Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 7 (Special Issue): 118-128 (2023) (http://www.wildlife-biodiversity.com/)

Research Article

Pharmacological value of *Agrocybe praecox* extract as a potential antifungal

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Received: 01 October 2023 / Revised: 19 November 2023 / Accepted: 25 November 2023/ Published online: 27 November 2023. Ministry of Sciences, Research, and Technology, Arak University, Iran.
How to cite: Abed, R.M., Mezher, M.A. (2023). Pharmacological value of Agrocybe praecox extract as a potential antifungal, Journal of Wildlife and Biodiversity, 7(Special Issue), 118-128. **DOI**: https://doi.org/10.5281/zenodo.10211330

Abstract

Wild mushrooms are considered one of the important sources of many bioactive compounds with different therapeutic properties. *Agrocybe praecox* is one of the mushrooms found in Iraqi forest habitats. In the present investigation, the presence of some phenolic compounds such as ferulic, gallic acid, quercetin, rutin, and falalic acid was detected in *A. praecox* extracts. To reveal the potential antifungal ability of *A. praecox* extracts for some yeasts isolated from sputum of patients with asthma and allergic bronchitis which were *Candida. albicans , C. lipolytica, C. tropicalis, C. krusei, C. lusittaniae, Cryptococcus laurentii* and *C. gattii*, ethanolic extract gave the highest results in inhibiting growth, and the inhibition rate increased with increasing concentration to reach the highest level in concentration.

Keywords: *Agrocybe praecox*, Candida, asthma, allergy, Cryptococcus

Introduction

Agrocybe praecox may colonize and thrive in leaf litter and higher soil wood chips with bark mulch (Hildén et al.,2014), and is a kind of edible fungus that is high in potassium, low in sodium, low in fat, and high in protein. (Fang et al.,2020), furthermore a fungus with anti-cancer (Shen et al., 2009) and antibacterial/fungal qualities (Suay et al., 2000). *A. praecox* is a common kind of fungus

found in North America, Europe, North Africa, and several Asian countries, including South Korea, Japan, New Zealand, Argentina, Mongolia, Siberia, and Sri Lanka. The first records of A. praecox were from Northern Iraq. by Suliaman et al. (2017) Additionally, Al-Khesraji (2018) provided the first report on this species from two Salahadin Governorate districts, Dujail and Tikrit.

Due to the tiny size of fungal spores, which may less easily enter the alveoli, fungal infections mostly affect the respiratory system, making the lungs and most other organs of the body vulnerable to infection. Chandler and colleagues, 1994). After bacterial and viral diseases, fungal infections that impact the respiratory system rank third (Wright et al., 1999). When examining the fungal origins of allergy and asthma, the majority of research reveal that patients have filamentous fungus with them (Al-Bader et al.,2013; Adeeb et al., 2021). Due to its ability to elicit allergic reactions, *Candida albicans* is involved in the pathophysiology of allergic disorders (Khosravi et al.,2009; Lagree et al., 2021). One may consider *Candida boidinii* to be the second (Crameri et al., 1998). key allergen caused by this fungus Nonetheless, there is ongoing debate over the therapeutic relevance of *Candida albicans* as an allergen responsible for allergic disorders. Studies showing the prevalence of *Candida* and other yeasts in asthma and allergy sufferers are still lacking, nevertheless (Buslau et al., 1990). Nevertheless, no research has been done on *Agrocybe praecox's* antifungal activity against yeasts. As a result, The purpose of this investigation was to find out how *well A. praecox* extract inhibited yeasts that were isolated from asthmatic and allergy sufferers.

Material and methods

The collection of Agrossip praecus fruiting body

In November 2019, fruiting bodies (basidiocarps) of *A. praecox* were collected from willow trees situated in certain regions of the Salah al-Din Governorate in Iraq and Dhuluiya. The samples were taken to the Department of Biology, Faculty of Science, Tikrit University's Mycology Laboratory to examine the macroscopic characteristics of the fruiting body, such as its dimensions, color, shape, and textures. The fertile layer's color and form were also examined at various stages of growth. The microscopic characteristics also included the kind of basidium, the size and form of the basidiospores, the germination holes' shape, and their diameters. All of these characteristics have been examined in accordance to (Mohanty et al.,2011; Keypour et al.,2014;Reilly,2016).

