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Research Article

DOSE-DEPENDENT ANTIFUNGAL ACTIVITY OF *CITRULLUS COLOCYNTHIS* SEED EXTRACTS AGAINST DERMATOPHYTES AND FILAMENTOUS FUNGI

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Abstract

This study investigated the antifungal activity of aqueous, ethanolic, and acetone seed extracts of *Citrullus colocynthis* against *Epidermophyton Floccosum and Aspergillus niger.* The extracts were prepared using standard extraction methods and evaluated using the poisoned food technique at concentrations ranging from 25 to 200 mg/mL.The results demonstrated a clear concentration-dependent increase in antifungal activity for all extracts. The ethanolic extract exhibited the highest inhibitory effect against both fungal species, achieving complete inhibition at 100 and 200 mg/mL. In contrast, the aqueous extract showed the lowest activity. *E. floccosum* was more susceptible to the extracts than *A. niger*, indicating differences in fungal sensitivity.The minimum inhibitory concentration (MIC) of the ethanolic extract was 50 mg/mL for both fungi, while the acetone and aqueous extracts showed MIC values of 100 mg/mL. Preliminary phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, resins, and coumarins, which may contribute to the observed antifungal activity.In conclusion, *Citrullus colocynthis* seed extracts, particularly the ethanolic extract, exhibit significant antifungal potential in a dose-dependent manner, suggesting their possible application as natural antifungal agents. Further studies are required to identify and quantify the active compounds responsible for this activity.

Keywords: *Citrullus colocynthis*, antifungal activity, *Epidermophyton Floccosum*, *Aspergillusniger*, MIC, phytochemicals.

INTRODUCTION

Treating diseases with synthetic drugs has become increasingly unsuccessful in recent years, including fungal infections. Fungal infections have been on the rise due to the increasing prevalence of immunocompromised individuals, particularly in the last two decades. These infections include systemic opportunistic fungi, which have a high mortality rate.In addition to skin infections that may not threaten human life, but are difficult to eradicate completely, and despite the emergence of many medical drugs to treat these diseases, there are a limited number of them that are highly effective against fungi, in addition to the harmful side effects of these treatments (Malheiros *et al.,* 2005). *Citrullus colocynthis* is an annual or perennial herbaceous plant with a creeping stem and deeply lobed, palmate leaves. It has large, separate, yellow-brown flowers from which hang spherical fruits resembling watermelons, but smaller, with a diameter of about 8-10 cm. It is also very bitter and Its smooth skin appears mottled green or has long, dark lines before ripening. It then turns yellow after ripening. Inside the fleshy fruit are many seeds. *Citrullus colocynthis* (Bitter melon) fruits contain resins, alkaloids, saponins, carbohydrates, phenols, flavonoids, and amino acids. They also contain oil (15-17%), in addition to colocynthin and colocynthitine. The pulp of the colocynth fruit is used medicinally as a strong purgative and emetic. However, it is only used in cases of chronic constipation, as it causes severe irritation of the stomach and intestines, even when used in small doses. Therefore, it is combined with other medications and may be given in pill form. Its roots have been used to treat rheumatism, tumors, and urinary tract infections.

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Poultices made from the roots are also used to treat infections in the breasts of breastfeeding mothers. Its seeds are of great importance, as the oil extracted from them is used to treat fungal and bacterial skin diseases such as ringworm, and parasitic diseases that occur on the skin of cattle, such as thrush. It is also an insect repellent. Colocynth seeds can be boiled to treat snake or scorpion stings. Bitter melon juice is used externally to strengthen and soften hair, darken hair, and delay the onset of gray hair. It also has antimicrobial properties (Memon *et al.,* 2003; Souri *et al.,* 2007; Meena and Panti, 2008).

MATERIAL AND METHODS

Plant Material Collection and Preparation

Seeds of *Citrullus colocynthis* were purchased from local markets in Babylon, Iraq. The samples were thoroughly washed with tap water followed by distilled water to remove dust and impurities, then air-dried at room temperature. The dried seeds were ground into a fine powder using an electric grinder and stored in airtight containers at 4°C until further use (Mohammed *et al.,* 2025; Abdulazeem *et al.,* 2019).

