtica urens L. had the highest flavonoid content (31.41 ± 0.31 mg CEs/200 g), followed by the water extract (29.56 ± 1.56 mg CEs/200 g). For flavonol content, the EtOH extract showed the highest amount (0.91 ± 0.03 mg rutin equivalents (REs)/200 g), followed by the water extract (0.51 ± 0.02 mg REs/200 g).

Flavonoids are known for their antioxidant properties and their significant impact on human nutrition and health. These findings suggest that phenolic compounds are an essential component of this plant and likely contribute to its pharmacological activity.

Regarding ortho-diphenolic content, the EtOH extract contained the highest amount (66 ± 4.20 mg caffeic acid equivalents (CAEs)/200 g extract), followed by the water extract (15.19 ± 0.84 mg CAEs/200 g extract). The tannin content was also highest in the EtOH extract (8.29 ± 0.3 mg CEs/200 g extract) compared to the water extract (4.05 ± 0.52 mg CEs/200 g extract) (Table 1).

Vitamin contents

The contents of vitamins D, C, and E in the EtOH and aqueous extracts of *Urtica urens L.* are presented in Table 2. In the EtOH extract, the levels of vitamin D, vitamin C, and vitamin E were found to be 1.45 ± 0.14 mg/100 g, 238 ± 2.95 mg/100 g, and 356 ± 0.15 mg/100 g, respectively. In the aqueous extract, the concentrations were lower, with vitamin D at 0.23 ± 0.04 mg/100 g, vitamin C at 160.55 ± 3.09 mg/100 g, and vitamin E at 2.3 ± 0.01 mg/100 g.

Antifungal activity

The antifungal activity of the aerial parts of *Urtica urens L.* was assessed using the gel diffusion method, employing both the disk and well techniques to measure the zones of inhibition for Candida strains (Table 3). Extracts of varying concentrations (0.03, 0.05, 0.07, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1, 3, 5 g/ml) were prepared from both EtOH and aqueous extracts. The results, shown in Table 3, indicate that both extracts exhibited significant antifungal activity starting from a concentration of 1 g/ml, with the well technique showing larger inhibition zones compared to the disk method. Specifically, the inhibition diameters for the EtOH and aqueous extracts ranged from 8 mm to 22 mm and 10 mm to 27 mm, respectively, at concentrations of 1 g/ml, 3 g/ml, and 5 g/ml.

The microdilution method was also employed to measure the inhibition percentages of both EtOH and aqueous extracts, as detailed in Tables 4 and 5. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that inhibits fungal growth by over 90%, determined using the microdilution method in a liquid medium.

Notably, the inhibition diameters obtained with the well method were larger than those observed with the disk method. This difference is likely attributed to the method itself, as the well can hold a larger volume of extract compared to the disk, which is only soaked in the extract (Figs. 1, 2, 3, 4, 5, and 6).

Table 1. Concentration of total phenols, flavonoids, tannins, flavonols, and ortho-diphenols in UU extracts (mean ± SEM).

Extract	*EtOH*	*Aqueous*
Total phenols (mg in gallic acid equivalent/g extract)	6.81±1.72	5.34± 0.21
Flavonoids (mg catechin equivalent/g extract)	31.41±0.31	29.56±1.56
Tannins (mg catechin equivalent/g extract)	8.29±0.3	4.05±0.52
Flavonols (mg in rutin equivalent/g extract)	0.91±0.03	0.51±0.02
Ortho-diphenols (mg in caffeic acid equivalent/g extract)	66±4.20	15.19±0.84

Table 2. Rates of vitamins D, C and E. Values are mean ± ESM (n = 3). *** p <0.001; α: Comparison with ethanol.

Extracts	*Vitamin D (mg/100 g)*	*Vitamin C (mg/100 g)*	*Vitamin E (mg/100 g)*
Ethanol	1.45±0.14	238±2.95	35±0.15
Aqueous	0.23±0.04	160.55±3.09$^{\alpha***}$	2.3±0.01$^{\alpha***}$

3. Discussion

In this study, we investigated the bioactive compounds of *Urtica urens L.* and its antifungal activity. Due to the diversity of antioxidants present in plant tissues, it can be challenging to measure each component individually. As a result, many studies utilize multiple extraction methods to maximize the extraction of available antioxidants [27].

