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Antimicrobial Activity of *Quercus infectoria* Gall and Its Active Constituent, Gallic Acid, against Vaginal Pathogens

Mozhgan Mehri Ardestani[1], Atousa Aliahmadi[2], Tayebeh Toliat[3], Abdolhossein Dalimi[4], Zohreh Momeni[4], Roja Rahimi[1,5]*

[1]*Department of Traditional Pharmacy, School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Biology, Medicinal Plants and Drug Research Institute, Shahid Beheshti University, Iran*
[3]*Department of Industrial Pharmaceutical Laboratory, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Department of Microbiology, Faculty of Sciences, Islamic Azad University, Karaj Branch, Karaj, Iran*
[5]*Evidence-Based Medicine Group, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran, Iran*

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Abstract

Vaginal infections are one of the most common reasons a woman visits a gynecologist. The increased resistance to conventional antibiotics is one of the main reasons for searching and developing new antimicrobial agents, especially those of natural origin. In traditional Persian medicine, the gall of *Quercus infectoria* has been claimed to eliminate vagina and cervix from excessive discharge. So, the aim of the present study was to evaluate the antimicrobial activity of ethanolic extract of *Quercus infectoria* gall as well as its active constituent, gallic acid, against some vaginal pathogens. In this study, the ethanolic extract of *Quercus infectoria* gall was obtained by maceration and standardized based on amount of gallic acid. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of this extract as well as its active compound, gallic acid, were determined against *Candida spp*., *Gardnerella vaginalis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Trichomonas vaginalis* and *Lactobacillus acidophilus*. The results demonstrated remarkable activity of ethanolic extract of *Quercus infectoria* gall against investigated pathogens with MIC and MBC in the range between 0.125 mg/ml and 16 mg/ml. The most inhibitory and bactericidal activity was observed on *Streptococcus agalactiae* and *Staphylococcus aureus*. The effects of gall dried ethanolic extract on *Trichomonas vaginalis* showed 100 % inhibition of the parasitic growth with concentration of 800 µg/ml after 24 h incubation. The antimicrobial and anti-trichomonas activity of extract was more than gallic acid.

*Corresponding Author: Roja Rahimi
Department of Traditional Pharmacy, School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel/Fax: +98-21-88990835
Email: rojarahimi@gmail.com

It seems that ethanolic extract of *Quercus infectoria* gall could inhibit the growth of vaginal pathogens. Further preclinical and clinical studies are required to confirm the efficacy of this natural extract in vaginitis.

Keywords: Quercus; Medicinal plant; Gallic acid; Vaginitis; Trichomoniasis; Vaginal candidiasis

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Introduction

Vaginal infection is among the most common reproductive tract infectious in women of reproductive age and may lead to pain, discomfort, anxiety, distress, embarrassment, dissatisfaction with sexual relationships, and absence from school or work [1]. Symptoms of vaginal problems such as discharge, itching, and odor are the leading cause for women to seek the care. In the United States, it is estimated that 10 million office visits per year are for vaginal symptoms. These symptoms are usually caused by one of 3 infections: bacterial vaginosis (BV), trichomoniasis, or vulvovaginal candidiasis (VVC) [1-3]. Worldwide prevalence of BV ranges from 11% to 48% of women of reproductive age [3]. Overgrowth of anaerobic bacteria especially *Gardnerella vaginalis* with reductions in *Lactobacillus* populations in the vagina is the main cause BV [4,5]. VVC is an infection caused by Candida species like *C. glabrata,C. tropicalis* and *C. krusei* that affects millions of women every year [6-10]. Trichomoniasis is caused by *Trichomonas vaginalis* (TV) and is the most common non-viral sexually transmitted infection (STI) with the baseline prevalence of 14.6%, and cumulative 6-month of 7.5% in the USA [11,12]. Mixed vaginitis occurs rarely (<5 %) but pathogen co-infection occurs frequently in women with vaginitis. Approximately 20 %–30 % of women with BV are coinfected with *Candida* species [13]. Vaginal infections can cause pelvic inflammatory disease (PID), postoperative infections, cervicitis, preterm labor and delivery, chorioamnionitis, premature rupture of membranes and low birth weight. It usually causes urogenital infection with positive urine cultures in neonates and neonates' respiratory tract infection [14-16]. Moreover trichomoniasis itself can increase the risk of acute endometritis, STIs, Human immunodeficiency virus (HIV), Herpes Simplex virus II (HSV-2) , and Human papilloma virus(HPV) infections as well as cervical neoplasia [12,15,17-20]. Azole-drug (metronidazole and tinidazole), nystatin and clindamycin are the standard treatments for vaginal

infections [2,18,21]. Insufficient treatment success (about 50 %) and increase recurrence rate (50 %) within 6–12 months after drug discontinuation cause displeasure in the majority of women [22-24]. Furthermore, allergic reaction and resistance to azole drugs are other problems encountered in the management of vaginitis [18,25]. The prevalence of resistance among VVC patients treated with fluconazole and itraconazole was more than 40% [26].