Figure 1. *Agrocybe praecox* : a, in its native environment, *Agrocybe praecox* is found on willow trees. b, The fungus's fruiting body, showing a portion along its length. c, spores.

phenolic compounds in Agrocybe praecox

Using a standard Folin-Ciocalteu reagent, the total quantity of phenolic components in the ethanolic extract was measured by HPLC based on (Zare et al., 2014).

Preparing an Agrocybe praecox extract

Fruiting bodies were made into a fine powder of 40 mesh after being dried. Three different kinds of organic solvents methanol, acetone, and ethanol were used to make the extract. The five gram samples were extracted twice using 100 millilitres of solvent 70:30 (in water) and stirring at 25 degrees Celsius and 130 revolutions per minute for three hours. The extraction process was then filtered using the Whatman N1 paper. Additional extractions failed to boost the yield. The mixed filtrates were weighted, freeze-dried, and concentrated using a rotary vacuum evaporator set at 40°C to remove the solvent. Before being used, the freeze-dried powders were kept in a freezer. (Carvajal et al., 2012) .

Collecting yeast sample

From March 2021 to July 2022, 100 sputum samples in triplicate were taken from individuals suffering from allergies and asthma. Every sputum specimen was processed using 10% KOH mount, culture, and Gram staining. The species of yeast were routinely recovered a pure culture from expectorate sputum samples that were taken two days in a row in the early morning. (Koneman and Roberts,1985). The sputum specimens were mounted in KOH and stained with Gram stain for microscopy. India ink was not made until capsulated budding yeast cells were evident on Gram staining.. Specimens were incubated at 25°C and 37°C after being infected in duplicate with chloramphenicol (16 mg/ml) on Sabouraud's Dextrose Agar (SDA). For 14 days, the cultures were checked every other day for development before being discarded as negative. The macrometric parameters of growth rate, colony form, texture, and surface pigmentation were

used to identify fungal growth. and the diagnosis was confirmed using the Vitek compact2 system diagnosis, according to the manufacturer's instructions. By using the germ tube test, chrom candida agar, and chlamydospore development on cornmeal agar, several species of *Candida* were identified. Gram staining was done when mucoid yeast-like growth was seen on SDA. Christiansen's urea agar stained with capsulated budding yeast cells and *Cryptococcus* Differential Agar was injected to detect *Cryptococcus* (Kurtzman et al.,2011) Additionally, Vitek Compact2 System Diagnosis was used to corroborate the diagnosis. (Jagdish, 2009) .

Yeast inoculum preparation

The inoculum was made from a 24-hour SDA medium-grown culture that contained 105 cells per millilitre. The yeast culture was prepared according to Mann and Markham's (1998) technique, in which, after growing for 24 hours, a part of the developing culture was transferred to a solid saproid medium, and the test tube containing 10 ml of liquid saproid media was then incubated for 16–18 hours at 30°C.

Assessing the efficacy of extracts from Agrocybe praecox

The three A. praecox fungal extracts were made in four concentrations: 25, 50, 75, and 100%. The Perez et al. (1990) technique was used to pour 25 millilitres of nutrient agar onto each plate in order to examine the impact of various extract concentrations on yeast growth. After comparing the inoculum's 0.1 ml using a sterile spreader from the yeast culture, which had 1.5x810) cells per millilitre, to a standard turbidity constant solution, the plates were allowed to dry at room temperature. A sterile cork borer was used to drill a 6 mm hole in the culture media. Then, using a sterile fine pipette, 0.2 ml of the produced graded quantities of the fungal extract was added. After three iterations of each treatment, the dishes were placed in the incubator and left there for 48 hours at 37 C°. The diameter of the inhibition zone—which is the area free of yeast growth—was measured to assess the effectiveness of each extract concentration. The disc's surrounding inhibition zones were also measured to interpret the results. (Amade et al., 1994).

