



ANTIFUNGAL POTENTIAL OF QUERCUS INFECTORIA GALLS AGAINST CANDIDA ALBICANS-AN INVITRO STUDY

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ABSTRACT

The promising potential of antimicrobial plant derived substances has attracted the attention of pharmaceutical and scientific communities during the last few years. The primary benefit of plant derived medicines is that they are relatively safer than their synthetic counterparts and offer profound therapeutic benefits and more affordable treatment. *Quercus infectoria* is a small tree or a shrub belonging to the *Fagaceae* (*Quercaceae*) family. The Gall of *Quercus infectoria* is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, anti-inflammatory, antipyretic, antiseptic, antistomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, local anesthetic, antiviral, antibacterial, larvicidal and antifungal activities. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid. This study was performed to assess the antifungal effects of the extracts of *Q. infectoria* Galls, against *Candida albicans* with cup-plate agar diffusion method. In the present study the extract the plant was tested for their bioactivity against *C. albicans*. Extract of *Q. Infectoria* Galls showed potential activity against the tested fungal isolate. According to the results of this study, the effect of Clotrimazole (positive control) was more than plant extracts effects, while the effect of antifungal drug Nystatin used in this study (Table 5) with the extract of *Q. Infectoria* Galls showed that Nystatin was effective in the treatment of *candida* infection with inhibition zone 28 mm at concentration 50 µg/ml, low activity at concentration 25 µg/ml with inhibition zone 14 mm and in active at concentration 12.5 µg/ml this means that *Q. Infectoria* Galls with more *anticandidal* potency than Nystatin chemotherapeutic antifungal agent used in this study. This study thus provides a useful database of the therapeutic bioactivity of *Quercus Infectoria* Galls against *C. albicans*, Thus effort must be making for isolation, standardization and clinical evaluation of such phytochemicals in order to obtain lead compounds for further new drug discovery.

KEYWORDS: Extract, Candidiasis, Bioactivity, Antifungal Activity.

INTRODUCTION

The potential for developing antimicrobials from higher plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within higher plants with their full spectrum of efficacy.^[1] The development of strategies to control fungal infections may be an effective means for therapeutic interventions. The majority of clinically used antifungals have various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to emergence of resistance strains. Antifungals based on synthetic chemicals cause severe and long-term medical problems are highly and acutely toxic. The primary benefits of using plant derived

medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Consequently, the aim of new antifungal strategies is to develop drugs that combine sustainability, high efficacy, restricted toxicity, safety for humans with low production cost. Since antifungals of biological and natural origin have been demonstrated to be specifically effective on target organisms and are also safe. For millions of people traditional medicine serves as the only opportunity for health care. Safety and lower side effects of many herbal extracts have also suggested them as sources of new pharmaceuticals.^[2] Recently, many plant extracts have shown to have therapeutic values with respect to oral diseases. Medicinal plants

remain a rich source of novel therapeutic agents. Many plant species are still unevaluated chemically or biologically. Several studies regarding the action of plant extracts against some pathogenic fungi have been performed. The quality and quantity of the biologically active compounds from the plant extracts significantly depend on the species, the plant organ and harvest time.^[2,3] There are global problems of multiple antifungals resistance as well as emergence of new and resurrection of previously eradicated diseases. Most of the current antimicrobial drugs simply reduce the level of growth of bacteria or fungi, and some of them are very toxic to the kidney, the hematopoietic and central nervous system. With the rising problems of side effects and limited efficacy of antifungal drugs, there is an urgent need for the development of alternative antifungal substances and researchers are nowadays turning to natural products from plants, as their main source of bioactive compounds with antifungal and antimicrobial properties, to complement the existing synthetic antifungal drugs that are gradually becoming less potent against pathogenic micro-organisms. Candidiasis, which holds a potential of life-threatening disease refers to the yeast infections caused by *Candida species* commonly from *Candida albicans* species.^[4] However, there has been a recent increase in yeast infections due to the *non-albicans* species such as *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis* and *Candida krusei*.^[5,6,7] Treatment of candidiasis is available; however, resistance towards many of the antifungal used to treat this condition is increasing. Patients receiving fluconazole are particularly at risk of developing infections due to fluconazole-resistant of *C. albicans* strain.^[7] Problems with antifungal resistance and the increasing number of infections caused by *non-albicans Candida* (NAC) have created a huge demand for new effective antifungal therapies.^[8]