Our work focused on organic extracts of *U. urens L.* (aqueous and ethanol extracts) and confirmed their fungicidal properties against various Candida strains, including *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida metapsilosis*, and *Candida orthopsilosis*. The results demonstrated that the extracts exhibited inhibitory effects against the Candida species tested. However, *Candida glabrata* showed resistance, particularly when tested with the disc method. This resistance could be attributed to the natural resistance of this particular strain [28].

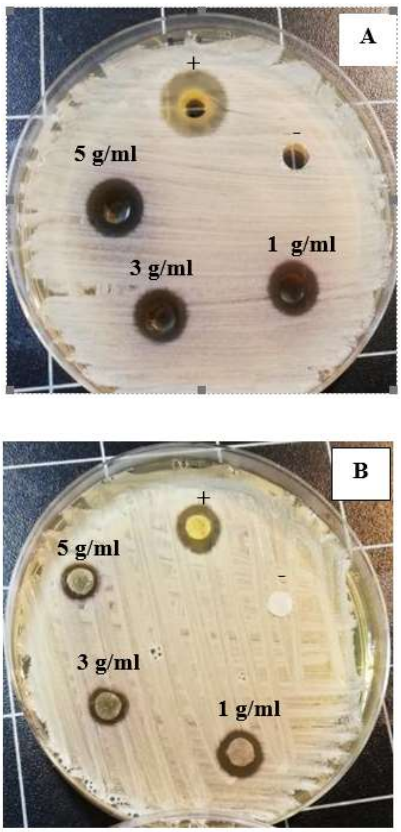

Fig.1. Inhibition diameter on *C. albicans* (aqueous extract).
A: Well method; B: Disk Method.

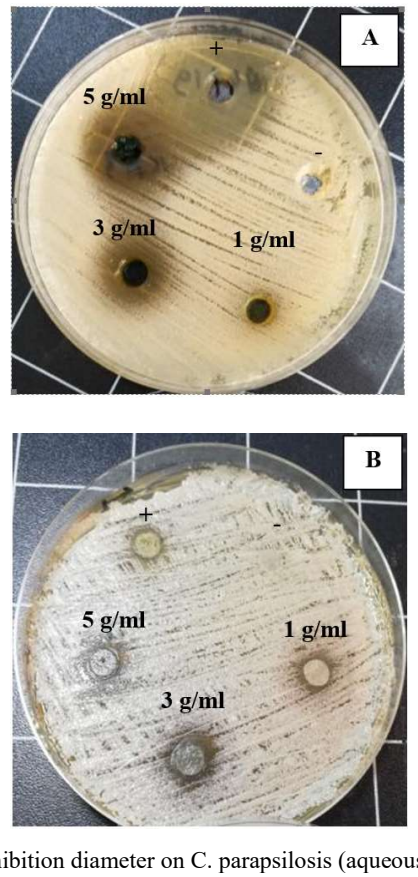

Fig.2. Inhibition diameter on C. parapsilosis (aqueous extract).
A: Well method; B: Disk Method.

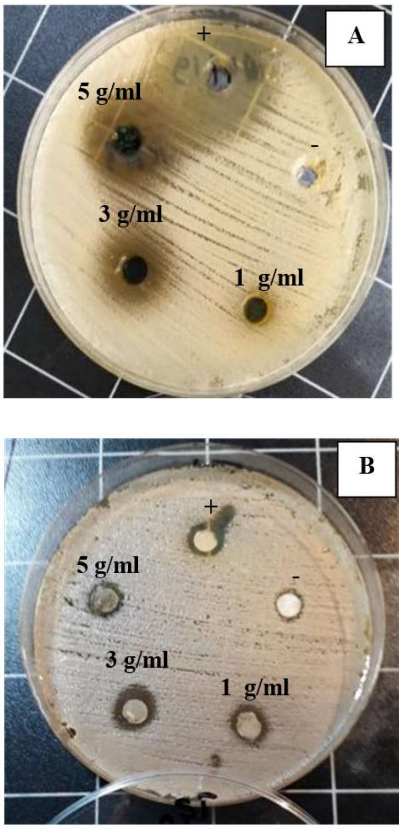

Fig.3. Inhibition diameter on C. metapsilosis (aqueous extract).
A: Well method; B: Disk Method.

