

Exploring the potent antifungal activity of Mazuj and Ghalghaf gall extracts against three *Candida* species and conducting compositional and characterization analysis of their extracts using HPLC-DAD and LC-ESI-MS/MS

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ABSTRACT

The development of novel antifungal agents has become increasingly crucial according to the antifungal resistance of *Candida* species. Using natural product compounds as alternatives to conventional drugs is being explored to advance a more effective treatment for *Candida* infections. This study aims to determine biological activity and the chemical compound characterization of the aqueous and acetonetic extracts obtained from Mazuj and Ghalghaf galls of *Quercus infectoria*. After the galls extraction, the extracts' antifungal properties were investigated using an agar well diffusion method and concentrations of minimum fungicidal (MFCs) and minimum inhibitory (MICs). We utilized the ABTS and MTT methods to determine gall extracts' antioxidant and cytotoxic properties. Additionally, we identified the chemical compounds present in the extracts using LC-ESI-MS/MS and HPLC-DAD. The study results showed that both acetonetic and aqueous extracts of Ghalghaf and Mazuj galls were efficient against species of three fungi species such as *Candida albicans*, *Candida glabrata*, and *Candida krusei*, and in particular, the acetonetic Mazuj extract demonstrated the highest effectiveness as an antifungal. Furthermore, the Mazuj extract exhibited elevated levels of total phenolics and the most increased antioxidant activity. Moreover, LC-ESI-MS/MS and HPLC-DAD analysis indicated the presence of various phenolic compounds in the extracts, including gallic acid, methyl gallate, and ellagic acid. Overall, the study's findings suggest that Mazuj and Ghalghaf galls demonstrate promising antifungal and antioxidant properties, where these compounds could potentially be used to develop natural and safe antifungal agents.

Keywords: Antifungal, *Candida albicans*, Mazuj, Ghalghaf, Gall, *Quercus infectoria*

INTRODUCTION

Candida species are opportunistic pathogens that cause global morbidity and mortality, threatening the public's health[1]. *Candida* species can also induce vaginitis, cutaneous candidiasis, candidemia, oral candidiasis, and systemic infections[2]. Vulvovaginal candidiasis (VVC) is a common fungal vaginal infection among women[3]. The leading cause of vulvovaginal candidiasis (VVC) is *Candida albicans*, which affects 138 million women worldwide every year and 492 million during their lifetimes[4].

Candidiasis induces vaginal itching, dysuria, and excessive vaginal discharge, causing considerable pain and discomfort[5]. Multiple risk factors for candidiasis include diabetes, pregnancy, immunodeficiency, and hormonal changes[6]. Several species of *Candida* are known to cause vaginal *Candida* infection, including *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*[7]. Polyenes, azoles, echinocandins, nucleoside analogs, and allylamines are among the compounds used to treat *Candida* infections with varying efficacy depending on the *Candida* species' type and sensitivity[8].

Growing concern about fluconazole-resistant *Candida* species outbreaks has recently been reported in several countries[9]. Studies have evidenced that azole antifungal agents such as fluconazole have been utilized more frequently in recent years[10]. These studies also show that widespread use of azoles leads to resistance to these drugs in the most commonly encountered isolates[11]. It is necessary to develop novel antifungal agents, including natural product compounds, to treat *Candida* infections as drug-resistant *Candida* species become more prevalent[12]. Accordingly, as a potential substitute for chemical antimicrobial agents, natural compounds are being developed to address issues concerning resistance to antimicrobials. Therefore, in recent years, medicinal plants have gained popularity as alternatives to chemical antimicrobials since they are less toxic and have a lower risk of causing microbial resistance[13, 14].