Medicinal plants are assumed as a valuable source of discovering and identifying new drugs for various disorders including vaginal infections [27].

In traditional Persian medicine, the extract of *Quercus infectoria* gall has been claimed to eliminate excessive discharge of vagina and cervix and used for treating vaginal infections [28]. The aim of this study was to evaluate the antimicrobial activity of *Quercus infectoria* gall extract as well as its active compound, gallic acid, against some vaginal pathogens.

Methods

Plant material

Quercus infectoria gall was purchased from herbal market of Tehran and authenticated by Dr G. Amin (Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences), and a voucher specimen (PMP-821) deposited at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran).

Preparation of ethanolic extract

Powdered galls were extracted with 80% ethanol by maceration for 7 days. The extract was filtered and evaporated under reduced pressure.

Quantification of free gallic acid

Rhodanine test was used for determination of gallic acid content of ethanolic extract. For this purpose, 0.1 mg dried extract were extracted with 70% acetone in an ultrasonic water bath for 20 min at room temperature The suspension was centrifuged for 10 min at 3000 rpm set at 4°C .200 µL of supernatant was transferred to a test tube (4 tube/sample) and dried under vacuum pressure. 600 µL of sulphuric acid (0.2 N) was then added to the tubes. To three tubes, 900 µL of rhodanine solution (0.667%) and to the fourth tube 900 µL of methanol was added as a blank. After 9 min 600 µL of potassium hydroxide solution (0.5 N) was added to all of the tubes and after 6 min 12.9 mL of distilled water was added. After 25 min the absorbance of the red-purple solution was measured at 520 nm against the blank. In order to draw calibration curve, five different concentrations (4-20 µg/5 mL) of gallic acid were used [29].

Antimicrobial activity

In vitro antimicrobial activity of the gall dried extract and gallic acid, were assessed against *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* PTCC 1768, *Escherichia coli* ATCC 25922, *Lactobacillus acidophilus* PTCC 1643, *Gardnerella vagina-*

lis ATCC 49145 and two pathogenic yeast; *Candida albicans* ATCC 10231, and *Candida krusei* PTCC 5295.

Broth micro-dilution susceptibility method was performed using 96 well trays to determine the minimum concentration of samples required for inhibition of visible growth of the test strains (minimum inhibitory concentration; MIC) and minimum concentration which could result in killing of 99.9% of tested microorganism in each experiment (minimum bactericidal/fungicidal concentration; MBC). Standard protocol of CLSI (Clinical Laboratory and Standards Institute) was used with some modifications and each experiment was done in triplicate. The inoculants of the microbial strains were prepared from freshly cultured strains, by using sterile normal saline which were adjusted to 0.5 McFarland standard turbidity and then were further diluted (1:100 for bacteria and 1:1000 for yeasts) just before adding to the wells containing a desired range of diluted samples in Mueller-Hinton broth medium. Samples were assessed in a concentration range of 64 to 0.03 mg/ml. Incubation of inoculated trays were done for 22 h at 37°C and then the results were recorded. For determination of MBC, 100 µl of wells with no visible growth was cultured onto proper agar containing media and results were recorded after 24 h incubation at 37°C. Also standard antibiotics were tested: Cefixime for *E. coli*, *S. aureus* and *S. agalactiae*; Clindamycin for *L. acidophilus* and *G. vaginalis* and Nystatin for to yeast strains. Other steps were same as determination of MIC and MBC values [30].

Anti-Trichomonas vaginalis activity

Anti-trichomonas activity of the *Quercus infectoria* gall extract was compared with the drug metronidazole which was supplied as positive control Trophozoites were cultured in TYM media at 24-well plates (5×105 cell/well) as triplicate and double blind. Different concentrations (37.5, 75, 150, 300, 600, and 1200 µl/ml) of *Quercus infectoria* gall extract and metronidazole (50 µg/ml) were added to each well separately. The number of parasites in each well plate was counted after 24 and 48h by trypan blue staining. In the negative control group, trophozoites were cultured in TYM media as triplicate without any drug.

