



# INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare &amp; Pharmascope Foundation

Journal Home Page: [www.ijrps.com](http://www.ijrps.com)

## Assessing the impact of quercetin isolated from *Ammi majus* seeds upon *Candida spp.* isolants isolated from different sources

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### Article History:

Received on: 01 Jun 2020

Revised on: 03 Jul 2020

Accepted on: 20 Jul 2020

### Keywords:

*Candida spp.*,  
Quercetin,  
*Ammi majus*,  
Inhibition,  
Antifungal,  
Vaginal infection,  
Mouth infection,  
Urinary tract infection

### ABSTRACT

Quercetin has been extracted from *Ammi majus* plant using ethanol via soxhlet system. Further, the substance was diagnosed by three binary and mono-layered chromatographical devices beside chromatographical quantitative separation. Total of 45 patients was inflicted with mouth infections and urinary tract in both genders. The momentary vaginal infections among different ages of people settled in different regions of Nineveh district / Iraq were considered for the study. The study patients were identified using microscopic testing, selective differential medium (CHROM candida agar) and Vitek system. The isolation results inferred that the mouth infection is caused by most yeasts such as *Candida parapsilosis* (the most frequent). The urinary tract infection is concerned, and most of the infections were reportedly caused by *C. parapsilosis*, *C. tropicalis* and *C. albicans*. with regards to the vaginal infections cases, *C. albicans* fungus has been the most frequent. Bio chemical tests were conducted using Vitek for the isolants studied, in which there were differences in the study results. Quercetin was extracted from *Candida spp.* The increase in the inhibition of quercetin is noted, whenever its concentration is increased. With regards to the anti-fungals Nystatin, for the concentration of 100IU/ml and Clotrimazole 30mg/ml had inhibited the yeast *Candida spp.*



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i4.3118>

Production and Hosted by

IJRPS | [www.ijrps.com](http://www.ijrps.com)

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### INTRODUCTION

Plants contain abundant numbers of different chemical compounds, whose discovery increases, thanks to the progress made in the field of multiple chemical analysis methods. This progress enables the detection of compounds and get acquainted to it

so as to harness its benefits. These compounds are of great importance because of the physiological effect and medicinal properties on human, animal and plant organs (Abboud and Waheed, 2017). One of these plants is *Ammi majus* L. which contains quercetin. *Ammi majus* L. falls under the family, umbelliferae. Being a common perennial herb, it grows in all the cultivated areas and is a native of Mediterranean Sea region, especially Africa. The interest towards *Ammi majus* L. was revived in the recent years after its value was established through many clinical investigations. It is a well-known fact that furocomairns exhibit photosensitizing activity and their derivatives have been found to possess antibacterial properties. In Northern Iraq, especially Mosul, Erbil and Zakho, these plants grow abundantly in different areas. The studies published so far proved that the fruits of *Ammi majus* L. plants contain glycoside and glycoside comarius while few studies reported the presence of flavonoids in L.

plants (Ayoub *et al.*, 1998).

This specific species and few other species under this family are considered as the source of khellin, which is used as a diuretic (Ziment, 1998). *Ammi majus* L. is used for grueling in teeth and palate diseases (Al-Janabi *et al.*, 2017). There are many diseases caused by fungi like yeasts and semi-yeasts including *Candida* genus while the latter consists of more than 200 species in it and morphologically, it looks similar to semi-yeasts and oval in shape. These cells are asexual ones i.e., breeding through budding or fission. *Candida* species descends from Saccharomycetaceae family, Saccharomycetales order, Saccharomyces class and Ascomycota phylum (Vazques and Sobel, 2003). This species causes infections in the mouth, urinary tract and vagina. The infection occurs as a causative one or accompanies the bacterial or viral infection or a side effect to weak immunity of the body or excessive use of antibiotics and change in the natural flora of infected area (Al-Hadithi *et al.*, 2007). The ability of some *Candida* species to develop the anti-medicine biomembranes is considered as the important factor that contributes to the virulence in human diseases (Rajendran *et al.*, 2010; Vila *et al.*, 2020). *Candida* is generally observed in mouth among patients with weak immunity. Especially, it exists in mouth up to 20 % to 40 % among healthy people (Habib *et al.*, 2015). Besides, *Candida* exists in respiration channel, vagina and in other such tracts (Lewis *et al.*, 2000).