Quercus species and their crops are believed to be among the most valuable medicinal plants[15]. Cynipid gall wasps, which cause galls, affect numerous organs of oak trees[16]. The formation of oak galls results from abnormal plant tissue growth caused by living organisms such as gall wasps that attack various parts of oak trees (*Quercus Infectoria*)[17]. Several phenolic compounds are found in oak gall tissue, including tannic and gallic acid[18]. These compounds possess antioxidant, antibacterial, anti-inflammatory, and antifungal properties[19]. In the present study, a Mazuj gall and a Ghalghaf gall were produced by the non-sexual activity of *Andricus strenlichti* and *Andricus quercustozae*, cynipid wasps in oak trees (*Quercus infectoria*)[20, 21]. This study aims to determine whether aqueous and acetonic extracts of Mazuj and Ghalghaf galls possess antifungal, antioxidant, and cytotoxic properties and to evaluate their chemical composition using HPLC-DAD and LC-ESI-MS/MS analysis.

MATERIAL AND METHODS

Preparation of Gall Extracts

Mazuj and Ghalghaf galls were collected from Zagros oak forests in Lorestan, Iran, in November 2019 and authenticated by M.T. (at the Research Center for Agricultural Sciences at Lorestan University of Medical Sciences, Khorramabad, Iran). In addition, a specimen of these galls was deposited in the Institute's Herbarium. This study obtained acetonic and aqueous gall extracts by macerating and evaporating gall under reduced pressure (50 °C) in a rotary evaporator. The extract was then transferred to a tared glass, dried in the fume hood, and freeze-dried as needed.

Fungi Strains

Three *Candida* species were used as model fungi: *Candida albicans* CBS 9120, *Candida glabrata* CBS 2175, and *Candida krusei* CBS 573.

Anti-Candida Activity Assays

Preliminary screening for antibacterial activity was conducted through the agar diffusion method. Briefly, 10 mg/ml extracts were prepared in distilled water. Afterward, a direct suspension of an overnight culture of test *Candida* spp. was prepared. The turbidity of the samples was measured to an equivalent 0.5 McFarland standard. Then, 100 µL of this microorganism sample was inoculated into Sabouraud dextrose agar (SDA) through the spread plate technique. Then, holes were created in the culture medium and were inoculated with 100 µL of each diluted extract, then incubated at 37 °C for one day. Finally, the size of the zone inhibition diameter was reported as a result[22].

Evaluation of MIC and MFC of Gall Extract

The MIC and MFC activity of gall extracts was evaluated by the Clinical and Laboratory Standards Institute (CLSI), which described the microbroth dilution method[23]. Gall extract stock solutions were prepared in a sterile RPMI-1640 medium. An aliquot of 100 µL of RPMI-1640 medium was dispensed into 96 wells and mixed with 100 µL of each gall extract sample to obtain 15 to 0.029 mg/mL concentrations. Subsequently, microorganism control was performed in Well 11 (broth containing only the organism), and negative control was achieved in Well 12 (broth devoid of any organism). We used fluconazole (128-0.125 µg/mL) as a positive control.

Determination of Minimum Fungicidal Concentration (MFC)

Aliquots from each well of the antifungal susceptibility assay were transferred to SDA plates. The plates were subsequently incubated for two days at 37 °C to determine whether the gall extracts had a fungicidal effect against *Candida* strains. After two days, the plates were examined to determine whether growth had occurred per the CLSI document[23].

ABTS assay

The gall extract's antioxidant assay was conducted by utilizing 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS as a cation radical, following the procedure outlined by Bach et al.[24]. After oxidizing seven mM ABTS with (2.45 mM) potassium persulfate, the solution was kept under dark conditions. After 12 to 16 hours, Phosphate buffer was used, after which the absorbance was calibrated to 0.70 ± 0.05 . 0.07 mL of extracts (0.48-250 µg/mL) and three mL of the ABTS were added to the sample. The reaction was measured after 6 min of storage using a spectrophotometer. Percentage inhibition of free radicals was calculated according to the below equation:

$$\text{A.A. (\%)} = (A_0 - A_1)/A_0 \times 100$$

To determine the anti-radical activity of the extracts, we used A.A. as the percentage of inhibition, A_0 to represent the control's absorbance, and A_1 as the sample's absorbance. The IC₅₀ parameter was used to compare the anti-radical activity of the extracts.