Evaluation of the extract from *Quercus infectoria* gall anti-T. vaginalis efficacy was done by Calculation of percent of growth inhibition according to the equation: Percent of growth inhibition = [(a – b)/a] × 100 where a = mean number of trophozoites in parasite control tubes and b = mean number of trophozoites in tested tubes[31-35]

Results

Gallic acid content of extract

The result showed that the amount of free gallic acid in100 g dried extract was8.68 g± .0.08

Antimicrobial activity

The results showed a good activity of extract against studied pathogens with mini-

mum inhibitory concentration) MIC) in the range from 0.125mg ml (-1) to 16mg ml (-1) and minimum bactericidal concentrations (MBC) in the range from 0.125 mg ml (-1) to 16 mg ml (-1). For gallic acid, the MIC was from 0.125 mg ml (-1) to 32 mg ml (-1). The most inhibitory and bactericidal activity was observed from the extract on *Streptococcus agalactiae* and *Staphylococcus aureus* (Table1).

Anti-Trichomonas vaginalis activity

Quercus infectoria gall extract showed 100% inhibition of the parasitic growth with concentration of 1200 µg/ml after 24 h incubation and 100% inhibition of the parasitic growth with concentration of 600 µg/ml after 48 h incubation (Table 2).

Gallic acid demonstrated 100 % inhibition of the parasitic growth with concentration of 4000 µg/ml after 24 h (Table 3).

Table 1. Anti-microbial activity of *Quercus infectoria* gall extract and gallic acid

Microorganism	Extract		Gallic acid		Standard antimicrobial*	
	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC µg/ ml	MBC µg/ml
Escherichia coli ATCC 25922	16	16	8	8	2	8
Staphylococcus aureus ATCC25923	0.125	0.125	0.125	4	0.06	0.125
Streptococcus agalactiae	0.125	0.5	4	8	0.03	0.06
Candida albicans ATCC10231	8	16	32	32	31.25	31.25
Candida krusei PTCC5295	8	16	8	32	7.81	7.81
Enterococcus faecium clinical strain	4	8	4	16	2	8
Lactobacillus acidophilus PTCC1643	0.5	1	8	16	4	8
Gardnerella vaginalis ATCC49145	4	8	32	32	>500	>500

* Cefixime for *E. coli*, *S. aureus* and *S. agalactiae*; Clindamycin for *L. acidophilus E.faecium* and *G .vaginalis*; Nystatin for *C. krusei and C. Albicans*

Table 2. Percent of growth inhibition after adding different concentrations of *Quercus infectoria* gall extract in two times on *Trichomonas vaginalis*

Concentration	24h	48h
Negative control	-	-
Metronidazole (50µg/ml)	100%	100%
37.5 µg/ml	38%	66%
75µg/ml	56%	75%
150µg/ml	62%	79%
300µg/ml	68%	85%
600µg/ml	80%	100%
1200µg/ml	100%	100%

Table3 . Percent of growth inhibition after adding different concentrations of gallic acid in two times on *Trichomonas vaginalis*

Concentration	24h	48h
Negative control	-	-
Metronidazole (50µg/ml)	100%	100%
250µg/ml	33%	70%
500µg/ml	65%	75%
1000µg/ml	84%	87%
2000µg/ml	89%	97%
4000µg/ml	100%	100%
8000µg/ml	100%	100%

Discussion

Vaginal infections are one of the most common reasons a woman visits a gynecologist [1-3]. A large number of antibiotics have been discovered from plant sources that have an important role in the treatment of infectious diseases [36,37]. The increased resistance and hypersensivity to conventional antibiotics for management of vaginitis are two reasons for searching and developing new antimicrobial agents, especially those of

natural origin [27]. Moreover, finding a drug that would be effective on all types of vaginitis may be a revolution in the treatment of vaginitis because if its potential to treatment of mix and co-infections [1-3].