The transformation of yeast from antiretroviral to an opportunist is due to its possession of aggressive factors like sticking to the surface of epithelial cells, production of digesting enzymes for fats and proteins and the development of production tube (Panagoda *et al.*, 2001). These yeasts are also found in tooth decay and mouth infections (Habib *et al.*, 2015; Al-Kaaby *et al.*, 2016). In addition, bacteria exist with the host round the mouth since it is important to have the ability to cause opportunist diseases so that they naturally exist in skin, nose, intestinal tract epithelium, and sexual organs. They contain a lot of antigens superficially and enzymes that enable the pathogen to penetrate body tissues (Nicholls *et al.*, 2011). *Candida spp.* are considered as the most common cause of urinary tract infections in which they affect the urinary tracts through urine stream followed by the bladder and then the bloodstream (Pfaller *et al.*, 2007b). These infections are the most dangerous health problems reported among millions of people; especially women, because they are likely to get affected than men. The rate of the spread of this disease differs according to different health and geo-

graphical conditions. The urinary tract infections may vary from case to case according to the patient, gender and their age than the symptoms of urinary tract infections (Al-Rubaie and Abdul-Hamza, 2010). More than 90% of the fungal cases are caused by *Candida albican*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (Pfaller *et al.*, 2007a). Nowadays, the increase in the number of anti-medicine yeasts and antifungal species are identified all over the world. Therefore, it is best to use the laboratory practical tests which may help the physician to choose the suitable remedy (Ingham *et al.*, 2012). In the present study, the technique of Vitek 2 Compact system was used (Aubertine *et al.*, 2006; Habib *et al.*, 2015; Badr and Abaas, 2017).

## MATERIALS AND METHODS

### Plant material

The plant was cultivated in Iraq using the seeds of *Ammi majus*, procured from local market. The seeds of *Ammi majus* L. that were originally collected from Al-Jazeera region were first cleaned and then ground.

### Preparation of plant extract

The plant was weighed upto 100 gms and extracted for 6 to 8 hours using soxhlet system with 1 litre of pet. Ether at 60°C to 80°C for delayed processing. This batch was again extracted with 70% of the ethanol. So, the ethanol got concentrated on vacuum votary that evaporated at 50°C to give 15 ml as a residue (Ayoub *et al.*, 1998).

A total of three batches of fifteen random samples each (total 45 samples) were collected from persons with a mouth infection, swab samples of patients affected with urinary tract infections and swab samples of women affected with vaginal infections. The samples were collected from patients who visited the clinics in Nineveh district and the swabs were cultured in a dish containing Sabouraud Glucose Agar (SGA) medium. All the samples were incubated at 37°C for 24 hours. The results were recorded, and the isolates were reserved in slanting mediums for further use (Habib *et al.*, 2015).

The isolated fungi were identified through mere observation and through microscopic testing in which the fungal colony shape and its structure were noted. The colonies were observed microscopically according to the method described earlier (Sood, 1994; Morello *et al.*, 2003). The fungi cultures were developed in differential medium chrome agar candida. This medium was used to identify different types of *Candida* genus depending on the enzymes that exhibit colour (Baumgartner *et al.*, 1996; Pfaller

*et al.*, 2007b; Ellis *et al.*, 2007). Quercetin, which was extracted from *Ammi majus* (Ayoub *et al.*, 1998) in the concentrations of 2, 3 and 4 mg/ml of the solved substance (DMSO), was prepared. The influence of concentrations of quercetin was tested by making a dig of 5 cm in diameter. In SGA medium, the isolated samples considered for testing were cultured with 0.1 ml concentration. Each dig was added the solution thrice and was incubated at 37°C for 24 hours. The results were recorded by measuring the diameter of the inhibition state, excluding the dig diameter (M R Mc Ginnis, 1980).