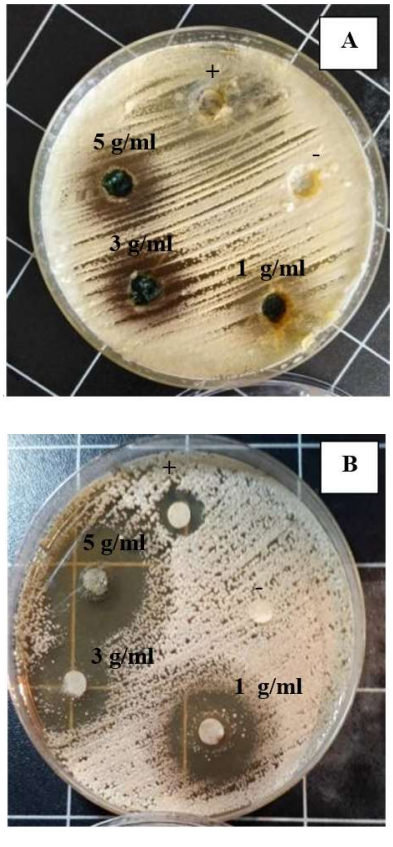

Fig.4. Inhibition diameter on C. orthopsilosis (aqueous extract).
A: Well method; B: Disk Method.

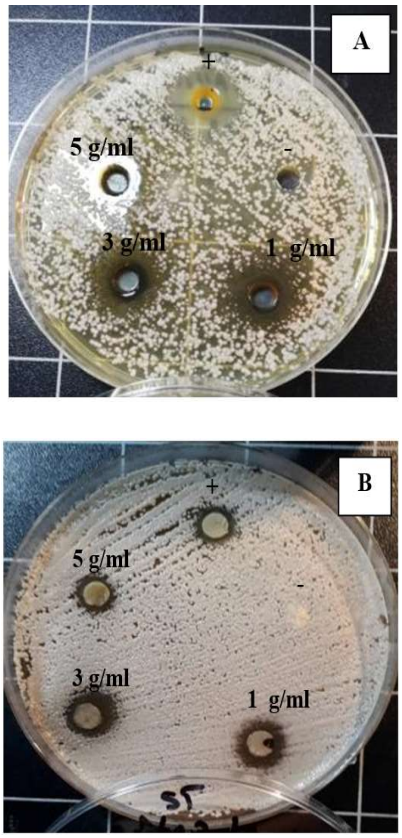

Fig.5. Inhibition diameter on C. glabarata (aqueous extract). A: Well method; B: Disk Method.

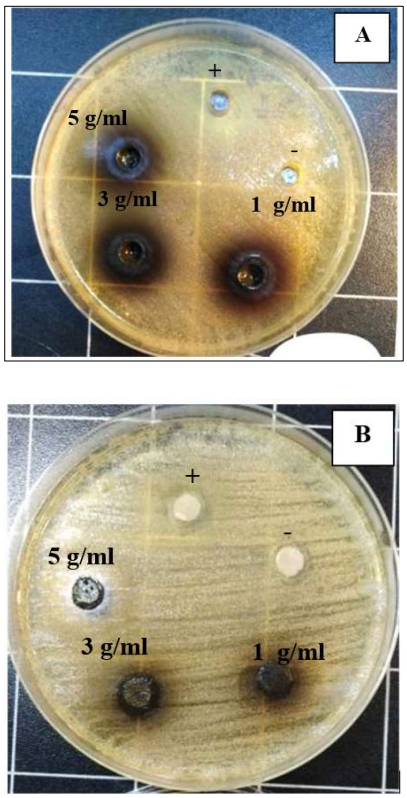

Fig.6. Inhibition diameter on C. tropicalis (aqueous extract). A: Well method; B: Disk method.

According to Ghaedi et al. [29], *Urtica dioica* exhibits stronger antifungal activity against *Aspergillus oryzae* and *Candida albicans* at concentrations of 100, 50, and 25 mg/ml, with inhibition diameters ranging from 6.85 mm to 12.40 mm. This difference in activity could be attributed to the variations in chemical composition between the two species of *Urtica* [26].

Recent research [30] has shown that *Urtica dioica* leaf extract displays notable antifungal activity, with inhibition zones between ~8 mm and ~20 mm against several fungal pathogens, including *Alternaria solani*, *Mucor indicus*, *Chaetomium globosum*, and *Tilletia indica*, at a concentration of 200 mg/ml.