Quercus infectoria is a small tree or a shrub belonging to the *Fagaceae* (*Quercaceae*) family. Galls of *Quercus infectoria* (QI) is known by different vernacular names, locally known as Ifas. The plant is found in Turkey, Syria, Persia, Cyprus and Greece.^[9] The various *Quercus species* originated in Iran, Iraq and Turkey, but are now widespread and particularly common in Asia Minor, Europe and North Africa.^[10] Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites.^[11] The QI galls are produced by the insect, *Cynipsquercufolii*, for depositing its eggs.^[12] The Gall of *Quercus infectoria* is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, antistomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, and wound healing.^[13-18] The galls of *Q. infectoria* have also been pharmacologically documented to possess antitremorine,

local anesthetic^[19], antiviral^[20], antibacterial^[21], larvicidal^[22] and antifungal^[23] activities. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid.^[24,25] The galls contain 50-70% of the tannin known as gallotannic acid. This is a complex mixture of phenolic acid glycosides varying greatly in composition. The galls also contain gum, sugar and essential oil.^[26] New alternative treatments to provide safe, cheap and effective antifungal agents are urgently needed. Due to the wide spectrum of anti-microbial properties^[27,28] of *Q. infectoria* galls, this plant might be potentially effective to treat the increasing cases of local and systemic candidiasis. Thus, this study was conducted to evaluate the *anti-Candida* potential of ethanolic extracts of *Q. infectoria* galls toward the commonly isolated *Candida species*.

MATERIALS AND METHODS

Plant materials collection and identification

The Gall of *Quercus infectoria* were collected. The plant was identified by a taxonomist at medicinal and aromatic plants institute, National Center for Research - Khartoum, Sudan. The tested plant part then ground into powder and was used for the subsequent experimentation.

Reagents: Chloroform (SD Fine India), Ferric Chloride (BDH England), Acetic anhydride (SD Fine England), Sulphuric acid (SD Fine India), Hydrochloric acid (Romile EU), Aluminium Chloride (BDH England), Potassium Hydroxide (Sharlau Spain), Hydrogen Peroxide (Sharlau Spain), Ammonium Hydroxide (SD Fine India), Benzene (Sharlau Spain), Sodium Chloride (Sharlau Spain), Gelatin salt (Sharlau Spain), Potassium chloride (BDH England), Mercuric iodide (BHD England), Ethanol (National Distillation Company).

Preparation of extract: Extraction was carried out according to method described by^[29]: 500 g of the plant sample was extracted by soaking in 2500 ml 80 % ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus, In order to obtain a completely dry extract, the resultant extract were transferred to glass dishes. The yield percentages were calculated as followed.
Weight of extract / weight of sample * 100

Phytochemical analysis

The phytochemical qualitative tests were carried out for ethanolic extract of *Q. infectoria* Galls to screen for the presence of tannins, saponins, coumarins, anthraquinones, alkaloids, flavonoids, sterols and triterpenes using standard procedure.^[30-34] These active compounds were commonly reported in plant extract with bioactivity.

Preparation of standard fungal organisms

The fungal standard cultures were obtained from the department of Microbiology and Parasitology, Medicinal and Aromatic Plant Research Institute, Khartoum and were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth were harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline and the suspension was stored in refrigerator till used.

Anti-Fungal activity: The *in vitro* screening of antifungal activity and determination of minimum inhibitory concentration (MIC) values are evaluated using cup-plate agar diffusion method.

In vitro testing of extract for antifungal activity

The cup-plate agar diffusion method^[35] was adopted, with some minor modifications; to assess the antibacterial activity of the prepared extracts (NCCLS 2000). In accordance with this method one ml of the isolated standardized and bacterial stock suspension (108-109 C.F.U per ml) were thoroughly mixed with 100 ml of sterile molten Mueller- Hinton agar which was maintained at 45°C. Twenty ml aliquots of the inoculated Mueller-Hinton agar were distributed onto sterile Petri-dishes. The agar was left to set, and in each of these plates, four cups (10 mm in diameter) were cut using a sterile cork borer (NO.4) and the agar discs were

removed. Alternate cups were filled with 100µL of samples of each of the extract, using standard fine adjustable automatic pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position, at 37°C for 18 hours. Two replicates were carried out for extract against the tested organisms. Simultaneously, positive control involving the addition of methanol instead of the extract was included. Upon the completion of incubation the diameter of the resultant inhibition zones were measured, averaged and then the mean values were tabulated.

Determination of Minimum Inhibitory Concentrations (MIC) by agar well dilution method

Extract was prepared in the series of decreasing concentrations in the following order 50, 25, 12.5, 12.5, 6.25 and 1.56 mg / ml. MIC is the least concentration of antifungal agent that completely inhibits the growth. Results were reported as MICs.

Antifungal activity of reference drugs: The antifungal drugs were also tested at different concentrations obtained by taking 0.1 g of each powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of 12.5, 25 and 50 µg/ml Nystatin against reference fungi *Candida albicans*. 5, 10 and 20 µg/ml Clotrimazole against the same organism.

RESULTS AND DISCUSSION

Table. 1: Yield percentages of ethanolic extracts.

Sample	Weight of sample	Weight of extract	Yield %
<i>Quercus infectoria</i> galls	500 g	76.3 g	15.26 %



Fig. (1): Rotary Evaporator apparatus.

Table. 2: Organoleptic and physical properties of extract.