## RESULTS AND DISCUSSION

The quercetin was extracted from *Ammi majus* as follows

### Separation of quercetin from ethanol extract

Crude ethanolic extract was obtained from the second step (preparation of plant extract) using 100 gms of seeds. This extract measured 15 ml. while 10 gm. of this crude ethanolic residue was applied onto a column packed with silica gel in the pet—ether 60–80°C.

The elution affected the petroleum ether followed by chloroform and chloroform: methanol. The fractions obtained were examined using Thin-Layer Chromatography on silica gel. The elution with 10% methanol in chloroform attained a yellow ppt, which was crystallized from ethanol to give 120 mg. Yellow needles. The material was made to undergo acid hydrolysis in 2NHCl for 30 minutes. This was followed by the usual work for the given residue that is identical to quercetin (m.p = 314–315) C° (lit 313–314) C° (Ayoub *et al.*, 1998) as shown in Table 1.

The Tables 2, 3 and 4 shows the data of isolates for 45 cases in both genders at different ages infected with the mouth, urinary tract infections while the samples also show the women infected with vaginal infections in Mosul district and its suburbs. The isolates were identified through microscopic testing and it has been observed that most of the pathogens belonged to candida genus as shown in Figure 1.

The species were identified through the medium (chrom candida agar) and the following isolants were found in the medium: *C. albicans* in green colour, *C. parapsilosis* in purple colour, and *C. tropicalis* in blue colour as shown in Figure 2 (Baumgartner *et al.*, 1996; Pfaller *et al.*, 2007b; Ellis *et al.*, 2007).

Then, the isolates were identified using Vitek system and their frequency for all the cases was observed as shown in the Tables 2, 3 and 4. The frequency can



Figure 1: *Candida spp.*

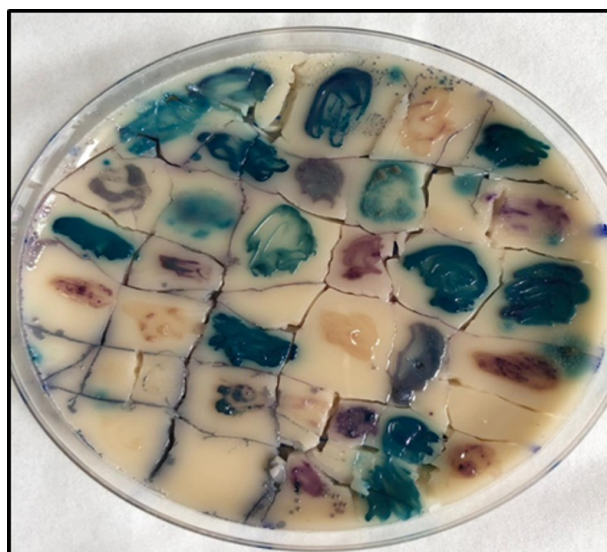


Figure 2: Colonies *Candida spp.* changed its colour on chrom agar candida medium

be observed in Table 2 containing age, gender, residents and the diagnosis of the patients with mouth infections. The regions lying between AL Harmat and Garage Al Shimal in Mosul district showed a difference in the frequency of infection. It has also been observed that the patients' ages varied from 18 years to 60 years and the most frequent ages were 26, 28, and 35 years. The affliction among the males was found to be more than females. It is obvious that the most frequent yeasts observed were *C. parapsilosis* and *C. tropicalis* which caused mouth infections in the Mosul region. The following yeasts such as *C. albicans*, *C. kefir*, and *Rhodotorula glutinis* took the next positions, respectively. These results support the study findings of (Al-Kaaby *et al.*, 2016) in which



**Table 1: Spectral data of flavonoids (Quercertin).**

	NMR( $\delta$ ppm) ( $d_6$ DMSO)	IR ( $\nu$ $\text{cm}^{-1}$ ).KBr			U.V ( $\lambda_{max}$ , nm) (MeOH)
		C=C	CO	OH	
Quercertin* (21)	12.6 (s, 1H, 5-OH); 9.6 (br, 4H, 3, 7, 3', 4', (OH); 7.9 (s, 1H, H-2'); 7.8 (d, 1H, J=4Hz, H-6'); 6.9 (d, 1H, J=4Hz, H-5'); 6.4 (d, 1H, J<1Hz, H-8); 6.2 (d, 1H, J<1Hz, H-6).	1600	1670	3400	256, 300 and 372

\*m. p 312-315, yellow ppt lit m. p(313-4°C).