Total Phenolic Content

This study determined total phenolic content using a modified version of the Singleton method[25]. To this end, Folin-Ciocalteu reagent and gallic acid were used as phenolic compounds' standards. The extract was initially combined with 2.5 mL of a 10% Folin-Ciocalteu and 2 mL of a Na₂CO₃ (7.5%) solution in test tubes. The reaction solution was subsequently shaken and stored for 25 min, at which point the reaction was read at 765 nm. Gallic acid equivalents (GAE) were expressed in one gram of dry extract (mg GAE/g DM).

Cytotoxicity Assay

To determine the cytotoxicity of gall extract, the MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was conducted in a 96-well plate, as described by Parra-Riofrío et al.[26]. The normal human dermal fibroblasts (NHDF) and HeLa cells used in the assay were acquired from the Iranian Pasteur Institute, following the manufacturer's instructions. After loading 104 cells per well, gall extracts were added to each well at 1000–15.6 µg/mL concentrations. After adding 50 µL tetrazolium salt (Sigma-Aldrich), producing a 5 mg/ml stock solution, 4 hours of plates were conducted at 37 °C. Then, 50 µL of DMSO was put into each individual well. The absorbance of the plate was read at 570 nm using an ELISA reader instrument (Bio-Tek, Epoch, USA) after 10 min of incubation. Finally, IC₅₀ values were calculated using the following equation for treated and control cells:

$$\% \text{ inhibition of cell viability} = \frac{(\text{control Abs} - \text{sample Abs})}{(\text{control Abs})} \times 100$$

HPLC-DAD Analysis

The Agilent HPLC 1200 Gradient System (CA, USA) was used to identify and measure ten different phenolic compounds based on the available references[27]. This system comprised a vacuum cleaner gas, an automatic sampling, and a diode-array detection (DAD) system. The results were subsequently analyzed using the Agilent HPLC ChemStation software. A mobile phase of distilled water with acetic acid (A: 1%) and methanol (B) was employed at a 0.7 mL/min flow rate. In total, 70 minutes were dedicated to the three-stage gradient program. Eight different wavelengths were utilized in the experiment. A 10 µL volume of samples was injected into the device at 25 °C after being filtered. Ten phenolic compounds were measured quantitatively using standard calibration curves. Several examples of these compounds include gallic acid, resorcinol, p-coumaric acid, caffeic acid, chlorogenic acid, vanillic acid, veratric acid, syringic acid, ellagic acid, and salicylic acid.

The Analysis Extracts by LC-ESI-MS/MS.

The gall compounds were analyzed by Waters Alliance instrument e2695 (Waters Corporation, MA, USA) and the Atlantis T3 C18 column (2.1 mm x 100 mm, 3 mm; Milford, Waters Corp., MA, USA)[28]. In addition, an electrospray ionization source (ESI) and a Quattro micro-API model were used to obtain the MS spectra (Waters Corp., Milford, MA, USA). The gall extracts obtained by 10 mg of acetonic gall extract solution in 10 mL

methanol (HPLC-grade) were filtered through a cellulose membrane filter. A blend of water, acetonitrile, and 1% formic acid was utilized to get the necessary resolution. The separation procedure lasted 20 minutes and consisted of two mixtures with varying ratios of eluents. The first mixture contained 5% eluent A and 95% eluent B and was used during the first 10 minutes. The second mixture had 95% eluent A and 5% eluent B and was used for the remaining 10 minutes. Additionally, we optimized other mass parameters, including the source temperature (300 °C), capillary voltage (3 kV), and cone voltage (20 V). The injection volume was five µL. The MS/MS decomposition study determined a collision energy of 30 eV[29]. Finally, the data and published articles were processed using the Waters software MassLynx (v. 4.1), and the PubChem and MassBank databases were used to identify compounds. All experiments were performed in triplicate.

Statistical Analysis

Treatment was compared using one-way and two-way ANOVA. All statistical analyses with a P-value of less than 5% were conducted using SPSS software (version 16).