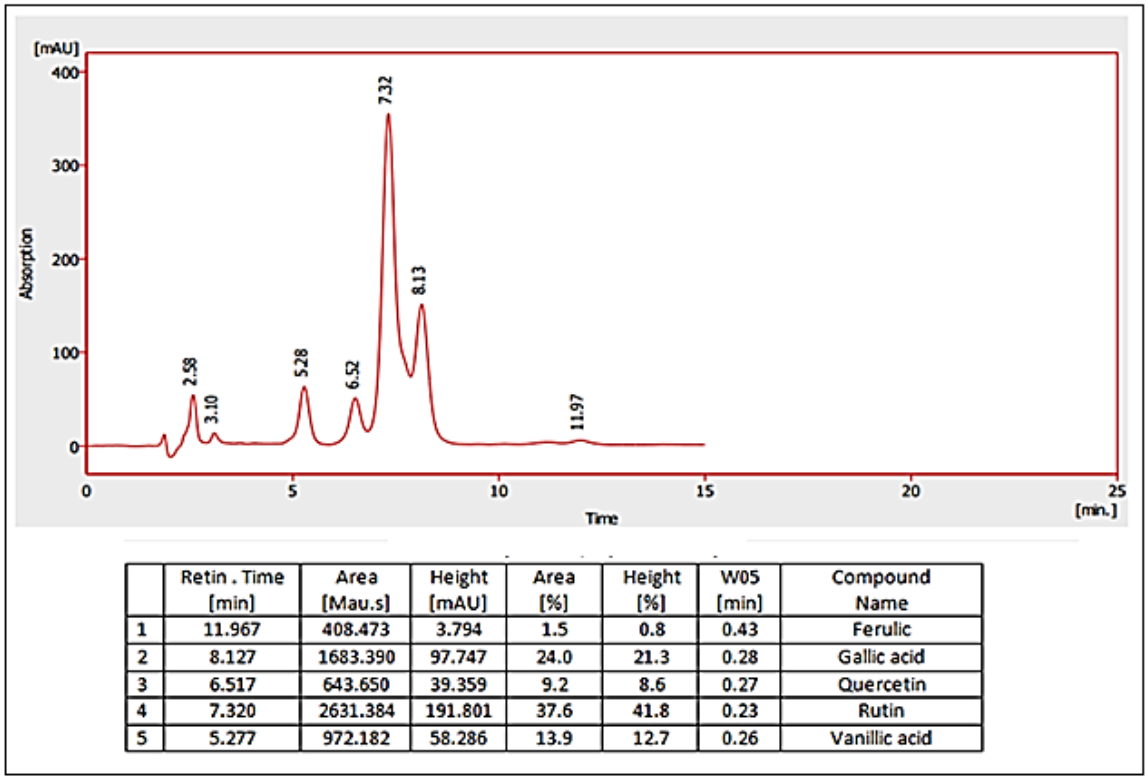
Statistical analysis

The means and standard deviations of the data were reported, and any statistically significant differences between the means were assessed using the Tukey test and unidirectional test variance (ANOVA). Mean differences were deemed significant at the 5% level ($P < 0.05$).

Results and discussion

Chemical components of Agrocybe praecox identified

By using HPLC/UV analysis, the extracts revealed the presence of five phenolics: rutin, vallic acid, gallic acid, quercetin, and ferulic acid (Fig. 2). Secondary metabolites called phenols are often present in fungus, plants, and other microbes. They have been shown to have a variety of biological effects, including antioxidant activity, therefore identifying the primary phenolic compounds in the fungus is crucial to understanding its nutritional makeup and functional characteristics. (Dimitrios, 2006; Kim et al., 2008). Fang et al. (2020) They verified that *Agrocybe praecox* had high levels of aspartic acid, glutamic acid, and methionine. Its essential amino acid concentration reached 44.85%. *Agrocybe praecox* is one kind of edible fungus that has a high protein, low fat, high potassium, and low salt content.