**Table 2: Age, Genus, region and the diagnosis of patients with mouth infections.**

Age	Genus	Region	Diagnosis		S.No
			Laboratory diagnosis	Diagnosis of Vitek	
35	Male	Al Harmat	<i>Candida sp.</i>	<i>Candida kefyr</i>	1
44	Male	Al Saddeiq	Ø	-	2
50	Male	Al Tahreer	<i>C.parapsilosis</i> <i>Rhodotorula</i>	+ <i>C.parapsilosis</i> <i>Rhodotorula glutinis</i>	3
30	Male	Adaan	Ø	<i>C. albicans</i>	4
23	Female	Garage Al Shemial	<i>C. tropicalis</i>	-	5
23	Male	Shiqaq alkadra	Yeast	Yeast not Candida	6
26	Female	Al Omawy	<i>C. tropicalis</i>	-	7
60	Female	Garage Al Shemial	Yeast	not Candida	8
28	Male	Garage Al Shemial	<i>C. albicans</i>	-	9
20	Mmale	Al Mohandeseen	<i>C. albicans</i>	<i>C. albicans</i>	10
26	Female	Al Noor	<i>C. tropicalis</i>	-	11
55	Male	Gogjaly	<i>C.parapsilosis</i>	-	12
28	Male	Summer	<i>C. parapsilosis</i>	-	13
35	Female	Al Jaza'aer	<i>C. parapsilosis</i>	-	14
18	Male	Garage Al Shemial	<i>C. tropicalis</i>	-	15

the semi-yeast *Candida spp.* was isolated as %10.4 from teeth and gum surfaces of patients of both genders at different ages.

Table 3 shows the urinary tract infections among the patients with information such as age, gender and residents. The patients identified with urinary tract infections reveal the fact that they were from different regions and the yeasts were isolated between Bahzani and Omar Kapchee regions. There was variations in the age range between 12 years and 60 years and the most frequent ones were 21, 25, 40 and 60 years. The table also reveals that the infection is high among females than the males. It is observed that the most common yeasts that cause urine tract infections in Mosul are *C. tropicalis*, *C. parapsilosis* and *C. albicans* (less frequent one).

These results support the findings of (Pfaller et al., 2007a) in which *Candida spp.* was found to be the causative agent in urinary tract infections.

With regards to the vaginal infections among women, Table 4 shows the age, and regions. The diagnosis reveals the fact that the regions were different from 17 Tammuz Region to Awainat Region. The age range of women varied between 19 years and 42 years whereas the most frequent ones were 29, 25 and 42 years. It is observed that *C. albicans* yeast was the common causal agent for vaginal infections in Mosul followed by *C.parapsilosis* yeast (less frequent), *C. triopicalis* and *C. stellatoide* yeast respectively.

These results are in agreement with the study findings of (Habib et al., 2007) in which the authors

**Table 3: Age, genus, regions, and the diagnosis of the patients infected with urinary tract infections .**

Age	Genus	Region	Diagnosis		S.No
			Laboratory diagnosis	Diagnosis of Vitek	
60	Female	Omar kapchee	<i>C. albicans</i>	<i>C. albicans</i>	1
45	Male	Meraky Bashiqa	<i>C. parasilosis</i>	–	2
20	Female	Bashiqa	<i>C. parasilosis</i>	–	3
21	Female	Bahzani	<i>C. tropicali</i>	–	4
12	Male	Bashiqa	<i>C. parasilosis</i>	–	5
46	Male	Bashiqa	Ø	–	6
40	Female	Al Omawy	<i>C. tropicalis</i>	–	7
60	Female	Omar kapchee	<i>C. albicans</i>	<i>C. albicans</i>	8
14	Male	Daramish	<i>C. albicans</i>	<i>C. albicans</i>	9
25	Female	Fadhliah	Ø	–	10
22	Female	Bashiqa	<i>C. tropicalis</i>	–	11
25	Male	Bahzani	Ø	–	12
52	Female	Bahzani	<i>C. tropicalis</i>	–	13
21	Female	Sumakiah	Ø	–	14
40	Female	Bahzani	Yeast	Not Candida	15