RESULTS

Anti-Candida Activities of Gall Extracts

Table 1 compares the diameters of the growth zones inhibition of various extracts from two galls. The extracts inhibited the growth of *Candida albicans* CBS 9120, *Candida glabrata* CBS 2175, and *Candida krusei* CBS 573. *Candida krusei* was the most sensitive sample to all gall extracts among the three strains. In addition, acetone extracts of both galls exhibited higher antifungal activity against these strains, with inhibition zones ranging from 15-23 mm. Both galls' aqueous extracts demonstrated similar antifungal properties based on the mean diameter of inhibition zones. Notably, the antifungal activities of the two galls differed significantly, with Mazuj extracts exhibiting significantly greater antifungal activity than Ghalghaf extracts ($P < 0.05$).

Table 1 The result of the agar well diffusion method of the Mazju and Ghalghaf extracts. AQEM Aqueous Extract of Mazuj/AEM: Acetonic Extract of Mazuj/ AQEG: Aqueous Extract of Ghalghaf/ AEG: Acetonic Extract of Ghalghaf

Fungi sp.	<i>Candida albicans</i> CBS 9120	<i>Candida glabrata</i> CBS 2175	<i>Candida krusei</i> CBS 573
Gall extracts 10 mg/ml			
AEM	20±1	17±1	23±2
AQEM	15±1	10±1	17±1
A E G	18±1	14±1	21±1
AQEG	12±1	8±1	15±2

The MIC values of various gall extracts against all *Candida* species ranged between 0.029 and 0.93 mg/ml (see Table 2). The acetonic extract of Mazuj showed notable activity against *C. krusei*, with MIC and MFC values of 0.029 mg/ml. Furthermore, this extract demonstrated the most potent anti-*Candida* activity among all extracts. As a result, both gall extracts exhibited antifungal properties against vulvovaginitis isolate strains and reference strains.

Table 2 The minimal inhibition concentration (MIC) and the minimal fungicidal concentration (MFC) of different gall extracts against the tested candida strains (mg/ml).

Gall extracts									Fluconazol	
	Mazuj				Ghalghaf					
	Candid strains	acetic		aqueous		acetic extract		aqueous		
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
<i>C. albicans</i> (PTCC 5027)	0.23	0.93	1.87	3.75	0.46	1.87	3.75	7.5	0.02	0.04
<i>C. krusei</i> (CBS 573)	0.029	0.23	0.46	0.93	0.11	0.46	1.87	3.75	0.005	0.01
<i>C. glabrata</i> (CBS 138)	0.93	1.87	3.75	7.5	1.87	3.75	7.5	15.2	0.08	0.16

Total Phenol Content and Antioxidant Activity of Gall Extracts

Table 3 details the total phenolic compound content analysis results for the samples, which ranged from 326 to 563 mgGAEs/g, estimated through the gallic acid standard curve ($y = 0.002x + 0.014$, $R^2 = 0.998$). The Mazuj acetonic extract had the highest total phenolic content (593 mgGAEs/g), and the Ghalghaf aqueous extract had

the lowest (306 mgGAEs/g). These differences in total phenolic content among all extracts were statistically significant (p-value <5%).

Additionally, the IC₅₀ values of the antioxidant activity of gall extracts using the ABTS method are shown in Table 3. The Mazuj acetic extract demonstrated the most potent antioxidant activity, with an IC₅₀ value of 41 µg/ml. In contrast, the Ghalghaf aqueous extract exhibited the lowest antioxidant peripeties, with the IC₅₀ value of 95 µg/ml (p-value <0.05%). The IC₅₀ of Trolox for the ABTS control is also provided in Table 3.