Additionally, Sayidi and Nematollahi [31] demonstrated that various concentrations of *U. dioica* extracts (500, 1000, 1500 mg/ml) significantly inhibited the growth of *Alternaria alternata*, with fungal mycelial growth inhibited by 40.25%, 53.08%, and 67.87%, respectively. Their findings indicated that the antifungal potential of the extracts was positively correlated with increasing concentrations in the growth medium.

In our study, the inhibition of yeast growth was also closely related to extract concentration. As shown in Figures 1, 2, 3, 4, and 6, the diameter of the inhibition zone was largest at a concentration of 5 g/ml compared to other concentrations. This observation aligns with the work of Bans and Jeremiah [32], who reported that higher concentrations of antimicrobial substances result in increased growth inhibition.

The results obtained (expressed as the diameter of the zone of inhibition, including the well diameter) demonstrated that both EtOH and aqueous extracts of *Urtica urens L.* exhibited variable antifungal activities depending on the strains tested at concentrations of 1 g/ml, 3 g/ml, and 5 g/ml. This is consistent with the findings of Mzid et al. [26], who showed that EtOH extract exhibited antibacterial activity against both Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Salmonella enteritidis* and *Pseudomonas aeruginosa*) at a concentration of 150 µg/ml.

The higher resistance of *Candida* (eukaryotic cells) compared to bacteria may be due to the structure of its membrane and outer cell wall. The notable resistance of *Candida* to our extracts is likely attributed to the presence of outer membranes, which surround the cell wall and limit the diffusion of hydrophobic compounds by covering lipopolysaccharides. In contrast, bacteria (prokaryotes) lack this additional barrier, allowing direct contact between the phenolic compounds and the bilayer phospholipids of the cell membrane, leading to increased permeability to ions, damage to intracellular bacterial enzymes, and disruption of essential cellular functions [33].

Furthermore, Candan et al. [34] noted that water-soluble substances exert weaker effects than non-water-soluble substances. This suggests that fat-soluble molecules have a greater ability to integrate into bacterial cell membranes and damage them. Phenolic diterpenoids, which are the major compounds in the apolar fraction of plant extracts, are highly lipophilic and are efficiently extracted using low-polarity solvents like hexane [35, 36]. This could explain the antimicrobial activity observed even at very low concentrations of the extracts against bacteria.

The results presented in Tables 3 and 4 indicate that the aqueous extract of *Urtica urens L.* exhibited greater antifungal activity than the ethanolic extract against the different strains tested. According to Fezan et al. [37], aqueous extracts of various plants, such as *Borreria latifolia*, *Borreria verticillata*, *Erigeron floribundus*, *Euphorbia hirta*, *Turraea heterophylla*, and *Vernonia colorata*, demonstrated superior antifungal activity compared to methanolic and dichloromethane extracts against a range of *Candida* species.

The relevance of Minimum Inhibitory Concentration (MIC) testing on solid medium has been criticized, as medium strength may influence the contact between inhibitor molecules and microbial cells due to diffusion within the agar [38, 39]. Additionally, some compounds may only have an immediate effect, hydrolyzing naturally once dissolved in aqueous solutions. This can lead to inaccurate measurements, as microorganisms may continue to multiply in areas not directly in contact with the active molecules. Consequently, determining the MIC in a liquid medium, which is considered the reference method, can provide more reliable results.

Regarding the antifungal activity of the aqueous extract of *U. urens L.*, microdilution tests revealed a variable inhibitory power depending on the *Candida* species. The MIC was 5 g/ml for *Candida albicans*, *Candida glabrata*, *Candida metapsilosis*, and *Candida orthopsilosis*, but its inhibitory power was lower than that of amphotericin B. Similarly, for the ethanolic extract, the MIC was also 5 g/ml for the same *Candida* species, but again, its inhibitory power was lower than amphotericin B (50 mg/10 ml).

Mikaeili et al. [40] demonstrated that both EtOH and aqueous extracts of *Urtica dioica* possess antifungal activity against *Microsporum canis*, with MICs of 30 mg/ml for EtOH and 20 mg/ml for aqueous extract. Our findings show that the EtOH extract of *U. urens L.* is richer in vitamins C, E, and D compared to the aqueous extract. This aligns with studies by Tatyana and Valentina [41], who reported that nettle leaves contain significant amounts of ascorbic acid (270 mg/100 g), carotenoids (50 mg/100 g), and vitamins B, D (200 mg/100 g), and E.