**Table 4: Age, region, and the diagnosis of vaginal infections among women.**

Age	Region	Diagnosis		S.No
		Laboratory diagnosis	Diagnosis of Vitek	
25	17 Tammuz	<i>C. stellatoidee</i>	–	1
35	Awainat	Yeast	–	2
20	Zummar	Yeast	Unidentified <i>not candida</i>	3
23	Thaljah cs	<i>C. parasilosis</i>	<i>C. parasilosis</i>	4
37	Alsokar	<i>C. parasilosis</i>	<i>C. parasilosis</i>	5
26	Hamam Aleel	<i>C. albicans</i>	Unidentified Organism	6
42	Rabia	<i>C. parasilosis</i>	<i>C. parasilosis</i>	7
29	Al Risalah	<i>C. albicans</i>	–	8
42	Mosul Jadeedah	<i>C. albicans</i>	–	9
40	Badush	<i>C. albicans</i>	–	10
25	Rabia	<i>C. albicans</i>	–	11
19	Al Yarmook	Yeast	–	12
29	Mosul Jadeedah	<i>C. albicans</i>	–	13
24	Al Harmaat	<i>C. albicans</i>	–	14
25	Awainat	<i>C. albicans</i>	–	15

**Table 5: Biochemical tests of the isolated fungi identified through Vitek system.**

<i>Rhodotorula glutinis</i>	<i>C.kefyr</i>	<i>C.parasilosis</i>	<i>Candida albicans</i>		
-	-	-	-	LysA	1
+	+	+	-	TyrA	2
+	+	+	+	dGLUa	3
-	+	-	-	dRAFa	4
-	-	-	-	IRHAa	5
-	-	+	+	dTURa	6
-	+	+	+	IGLTa	7
-	+	+	+	IPROa	8
(+)	(+)	(+)	(+)	IMLTa	9
+	-	-	-	BNAG	10
-	+	-	-	LACa	11
-	-	-	+	NAGA1	12
-	+	+	+	XLTa	13
+	-	+	+	dTREa	14
-	-	+	+	dXYLa	15
-	-	+	+	2KGa	16
+	+	+	+	LeuA	17
-	-	-	-	ARBa	18
-	-	+	+	MAdGa	19
+	+	+	+	dMNEa	20
-	+	+	+	dSORa	21
-	-	-	-	NO3a	22
-	+	-	+	LATa	23
-	-	-	+	NAGa	24
-	-	+	+	ARG	25
-	+	-	-	AMYa	26
-	-	-	-	dCELa	27
-	-	-	-	dMELa	28
+	+	+	+	SACa	29
-	-	+	-	IARAa	30
+	+	+	+	ACEa	31
-	-	+	+	dGNTa	32
-	-	-	-	ERYa	33
-	+	+	+	dGALa	34
-	-	-	-	GGT	35
-	-	-	-	dMLZa	36
-	-	-	-	URE	37
-	-	-	-	dGATa	38
-	+	-	+	CITa	39
-	-	+	+	GLYLa	40
-	-	-	-	GENa	41
+	-	+	+	dMALa	42
-	-	-	-	ISBEa	43
+	-	+	+	AGLU	44
-	-	-	-	ESC	45
-	-	+	-	GRTas	46

**Table 6: Effect of quercetin with different concentrations (2, 3, 4) mg/ml along with DMSO and antifungal Nystatin, Clotrimazole .**

Clotrimazole 30 mg/ml	Nystatin 100 Iu/ml	Stander DMSO	Quercetin substans 4 mg/ml	Quercetin substans 3 mg/ml	Quercetin substans 2 mg/ml	<i>Candida spp.</i>
1.9	2.8	F	2.1	1.5	1.1	<i>C.albicans</i>
1	2.3	F	2	1.6	1	<i>C. parapsilosis</i>
1.5	1.8	F	1.3	0.7	0.4	<i>C.tropicalis</i>