	Retin . Time [min]	Area [Mau.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11.967	408.473	3.794	1.5	0.8	0.43	Ferulic
2	8.127	1683.390	97.747	24.0	21.3	0.28	Gallic acid
3	6.517	643.650	39.359	9.2	8.6	0.27	Quercetin
4	7.320	2631.384	191.801	37.6	41.8	0.23	Rutin
5	5.277	972.182	58.286	13.9	12.7	0.26	Vanillic acid

Figure 2. Phenols content in *Agrocybe praecox* fruiting body by HPLC

Patients with allergies and asthma have yeasts in their sputum.

The number of yeast isolates obtained from 100 sputum samples from allergy and asthma patients is shown in Table 1. Of these isolates, 85 belonged to the genera Candida and Cryptococcus. Candida is a genus that includes five species: *Candida albicans*, *Candida lipolytica*, *Candida tropicalis*, *Candida krusei*, and *Candida lusittaniae*. The number of isolates for each species was 13, 23, 16, 7, 2, and so on. There have been 18 isolates of each of the two *Cryptococcus* species

C. laurentii and *C. gattii* respectively. Out of all the isolates, *C. lipolytica* had the highest proportion (27.06%), followed by *C. laurentii* (21.18%).

The genus *Candida* contains the yeasts that are most frequently discovered in bronchiectasis, with *Candida albicans* being the most prevalent species. Recent data analysis from normal microbiological cultures indicates in several Spanish hospitals, 45.2% of the patients studied had *C. albicans* isolated (Máiz et al., 2015). Kandati et al. (2016) found that the most common fungal pathogen was *Candida* sp. (16.45%), followed by *Aspergillus* sp. (12.83%) and *Cryptococcus* sp. (1.97%). Additionally, a different study discovered that the primary causative fungus causing the symptoms of lung illnesses is *C. albicans* (Talle et al., 2017). That aligns with the findings of the present investigation.

Table 1. Taxa of yeasts from asthma and allergy patients' sputum

Yeasts	Number	%
Candida albicans	13	15.29
C. lipolytica	23	27.06
C. tropicalis	16	18.82
C. krusei	7	8.24
C. lusittaniae	2	2.35
Cryptococcus laurentii	18	21.18
C. gattii	6	7.06
Total	85	100

Percentages of related yeast infections based on research groups

With 51.76% of males and 48.24% of females infected, Table 2 shows that the percentage of males with yeast infection was higher than that of females. Fungal infection and sex are unrelated, according to statistical research that showed no significant correlation. This aligns with previous studies. (Taura et al., 2014; Talle et al., 2017). According to Table 3's findings, the infection rate that was highest was The percentage for the age group (51–60) was 31.76%, while the percentages for the age groups (41–50, 31–40, 21–30, and 11–20) were 28.24, 16.47, 14.12, and 9.41%), respectively. An age-related association was found in the age distribution statistical analysis, and it was significant ($P<0.05$). This bolsters the findings of (Aluyi et al., 2010) contradicting, however, research by Talle et al. (2017), which discovered that young persons had a greater incidence of yeast infection while those between the ages of 51 and 60 had a lower frequency.

Table 2. The incidence of yeast infection by gender.

Gender	Number	%

Male	44±6.32	51.76
Female	41±3.48	48.24
Total	85	100
P-value = 0.855 (≥ 0.05)		

Table 3. The prevalence of yeast infections by age group.