Moreover, our study revealed that *U. urens L.* leaves are rich in bioactive compounds such as total phenols (6.81 ± 1.72 mg EAG/g extract), flavonoids (31.41 ± 0.31 mg EC/g extract), tannins (8.29 ± 0.3 mg EAG/g extract), flavonols (0.91 ± 0.03 mg ER/g extract), and ortho-diphenols (66 ± 4.20 mg EAC/g extract). Naturally occurring phenolic compounds, including phenolic acids, flavonoids, and tannins, have been shown to possess significant biological activities, particularly antioxidant properties, which contribute to scavenging free radicals and reactive oxygen species [42]. The phytochemical and pharmacological data obtained in this study indicate that *U. urens L.* extract is not only rich in vitamins and phenolic compounds but also exhibits considerable biological activity. Specifically, the EtOH extract was found to have the highest concentration of ortho-diphenols (66 ± 4.20 mg CAEs/200 g extract), followed by the aqueous extract (15.19 ± 0.84 mg CAEs/200 g extract). Similarly, the EtOH extract contained higher levels of tannins (8.29 ± 0.3 mg CEs/200 g extract) compared to the aqueous extract (4.05 ± 0.52 mg CEs/200 g extract).

The antifungal activity observed in *Urtica urens L.* could be attributed to the presence of several antioxidant compounds, including phenols, flavonoids, tannins, and vitamins. These compounds have long been recognized for their anti-fungal properties. Specifically, the antifungal activities of flavonoids [43], tannins [44], alkaloids [45], triterpenoids [46], steroids [47], anthocyanins [48], coumarins [49], and saponins [50], which are also found in *U. urens L.*, have been widely reported. Previous studies have shown that plant extracts and essential oils possess antifungal activity [51].

Tegegne et al. [52] demonstrated that various natural substances, such as plant extracts, exhibited growth-inhibiting effects against fungi like *Alternaria*, *Fusarium*, *Botrytis*, and *Rhizoctonia*. Additionally, Rodino et al. [53] reported the in vitro antifungal properties of ethanolic and aqueous extracts of traditional medicinal plants (*Artemisia absinthium*, *Rosmarinus officinalis*, *Datura stramonium*, and *Xanthium strumarium*) against phytopathogenic fungi, specifically *Alternaria alternata*.

While the exact mechanisms underlying the antifungal activity of plant extracts and essential oils remain unclear, several studies have investigated their modes of action. It has been proposed that phenolic compounds play a key role in the inhibition of fungal growth. Studies suggest that these compounds may exert toxic effects on fungal cell membranes, disrupting their functionality and structure [54, 55]. This disruption may lead to the collapse and death of mycelium, causing the loss of rigidity and integrity of the hyphal cell wall, ultimately leading to the leakage of cellular components from the cytoplasm [56].

In recent years, considerable research has focused on reducing the use of synthetic fungicides, aiming to exploit plant compounds as safe and eco-friendly alternatives for commercial use [57, 53].

Table 3. Antifungal activity of aqueous and ethanol extracts of *U. urens L.* sheets based on diameter of the inhibition zone (mm) : using well method. (values represent mean ± SEM)

Strains	**Aqueous extract**						**Ethanol extract**					
		Concentrations (g/ml)						**Concentrations (g/ml)**				
	+	**-**	**0.8**	**1**	**3**	**5**	**+**	**-**	**0.8**	**1**	**3**	**5**
Candida albicans	21±0.56	7±0.5	7±0.5	12±0.6	16±0.89	19±0.04	25±0.66	7±0.5	7±0.5	9±0.67	12±0.73	15±1.55
Candida parapsilosis	27±0.5	7±0.5	7±0.5	17±0.5	20±0.5	25±0.5	32±0.89	7±0.5	7±0.5	13±0.45	18±0.88	20±1.56
Candida metapsilosis	27±0.78	7±0.5	7±0.5	17±0.34	21±0.39	25±0.78	25±1.09	7±0.5	7±0.5	7±0.09	17±0.67	22±1.72
Candida orthopsilosis	30±0.67	7±0.5	7±0.5	19±0.65	20±0.69	24±0.23	28±1.89	7±0.5	7±0.5	18±1.09	20±0.69	21±0.92
Candida glabarata	16±1.22	7±0.5	7±0.5	11±0.98	11±1.18	13±0.95	38±3.17	7±0.5	7±0.5	13±1.11	18±1.90	19±2.1
Candida tropicalis	28±1.67	7±0.5	7±0.5	20±0.87	23±2.89	27±0.34	25±2.95	7±0.5	7±0.5	8±0.66	20±0.42	22±1.75