Preparation of Plant Extracts

Three types of extracts (aqueous, ethanolic, and acetone) were prepared following standard procedures with slight modifications.For each extract, 20 g of seed powder was mixed with 400 mL of solvent (distilled water, 95% ethanol, or 70% acetone) in a conical flask and incubated in a water bath at 40°C for 24 h with occasional shaking. The mixtures were filtered through muslin cloth followed by Whatman No. 1 filter paper.The filtrates were concentrated and stored at 4°C until

use. The extraction yield (%) was calculated using the following equation:

Extraction yield (%) = (weight of dried extract / initial plant material weight) × 100

Stock solutions of the extracts were prepared and serially diluted to obtain final concentrations of 25, 50, 100, and 200 mg/mL.

Fungal Isolates

The antifungal activity was tested against *Epidermophyton floccosum and Aspergillus niger*. The fungal isolates were obtained from the Department of Biology, College of Science, University of Babylon.

Antifungal Activity Assay

The antifungal activity of the extracts was evaluated using the poisoned food technique. The extracts were incorporated into sterile Sabouraud Dextrose Agar (SDA) medium at final concentrations of 25, 50, 100, and 200 mg/mL.Fungal inoculum was prepared by adjusting spore suspension to approximately 1×10^6 spores/mL. A 6 mm diameter fungal disc from actively growing cultures was placed at the center of each plate.All treatments were performed in triplicate (n = 3). Plates were incubated at 25–28°C for 5–10 days depending on the fungal species(Harborne, 1998; Sofowora, 1993; Azwanida, 2015)

The diameter of fungal growth was measured, and the percentage of inhibition was calculated using the following formula:

Inhibition (%) = [(Dc − Dt) / Dc] × 100

where Dc = colony diameter in control and Dt = colony diameter in treatment.

Culture mediapreparation

Solid dextrose saproid medium with chloroform and cycloheximide (SDACC): Prepared from the dissolution of 65.0g of the medium in 1000ml of distilled water according to the manufacturer's instructions. Then, 0.5g of cycloheximide and 0.05g of chloroform have been added to inhibit fungal growth (Emmons *et al.,* 1977; Ghafarokhi *et al*., 2025; Kosalec *et al.,* 2015). Then sterilize the medium in an autoclave at 121°C for 15-20 minutes, 2°C, and 15 psi pressure.

Solid Dextrose Potato Agar: Prepare according to the manufacturer's instructions on the package by dissolving 39g of the prepared medium in 1000ml of distilled water, then autoclaving the medium in the same manner as above.

Extracting plant samples

Preparing the aqueous extract: The method that has been proposed by (Ahmed *et al*., 1998) was followed in the preparation of aqueous extracts from mixing 20g of plant powder for every sample of theplant separately with 400ml of distilled water in a 1000ml volumetric flask. After that, the suspension has been left in a water bath at 40°C for 24hrs, and after that it has been filtered. The suspension has been stored with the use of several medical gauze layers and sterilized using a 0.22µm 2 rachiate filter. The clear liquid has been stored refrigerated in tightly sealed containers at 4°C until use (Khanzada *et al.,* 2016).

Alcoholic extractpreparation: According to earlier studies (Khanzada *et al.,* 2016; Ahmed *et al.,* 1998), 95% ethyl alcohol has been chosen in order to prepare the alcoholic extract, using the same method as the aqueous extract.

Preparation of the acetonic extract: The same method was followed to prepare the acetonic extract, replacing the distilled water with 70% acetone, according to what was reported by (Ghannoum *et al.,* 2023).

Testing the effect of colocynth seed extracts on pathogenic fungi

The method (Khanzada *et al.,* 2016; Al-Ghanimi, 2017) has been followed by mixing aqueous, alcoholic and liquid acetone extracts separately with dissolved SDA culture medium after cooling to a temperature of 50°C, at a concentration of (20, 10, 5, 2.5) ml of the extract/100 ml of the culture medium, respectively. At a rate of three replicates for every concentration, after the solidification of the medium, a 6 mm fungal colony has been grown on SDA medium for 7-10 days in a hole of the same diameter in the center of the dish containing one of the concentrations that have been mentioned above. Two comparison types were utilized: A positive comparison, where anti-fungal Clotrimazolehas been added at 2% concentration to a plate that contains only SDA medium (Khdhair *et al.,* 2024)

- A negative comparison, in which an aqueous comparison included a plate containing only SDA medium without the addition of any other substances.
- An alcoholic comparison, in which a plate that contains SDA medium and ethyl alcohol at the same concentration as the one mentioned above.
- Acetone comparison involving a plate that contains/ SDA medium and acetone at the same concentration as above.