In this study, the antimicrobial effects of *Q. infectoria* gall extract and its active compound, gallic acid, was evaluated against different vaginal pathogens. The results showed that ethanolic extract of *Q. infectoria* gall have a inhibitory effect on the growth of *Gardnerella vaginalis*, *Staphylococcus aureus*, *Streptococcus agalactia*e as well as *Candida* spp. Abdul Qadir et al. investigated the antimicrobial activity of oak extracts in terms of zone of inhibition on four pathogenic bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Pasteurella multocida*, and *Escherichia coli*) and four fungal strains (*Aspergillus flavus*, *Rhizopus solani, Aspergillus niger*, and *Alternaria alternate*). The antibacterial activity of extracts was more than their antifungal [38]. Vermani A. showed anti-bacterial activity of gall methanolic extract against dental pathogens. The most susceptible bacteria were *S. sanguis* followed by *S. aureus*, *S. mutans*, *S. salivarius* and *L. acidophilus* [39]. An ethanolic extract of *Q. infectoria* demonstrated potent inhibitory and bactericidal effects on *Escherichia coli* [40]. The extract from galls of *Q. infectoria* in combination with vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) were shown synergistic effect. The time-kill curves showed that the interaction was additive with a more rapid killing rate [41]. Also the results of the susceptibility test on the *Candida* spp. Indicated that gall dried extract have better activity against *Candida albicans*, *Candida krusei* than gallic acid. Baharuddin NS et al. showed that the methanol and aqueous extracts of *Q. infectoria* galls exhibited antifungal activities against *Candida albicans*, *Candida krusei*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*. The MIC was between 0.03 mg/ml - 16 mg/ml [42]. The effects of gall dried ethanolic extract on *Trichomonas vaginalis* showed 100 % inhibition of the parasitic growth with concentration of 800 µg/ml after 24 h incubation. Given that the amount of gallic acid in ethanolic extract of oak is 8/68 g ,100/it is expected that gallic acid showed 100 % inhibition of the parasitic growth at a concentration between 60-70 µg/ml after 24 h incubation, but it showed complete inhibitory activity with concentration of 4000 µg/ml .These results show that gallic acid is not exclusively responsible for antifungal activity of gall extract and this activity may be enhanced by other constituents of extract via their synergistic activity or enhancing penetration of gallic acid into phatogens. Some of other compounds identified in gall of *Q. infectoria* rather than gallic acid are ellagic acid, gallic acid, and methyl gallate, pyrogallol, syringic acid, difluoroheptacosanoic acid and γ-sitosterol [42-46]. Treatment with *Q. infectoria* extract and gallic acid and tannic acid results in hypersensitivity to low and high osmotic pressure and finally eradication of MRSA cells [45]. Gallic acid has demon-

strated inhibitory activity on the growth and biofilm formation of *Escherichia coli, Streptococcus mutans*, *S. aureus*, *Pseudomonas aeruginosa* and *C. albicans* under different conditions. Adhesion of *Staphylococcus aureus* and *P. aeruginosa* was reduced when these bacteria were exposed to gallic acid [47-49]. The most sensitive Candida species to gallic acid was *Candida albicans* and the most sensitive filamentous species was *Trichophyton rubrum* which was comparable to fluconazole. Gallic acid reduced the activity of sterol 14α-demethylase P450 in *Candida* spp. and squalene epoxidase in the *T. rubrum* membrane. The intraperitoneal injection of gallic acid significantly enhanced the cure rate in mice [50].

A combination of ellagic acid and tetracycline (ETC; 250 μg ml(-1) + 0.312 μg ml(-1)) was determined to effectively inhibit biofilm formation by *Propionibacterium acnes* without affecting its growth and reduce the production of extracellular polymeric substances to make more susceptible to the human immune system and antibiotics [51]. The botanical extract from the root of *Rubus ulmifolius* rich in ellagic acid and its derivatives can be used to inhibit *Staphylococcus aureus* biofilm formation to a degree that can be correlated with increased antibiotic susceptibility without toxic effects on normal mammalian cells. It can enhance susceptibility to the functionally-distinct antibiotics daptomycin, clindamycin and oxacillin [52].

Treatment with *Q. infectoria* extract and gallic acid and tannic acid results in hypersensitivity to low and high osmotic pressure and finally eradication of MRSA cells [45]. Pyrogallol showed a synergistic effect with fluconazole against *Candida albicans* and *Candida tropicali*s. It was reduced the MIC of *S. aureus* with gentamicin and norfloxacin [53]. Conclusively, *Q. infectoria* gall dried ethanolic extract is a potent inhibitor of the growth of all of *vaginal pathogens* including bacterial, fungal and parasitic pathogens *in vitro* and thus can be assumed as a promising medicine for management of all types of vaginal infection including BV, VVC, and trichomoniasis. Also, other components of extract may increase the effect of gallic acid against *Trichomonas vaginalis*. Further investigations are needed to confirm the efficacy and mechanism of action from *Q. infectoria* gall in vaginitis.

Conflicts of interest

The authors confirm that this article content has no conflicts of interest.

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