Table 4. Antifungal activity of aqueous and ethanol extracts of *U. urens L.* sheets based on diameter of the inhibition zone (mm) : using disk method. (values represent mean ± SEM)

Strains	**Aqueous extract**						**Ethanol extract**					
		Concentrations (g/ml)						**Concentrations (g/ml)**				
	+	**-**	**0.8**	**1**	**3**	**5**	**+**	**-**	**0.8**	**1**	**3**	**5**
Candida albicans	21±0.56	7±0.5	7±0.5	12±0.6	16±0.89	19±0.04	25±0.66	7±0.5	7±0.5	9±0.67	12±0.73	15±1.55
Candida parapsilosis	27±0.5	7±0.5	7±0.5	17±0.5	20±0.5	25±0.5	32±0.89	7±0.5	7±0.5	13±0.45	18±0.88	20±1.56
Candida metapsilosis	27±0.78	7±0.5	7±0.5	17±0.34	21±0.39	25±0.78	2±1.09	7±0.5	7±0.5	7±0.09	17±0.67	22±1.72
Candida orthopsilosis	30±0.67	7±0.5	7±0.5	19±0.65	20±0.69	24±0.23	2±1.89	7±0.5	7±0.5	18±1.09	20±0.69	21±0.92
Candida glabarata	16±1.22	7±0.5	7±0.5	11±0.98	11±1.18	13±0.95	38±3.17	7±0.5	7±0.5	13±1.11	18±1.90	19±2.1
Candida tropicalis	28±1.67	7±0.5	7±0.5	20±0.87	23±2.89	27±0.34	25±2.95	7±0.5	7±0.5	8±0.66	20±0.42	22±1.75

Table 5. Percent inhibition of aqueous extract on different strains of *Candida*.

	Concentrations (g/ml)				**% inhibition (Positive control)**	**Concentrations (g/ml)**				**% inhibition (Positive control)**
	0.8	**1**	**3**	**5**		**0.8**	**1**	**3**	**5**	
Candida albicans	0	46.89%	63.89%	95.89%	97.98%	0	33.09%	52.88%	77.77%	96.66%
Candida parapsilosis	0	54.67%	58.89%	85.89%	98.09%	0	7.81%	34.89%	58.85%	97.86%
Candida metapsilosis	0	35.89%	44.78%	92.78	96.89%	0	7.03%	23.96%	90.58%	98.05%
Candida orthopsilosis	0	53.45%	75.89%	93.78%	98.67%	0	34.7%	35.01%	91.33%	98.06%
Candida glabarata	0	24.67%	42.67%	95.87%	99.06%	0	22.76%	35.71%	92.24%	97.52%
Candida tropicalis	0.67%	64.89%	76.78%	86.91%	99.05%	0.61%	11.09%	27.89%	51.01%	96.93%

In our study, the antifungal activity of both ethanolic and aqueous extracts of *U. urens L.* was evaluated through in vitro contact tests. The antifungal screening revealed that *U. urens L.* extracts, at a concentration of 1 g/ml, significantly inhibited the mycelial growth of the tested pathogens.

Conclusion

This study highlights the promising potential of *Urtica urens* extracts at various concentrations in inhibiting Candida strains, suggesting the viability of using natural plant extracts in disease control. Moreover, the organic extracts of medicinal plants could serve as alternative industrial products to synthetic antifungals, providing a more sustainable option for agro-industries. This approach opens the possibility of developing new, selective, and natural antifungals for biological control in combating numerous pathogenic agents affecting agricultural plants, thus reducing significant crop losses.

Consent for publication

Not applicable.

Ethical considerations

Not applicable.

Conflict of interest

All authors declare that they have no conflict of interest.

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Authors' contribution

All authors have read and approved the final manuscript.

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