Table 3 shows the total phenol content, antioxidant activity, and cytotoxicity assay results of the Mazuj and Ghalghaf extracts. Acetic Extract of Mazuj/ AQEG: Aqueous Extract of Ghalghaf/ AEG: Acetic Extract of Ghalghaf

Type of tests	TPC	ABTS	HDF normal cell	HeLa cell
Extract	mgGAE/0.1-gram extract	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
AEM	593	41	850	60
AQEM	388	78	nd	nd
A E G	512	57	740	52
AQEG	306	95	nd	nd
TOROLOX	nd	10	nd	nd

Cytotoxicity Assay

The percentage of viable cells demonstrated a negative correlation with the dose in a 24-hour toxicity test using gall extract on HDF and HeLa cells. The IC₅₀ values for Mazuj and Ghalghaf extracts on HDF cells were 850 µg/ml and 740 µg/ml, respectively (Table 3). For HeLa cells, the IC₅₀ values for both extracts were 60 µg/ml and 52 µg/ml for Mazuj and Ghalghaf, respectively.

HPLC DAD Analysis

The present study utilized the HPLC-DAD technique to analyze the acetic extracts of Mazuj and Ghalghaf galls for their phenolic content (Table 4). Nine phenolic compounds were identified and quantified using various wavelengths, retention times, and standard calibration curves. As shown in Table 4, the separation of 9 phenolic compounds was effectively achieved. The acetic extract of Mazuj and Ghalghaf exhibited gallic acid content of 308.4 and 250 µg/L, respectively. Moreover, ellagic acid was detected in the acetic extract of Mazuj and Ghalghaf at concentrations of 117.6 and 578 µg/L, respectively.

Table 4 detected Phenolic compounds in Mazuj and Ghalghaf acetic extracts by HPLC-DAD. ND: not detected

STD Compound	TR (min)	λ(nm)	Mazuj (µg/L)	Ghalghaf (µg/L)
Gallic acid	5.5	278	308.4	250.9
Resorcinol	8.9	278	14.38	ND
Vanillic acid	30.7	260	214	181.5
Veratric acid	42.3	260	12.2	ND
Salicylic acid	44.2	300	178.8	ND
Coumaric acid	45.2	300	156.1	130
Catechin	47.5	278	ND	329
Ellagic acid	53.0	254	117.6	578
Quercetin	63.6	254	12.3	15.5

LC-ESI-MS/MS Analysis

The LC-ESI-MS/MS analysis was employed to determine the compounds in the total acetic extract qualitatively. Table 5 summarizes the LC-ESI-MS/MS results for the identified and proposed compounds. Tentative identification of the detected compounds was performed using MassBank and PubChem databases. The obtained mass spectrum facilitated the preliminary identification of 16 compounds for Mazuj and 16 for Ghalghaf. Several phenolic compounds and their derivatives were identified during the analysis. In addition, the study detected various compounds for Mazuj, such as gallic acid, methyl gallate, ellagic acid, quinic acid, beta-glucogallin, methyl digallate, and gallic acid-methyl gallate-gallic acid. The analysis also detected several compounds for Ghalghaf, including gallic acid, methyl gallate, ellagic acid, quinic acid, brevifolincarboxylic acid, digallic acid, methyl 3,6-di-O-galloyl-α-D-glucopyranoside, and methyl digallate. Notably, A few compounds were present in both galls.

Table 5 Characterization of significant compounds from gallnuts identified using LC-ESI-MS/MS. M.W.: molecular weight [M-H]⁻: *m/z* of the molecular ion; MS/MS ions (at 30 eV): product ions for each compound; (R.I. %): relative intensities %; Presence or absence of identified secondary metabolites in each sample (MA, GH) is indicated respectively by + and –