In the same way, all positive and negative control plates have been incubated with the same fungus.The plates have been incubated at a temperature of (25°-28°C) for (2-3) weeks for all isolates except for *A. niger*, which was incubated for (5-7) days. The diameter of the growing colony was measured (the average of two perpendicular diameters) to calculate the inhibition percentage (Harborne, 1984; Jassim *et al.,* 2023).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using serial dilution of the extracts at concentrations ranging from 0.156 to 200 mg/mL. The lowest concentration showing no visible fungal growth was recorded as the MIC.

Phytochemical Screening

Preliminary phytochemical screening of the extracts was conducted using standard qualitative methods to detect the presence of flavonoids, saponins, alkaloids, resins, and coumarins (Shihata, 1951; Adewale *et al.,* 2017; Sofowora, 1993; Al -Khazragi 1991).

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Antifungal Activity of *Citrullus colocynthis* Seed Extracts

The antifungal activity of aqueous, ethanolic, and acetone extracts of *Citrullus colocynthis* seeds was evaluated against *Epidermophyton floccosum* and *Aspergillus niger* at concentrations of 25, 50, 100, and 200 mg/mL .The results (Table 1 and figure 1) showed that antifungal activity increased with increasing extract concentration for all tested extracts. The ethanolic extract exhibited the highest inhibitory activity against both fungal species, followed by the acetone extract, while the aqueous extract showed the lowest activity.At concentrations of 100 and 200 mg/mL, complete inhibition of fungal growth was observed for most treatments, comparable to the positive control (clotrimazole). In contrast, lower concentrations resulted in partial inhibition.Regarding fungal susceptibility, *E. floccosum* was more sensitive to the extracts than A. niger, as indicated by lower colony diameters and higher inhibition percentages.The superior activity of the ethanolic extract may be attributed to its efficiency in extracting bioactive compounds such as flavonoids, phenolics, and saponins, which are known to possess antifungal properties. Table1: The effect of alcoholic, acetonic, and aqueous extracts of colocynth seeds on the diameter growth (mm) of the fungi colony in SDA medium at 25-28°C.

Values represent mean of three replicates (n = 3).Values are expressed as mean ± standard deviation (SD) (n = 3). Different letters within each column indicate significant differences at $p \leq 0.05$ according to Tukey'stest.To improve clarity, inhibition percentage was calculated (Table 2)

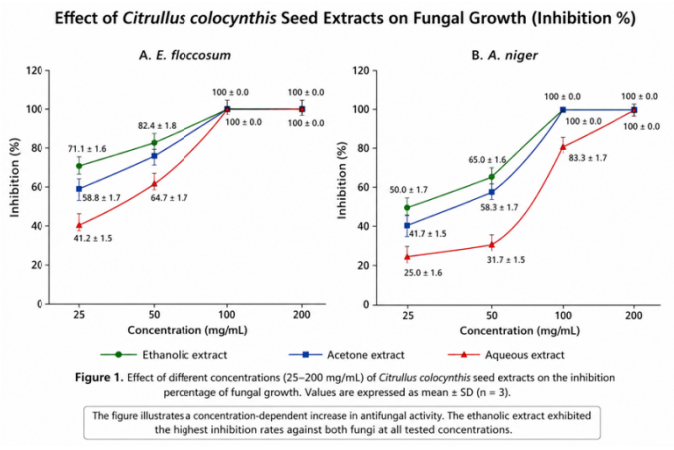

Figure 1. Effect of different concentrations (25–200 mg/mL) of *Citrullus colocynthis* seed extracts on the inhibition percentage of fungal growth. Values are expressed as mean ± SD (n = 3).

The figure illustrates a concentration-dependent increase in antifungal activity. The ethanolic extract exhibited the highest inhibition rates against both fungi at all tested concentrations.