Weight of sample	Weight of extract	Yield %	Appearance	Color	Odor	Taste
500 g	76.3 g	15.26 %	Powder	Light Brownish yellow	Pungent	Bitter

Table 3: Phytochemical screening of *Quercus infectoria* galls.

Test	Results	Observation
Saponins	++	Foam
Cumarins	+	UV absorption
Alkaloids	-	No observation
Anthraquinones	++	Pink colour
Tannins	+++	blue colour
Flavonoids	+++	Yellow color
Sterols	-	No observation
Triterpenes	++	Purple colour

Table 4: Antifungal activity of the extract against Standard Organisms.

Sample	Concentration	Standard tested organism* /M.D.I.Z(mm)**
		C. a
<i>Quercus Infectoria</i> Galls Extract	10 %	28
	5 %	25
	2.5 %	22
	1.25 %	21
	0.65 %	20

*Standard organism tested: C.a= *Candida albicans*.

Table 5: Antifungal activity of reference antibiotics against standard microorganisms.

S. No	Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)
			Tested fungi used(M.D.I.Zmm)
			C.a
3	Clotrimazole	40	42
		20	40
		10	33
		5	30
4	Nystatin	50	17
		25	14
		12.5	-

DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening was performed to establish the profile of gall extracts for its chemical composition. The *Q. infectoria* gall extracts showed the presence of tannins, flavonoids, saponins, anthraquinones, triterpenes and coumarins. Alkaloids and sterols gave negative results (Table 3). These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. Tannins or tanning agents are natural occurring phenolic plant compounds. Their main operation area is to support the healing process of inflammations, abscesses, incinerations, wounds^[36], atopic skin^[37] as well as quinsy.^[38,39] The effect of tannins is antibacterial, antiviral^[40], antifungal^[41] anti-inflammatory, astringent and toxin neutralizing. Anthraquinones and anthrones are very reactive and have a broad pharmacological activities including, they are potent anticancer, antidiabetic,

antimicrobial, antiinflammatory, and cathartic properties as well as its cardio-, hepato-, and neuroprotective qualities.^[42] Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Plant sterols are known to be important for their cardiotoxic, insecticidal and anti-microbial properties. Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotoxic in nature and are reported to have anti-diabetic and anti-fungal properties.^[43,44] Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages.^[45] Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Plant sterols are known to be important for their cardiotoxic, insecticidal and anti-microbial properties. They are also used in nutrition, herbal medicines, cosmetics and they are routinely used in medicine because of their profound biological

activities.^[46] A large number of studies have been done in recent years on the antifungal and antibacterial activity of terpenoids of natural origin. The mechanism of action of triterpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic nature.

Antifungal activity

The effectiveness of ethanolic plant extract under study toward the tested isolate

Antifungal activity (assessed in terms of inhibition zone) of the extract of *Q. Infectoria* was recorded (Table 4). In the present study the extract of the plant was tested for its bioactivity against *C. albicans*. Extract of *Q. Infectoria* Galls showed potential activity against the tested fungal isolate at concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml (Table 4). The tested fungal isolate they were sensitive to extract of *Q. Infectoria* Galls. The highest sensitivity was observed as 28 mm at concentration of 100mg/ml and 20 mm at 6.25mg/ml. MIC value (Table 4) was evaluated for the extract, which was showing activity in diffusion assay. The study results showed the effectiveness of plant extract under study toward the isolate tested (*C. albicans*) and generally have attributed the cause to the use of ethanol as a solvent polar extraction plants study which serves to pull the polar active compounds. As shown in Table (3) example (flavonoids, tannins and saponins) where these compounds operate on Union with protein deposition and cell altering the nature and function as a good solvent for fatty substances, that is lead to analyzed the membranes of living cells and as a result he graduated cellular components inside to outside and die the yeast cell.^[47] According to the results of this study, the effect of Clotrimazole (positive control) was more than plant extracts effects, while the effect of antifungal drug Nystatin used in this study (Table 5) with the extract of *Q. Infectoria* Galls showed that Nystatin was effective in the treatment of *candida* infection with inhibition zone 28 mm at concentration 50 µg/ml, low activity at concentration 25 µg/ml with inhibition zone 14 mm and in active at concentration 12.5 µg/ml this means that *Q. Infectoria* Galls with more *anticandidal* potency than Nystatin chemotherapeutic antifungal agent used in this study. The antimicrobial activity of plant extracts has been linked by many researchers to be due to the presence of phytochemicals in them.^[48,49] Reported that crude extracts of plant materials may contain inactive substances which may also antagonize the antimicrobial actions of one another.

CONCLUSIONS

It is intriguing to note that crude extracts of *Q. infectoria* galls hold an *anti-Candida* potential, and contain more active compounds allowing recommended therapeutic alternatives to antifungal chemical drugs. Further investigations are needed to develop a standardized *Q. infectoria* gall extract and to understand its mechanism of *anti-Candida* activity.

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