no.	Formula	Ion mode	MW	[M-H] ⁻ <i>m/z</i>	RT (min)	Product ions (R.I. %)	Tentative identification	Samples MA GH		Ref.
1	C ₇ H ₆ O ₅	[M-H] ⁻	170.02	169.198	6.37	124 (30), 79 (85), 69 (25), 51 (100)	Gallic acid	+	+	[43]; [44]; [45]; [46]; [47]; [48]; [49]; [50]; [51]; [52]; [53]; [54]; [55]; [56]; [57]
2	C ₈ H ₈ O ₅	[M-H] ⁻	184.04	183.173	11.55	124 (100), 106 (15) 78 (60)	Methylgallate	+	+	[41]; [58]; [59]
3	C ₇ H ₁₂ O ₆	[M-H] ⁻	192.06	191.218	3.13	93 (75), 85 (100), 59 (25)	Quinic acid	+	+	[60]; [58]; [61]; [62]; [63]
4	C ₁₃ H ₈ O ₈	[M-H] ⁻	292.02	291.283	11.75	247 (70), 219 (22), 191 (65), 175 (70), 102 (100)	Brevifolincarboxylic acid	-	+	[62]; [64]
5	C ₁₄ H ₆ O ₈	[M-H] ⁻	302.01	301.245	14.61	284 (50), 201 (15), 185 (35), 173 (15), 145 (12)	Ellagic acid	+	+	[41]; [65]; [58]; [66]; [67]; [59]; [68]; [61]; [62]; [64]; [69]; [70]; [71]
6	C ₁₄ H ₁₀ O ₉	[M-H] ⁻	322.03	321.377	10.66	169 (100), 125 (37)	Digallic acid	-	+	[65]; [72]; [58]; [38]
7	-*	[M-H] ⁻	-	329.567	17.54	230 (15), 211 (80), 183 (25), 171 (100), 167 (25), 155 (20), 139 (30), 127 (45), 99 (28)	Unknown compound	+	-	-
8	C ₁₃ H ₁₆ O ₁₀	[M-H] ⁻	332.07	331.410	4.68	211 (20), 169 (65), 151 (35), 125 (55), 123 (100), 89 (70), 71 (20), 59 (100)	1-O-Galloyl-beta-D-glucose (Beta-glucogallin)	+	-	[58]; [73]; [68]; [61]; [64]; [69]; [71]; [38]
9	C ₁₅ H ₁₂ O ₉	[M-H] ⁻	336.05	335.300	13.55	183 (100), 168 (10), 124 (30)	Methyl digallate	+	+	[58]
10	-*	[M-H] ⁻	-	405.814	19.39	405 (55), 361 (20), 344 (30), 343 (100), 327 (10)	Unknown compound	-	+	-
11	C ₂₀ H ₂₀ O ₁₄	[M-H] ⁻	484.09	483.378	10.83	331 (15), 313 (45), 271 (100), 211 (45), 169 (88)	1,6-Bis-O-Galloyl-beta-D-Glucose	+	-	[72]; [66]; [74]; [73]; [68]; [64]; [69]; [38]
12	C ₂₂ H ₁₆ O ₁₃	[M-H] ⁻	488.06	487.407	14.07	183 (100)	Methyl p-trigallate (gallic acid-methylgallate-gallic acid)	+	-	[75]; [58]
13	C ₂₁ H ₂₂ O ₁₄	[M-H] ⁻	498.10	497.718	14.32	452 (100), 368 (10), 226 (55)	methyl 3,6-di-O-galloyl-alpha-D-glucopyranoside	-	+	[76]; [77]; [58]
14	C ₂₃ H ₁₈ O ₁₃	[M-H] ⁻	502.07	501.815	19.79	501 (100), 457 (25), 440 (17), 395 (5)	Ethyl m-trigallate (gallic acid-ethylgallate-gallic acid)	-	+	[78]; [58]