**Figure 3: Effect of quercetin of different concentrations (2, 3, 4) mg/ml along with DMSO, antifungal Nystatin and Clotrimazole**

noted that the vaginal infections were mostly caused by candida spp. Yeast. Henceforth, it is noticed that the yeasts have a great role in mouth, urinary tract and vaginal infections. They have an important relationship with natural flora that creates an environmental imbalance in the human body (Al-Hadithi et al., 2007). In other words, the environment plays a vital role in infection and the progression of disease in the isolant. Yeasts are the main causative agents of infection that differ in their capability of causing the disease from one type to another. The capability of the island in developing diseases also differs from one type to another.

Further, the position of affliction also has an important role in disease progression. It has been observed that most of the infections are dependent on the region and age (van den Braak et al., 2001; Badr and Abaas, 2017). This observation is supported by the study findings of (Habib et al., 2015) in which the author inferred that candida infection is not confined to a specific geographical location. As per the Table 5, biochemical tests of both isolated and identified fungi were accomplished by Vitek system as follows,

The tests results for different species such as *C.albicans*, *C.parapsilosis*, *C.kefyr* and *Rhodotorula glutinis*, were arrived at, and each fungus has been found among the materials used in the above test. But IMLTa, dMNEa and SACa were observed to be positive in the isolated and diagnosed yeasts such as *C.albicans*, *C.parapsilosis*, *C.kefiy* and *Rhodotorula glutinis*.

With regards to the assessment of effect of quercetin isolated from *Ammi majus*, (Ayoub et al., 1998) concentrations (2,3,4 mg./ml), it can be observed that the inhibition (A) got increased with increase in the concentration of the inhibited material. It inhibited 2.1 cm at a concentration of 4 mg /ml in the fungus *C.albicans*. Further, the inhibition was measured at 2 cm when the concentration was 4 mg/ml for the fungus *C. parapsilosis* and 1.3 cm for *C. tropicalis* fungus of the similar concentration. There was no significant effect of stander DMSO on the previous yeasts whereas the antifungal Nystatin exhibited high inhibition rate (2.8cm, 2.3 cm. and 1.8 cm.) for the concentration (1001U/ml) in both *C. parapsilosis* and *C.albicans* respectively. Meanwhile, the antifungal Clotrimazole, at a concentration of 30.mg/ml inhibited the yeasts and measured at 1.9 cm for *C. albicans* fungus, 1 cm for *C.parapsilosis* fungus and 1.5 cm for *C. tropical* as shown in the and Figure 3.

Henceforth, it can be inferred that quercetin has inhibitory effect on yeasts. This inference is supported by the findings of (Al-Sanafi, 2013) in which the quercetin plant consists of second oils with 18% and coumarin 0.5- 0.2%. It also consists of quercetin in which these substances are considered effective in healing psoriasis. It is germicidal in nature and act against fungi, particularly, *Tinia versicolors*. They are also effective in treating skin cancer (Al-Janabi et al., 2017; Kasper et al., 2015) mentioned that Clotrimazole has a fatal effect on the cells of *C.albicans* fungus in its growth and formation stages.

The antifungal substance, Nystatin exhibits strong inhibition upon *Candida spp.* especially *C.albicans* (Yahya and Altae, 2013) Nystatin consists of polyene which helps in the prevention of

membranes that form *Candida* species. It further inhibits its growth and impact it by replacing the ergosterol in the membrane of yeasts. As a result, it stops the growth of yeast membrane, change the osmosis, free transportation, ionic exchange and consequently death of the yeast cell (Carrillo-Munoz et al., 2004; Zotchev, 2003; Carrillo-Muñoz et al., 2001).

## CONCLUSIONS

The significance in this study is that,

1. The quercetin has an inhibitive effect on these yeasts. Thus, it is necessary to explore the substance in detail so as to use it as an antifungal medicine in the diseases caused by *Candida* sp. Quercetin can be used as a drug or as mouth wash too.
2. It has been noticed through research studies that the inhibition by Nystatin is superior to inhibition by Clotrimazole in yeasts.

## Source of funding

Self Funding.

## Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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