	Age groupings (years)	N.	%
Study groups	11-20	8±1.24	9.41
	21-30	12±1.91	14.12
	31-40	14±1.83	16.47
	41-50	24±3.61	28.24
	51-60	27±3.34	31.76
	Total	85	100
P-value = 0.017 (≤ 0.05)			

Agrocybe praecox extracts' efficacy against Cryptococcus and Candida species

The main objective of this investigation was to assess and contrast how different extraction methods affected the growth of pathogenic yeasts that were isolated from asthma and allergy patients. According to the results, the fruiting body of *Agrocybe praecox* ethanolic extract inhibited growth the most, and the rate of inhibition rose as concentration increased, reaching a maximum of 100%. At different concentrations, every extract demonstrated a notable impact in preventing the development of yeasts. Results also show that the yeasts' responses to the three Agrocybe praecox extract treatments did not differ significantly from one another. as seen in Table 4. This result is in agreement with a study carried out by Suay et al. (2000), which showed that 45% of the fungal isolates from Basidiomycetes 109 species out of 204 had extracts that demonstrated their capacity to create physiologically active chemicals. The present study's findings, which indicated that the fungus includes some phenolic chemicals with anti-fungal characteristics, support this.

Table 4. *Agrocybe praecox* extracts' efficacy against *Cryptococcus* and *Candida* species

Ethanolic extract					
Yeasts	**0%**	**25 %**	**50%**	**75%**	**100 %**

Candida albicans	0	3.55±٣,٠٤	5.63±٢,٦٢	15.01±٦,١١	20.47±٧,٨٨
C.lipolytica	0	3.28±٢,٤٦	5.27±٤,٨٣	14.27±٤,٤٠	26.73±١٠,٧٧
C. tropicalis	0	3.25±4.87	5.77±4.12	14.65±6.32	22.17±3.49
C. krusei	0	1.78±2.88	3.72±2.38	15.8٤±6.79	24.58±7.77
C. lusittaniae	0	2.42±2.26	4.75±5.01	7.89±7.44	9.81±8.62
Cryptococcus laurentii	0	1.25±1.86	7.32±6.67	16.37±9.60	28.95±5.88
C. gattii	0	0.95±1.72	1.54±1.86	9.55±6.16	25.11±11.50
Acetone extract					
Yeasts	**0%**	**25 %**	**50%**	**75%**	**100%**
Candida albicans	0	3.12±٥.89	4.54±4.53	6.42±6.40	10.98±9.80
C.lipolytica	0	5.70±5.08	9.78±3.43	14.58±4.35	17.54±6.55
C. tropicalis	0	1.14±3.63	1.04±2.40	2.30±3.71	8.62±9.36
C. krusei	0	6.62±4.12	9.24±6.78	12.77±6.97	19.35±8.32
C. lusittaniae	0	0.72±1.71	1.72±1.39	1.90±2.5	9.09±4.13
Cryptococcus laurentii	0	4.90±5.90	7.27±7.55	9.81±9.01	12.68±10.30
C. gattii	0	0.00	0.00	0.00	0.00
Methanolic extract					
Yeasts	**0%**	**25 %**	**50%**	**75%**	**100%**
Candida albicans	0	0.23±1.83	0.69±2.61	0.60±2.38	2.76±2.22
C.lipolytica	0	0.20±1.74	0.72±3.76	1.07±3.07	2.11±3.11
C. tropicalis	0	0.56±2.41	0.50±2.36	1.31±2.05	2.24±4.08
C. krusei	0	0.94±0.33	1.55±0.59	2.05±1.45	8.52±3.31
C. lusittaniae	0	2.51±1.73	1.95±1.33	3.24±4.09	4.25±4.61
Cryptococcus laurentii	0	1.02±1.72	1.70±3.49	2.22±4.89	3.41±8.03
C. gattii	0	3.03±2.09	5.21±3.38	10.38±5.72	15.95±9.21
Pvalue	**Extraction = 0.000 (≤ 0.05)**				
	Concentrations = 0.000 (≤ 0.05)				
	Yeast = 0.721 (≥ 0.05)				

Conclusions

According to the study, a significant proportion of patients with allergies and asthma also had concurrent yeast infections, particularly with Candida and Cryptococcus species. It was shown that men were more likely than women to get yeast infections, and that the age range of 41 to 50 had

the greatest overall incidence of yeast infections.. The fungus *Agrocybe praecox* has demonstrated remarkable efficacy in decreasing the pace of yeast development, rendering it a viable tool for mitigating the prevalence of yeasts linked to individuals suffering from asthma and allergies.

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