15	C27H24O18	[M-H] ⁻	636.10	635.998	11.72	484 (65), 466 (100), 313 (17), 169 (15)	1,2,6-Trigalloylglucose	+	-	[65]; [72]; [58]; [66]; [79]; [73]; [68]; [61]; [64]; [69]; [80]; [38]
16	-*	[M-H] ⁻		671.779	13.55	335 (10), 183 (100)	Phyllanemblinins isomer	+	-	[61]
17	-*	[M-H] ⁻		680.041	16.45	680 (100), 517 (20)	28-β-D-Glucopyranosyl-2α,3b,19α-trihydroxyolean-12-ene-24,28-dioic acid	+	-	[71]
18	-*	[M-H] ⁻	-	724.489	15.39	697 (10), 678 (100)	Kaempferol-O-dipcoumaroyl deoxyhexoside	+	+	[81]
19	C34H28O22	[M-H] ⁻	788.11	787.365	11.87	787 (50), 636 (55), 617 (100), 580 (35), 465 (100)	Tetra-O-galloylhexoside	-	+	[72]; [58]; [66]; [79]; [73]; [68]; [61]; [64]; [69]; [80]; [38]
20	C41H26O26	[M-H] ⁻	934.07	933.683	9.96	933 (100), 915 (30), 631 (50), 424 (20), 301 (20),	Castalagin	-	+	[80]; [82]; [71]
21	C41H30O26	[M-H] ⁻	938.10	937.435	11.37	937 (100), 786 (10), 301 (10)	Eugeniin (Tellimagrandin II)	-	+	[72]; [67]
22	C41H32O26	[M-H] ⁻	940.12	939.550	12.56	787 (100), 636 (5), 618 (7)	Pentagalloyl-hexoside	+	+	[41]; [72], [58]; [66]; [79]; [73]; [68]; [64]; [71]; [38]
23	C41H30O28	[M-H] ⁻	970.09	967.385	10.83	483 (100)	Phyllanemblinin C	+	-	[83]
24	C48H36O30	[M-H] ⁻	1092.13	1091.098	12.66	940 (100), 787 (45)	Hexagalloyl-glucopyranose	+	+	[58]; [66]; [79]; [38]

*-: Unknown compound.

DISCUSSION

In recent decades, opportunistic fungi, including *Candida albicans* yeast, have caused a dramatic increase in infections[30]. According to reports, the most notable obstacles to candidiasis treatment are limitations, including limited antifungal medications effective against *Candida* species, their toxic effects on human cells, and reduced sensitivity to some *Candida* species sensitivity to them[31].

Like other fungal infections, vulvovaginitis is treated with azole antifungal medications. Still, the excessive use of fluconazole and other azoles can lead to the colonization of resistant species, such as *Candida glabrata* and *Candida krusei*, which results in treatment failure and relapse[32]. To this end, researchers look for new antifungal medications, especially medicinal plants. The findings by Nori et al. on galls' antifungal effects[33] are consistent with the results of the present study, which found that Mazuj and Ghalghaf galls extracts demonstrated significant antifungal effects[33]. Oak gall extract smeared on a cotton swab showed significant inhibitory effects of its anticandidal properties on *Candida albicans*[34]. All types of extract (aqueous, acetone) of both galls showed anti-*Candida* activities. However, the aqueous extract of Ghalghaf gall showed minimal anti-*Candida* activity in agar well diffusion tests. In the present study, all *Candida* strains demonstrated sensitivity to gall extracts, and this sensitivity to extracts significantly differed based on the type of *Candida* strains. The agar well diffusion method revealed that aqueous and acetone extracts of Mazuj and Ghalghaf galls have distinct anti-*Candida* effects on all *Candida* strains. Extracts' analyses of LC-ESI-MS/MS detected several compounds, such as quinic acid[35], gallic acid and its derivatives[36], brevifolincarboxylic acid[37], digallic acid[38], and ellagic acid[39], that possess several medicinal effects, such as antimicrobial property inhibits various fungal and bacterial strains, significant antioxidant activity [40, 41], and anticancer activity. Future studies should examine all chemical compounds in Mazuj and Ghalghaf galls to determine their potential as antifungal agents. By industrializing the extraction of significant antifungal compounds for treating candidiasis, new antimicrobial agents may be developed with fewer adverse effects, specifically for hospitalized or immunocompromised patients. Moreover, ingredients found in the extracts have other positive effects, including anti-diabetic and anti-inflammatory activities, making them potentially useful for treating different medical conditions[42]. To conclude, fungal infections from opportunistic fungi, specifically *Candida albicans*, have become increasingly prevalent, making them a significant concern for human health. New therapeutic options are necessary due to the limitations of the available antifungal medications. According to our findings, Ghalghaf and Mazuj galls are potential sources of new antifungal agents. There is a need for further studies to determine active compounds contributing to these antifungal effects as well as to determine their clinical and preclinical safety and efficacy.

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Conflicts of Interest

The authors declare no conflicts of interest in this study

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