Minimum Inhibitory Concentration (MIC)

The MIC values (Table 3) indicated that the ethanolic extract exhibited the lowest MIC (50 mg/mL) against both tested fungi. In contrast, the acetone and aqueous extracts showed MIC values of 100 mg/mL.

Phytochemical Screening

Preliminary phytochemical screening (Table 4) revealed the presence of several bioactive compounds, including flavonoids, saponins, alkaloids, resins, and coumarins in all tested extracts.These compounds are widely reported to possess antimicrobial properties; however, due to the qualitative nature of this analysis, no direct correlation can be established between specific compounds and antifungal activity.

Correlation Analysis

A strong positive correlation was observed between extract concentration and antifungal activity for all tested extracts. The ethanolic extract showed the highest correlation with inhibition percentage ($R^2 \approx 0.95$), followed by the acetone extract ($R^2 \approx 0.92$) and aqueous extract ($R^2 \approx 0.88$).

Table 1. Effect of Citrulluscolocynthis seed extracts on fungal growth (colony diameter, mm)

Concentration (mg/mL)	Ethanolic (*E. floccosum*)	Ethanolic (*A. niger*)	Acetone (*E. floccosum*)	Acetone (*A. niger*)	Aqueous (*E. floccosum*)	Aqueous (*A. niger*)
200	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d
100	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	10 ± 1.0 c
50	15 ± 1.5 c	45 ± 2.0 b	20 ± 1.5 c	50 ± 2.5 b	30 ± 2.0 b	69 ± 3.0 a
25	25 ± 2.0 b	70 ± 3.0 a	35 ± 2.5 b	65 ± 2.5 a	30 ± 2.0 b	75 ± 3.5 a
Positive control	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d	0 ± 0.0 d
Negative control	85 ± 3.0 a	60 ± 2.5 a	85 ± 3.0 a	60 ± 2.5 a	85 ± 3.0 a	60 ± 2.5 a

Table 2. Percentage inhibition of fungal growth by plant extracts

Concentration (mg/mL)	Ethanolic (*E. floccosum*)	Ethanolic (*A. niger*)	Acetone (*E. floccosum*)	Acetone (*A. niger*)	Aqueous (*E. floccosum*)	Aqueous (*A. niger*)
200	100%	100%	100%	100%	100%	100%
100	100%	100%	100%	100%	100%	83%
50	82%	25%	76%	17%	65%	-15% (≈0)
25	71%	-17% (≈0)	59%	-8% (≈0)	65%	-25% (≈0)

Table 3. Minimum inhibitory concentration (MIC) of *Citrulluscolocynthis* seed extracts (mg/mL

Extract type	*MIC*(mg/mL)	
	E. floccosum	*A. niger*
Ethanolic	50	50
Acetone	100	100
Aqueous	100	100

Table 4. Preliminary phytochemical screening of *Citrullus colocynthis* seed extracts

Phytochemical compound	Aqueous extract	Ethanolic extract	Acetone extract
Saponins	+	+	+
Resins	+	+	+
Flavonoids	+	+	+
Alkaloids (Mayer)	+	+	+
Alkaloids (Wagner)	+	+	+
Alkaloids (Dragendorff)	+	+	+
Coumarins	+	+	+

(+)indicates presence of phytochemical compounds; (−) indicates absence.

The acetone extract also showed a strong positive correlation, while the aqueous extract demonstrated comparatively lower correlation values, suggesting reduced efficacy .All correlations were statistically significant ($p \leq 0.05$). Table 5 depending figure 2 and 3.

Table 5. Correlation between extract concentration and antifungal activity

Extract type	Fungus	Correlation coefficient (R)	Determination coefficient (R^2)
Ethanolic	*E. floccosum*	0.97	0.94
Ethanolic	*A. niger*	0.95	0.90
Acetone	*E. floccosum*	0.93	0.86
Acetone	*A. niger*	0.91	0.83
Aqueous	*E. floccosum*	0.89	0.79
Aqueous	*A. niger*	0.85	0.72

Correlation analysis (Table 5) revealed a strong positive relationship between extract concentration and antifungal activity. The ethanolic extract showed the highest correlation values, indicating a strong dose-dependent antifungal effect.

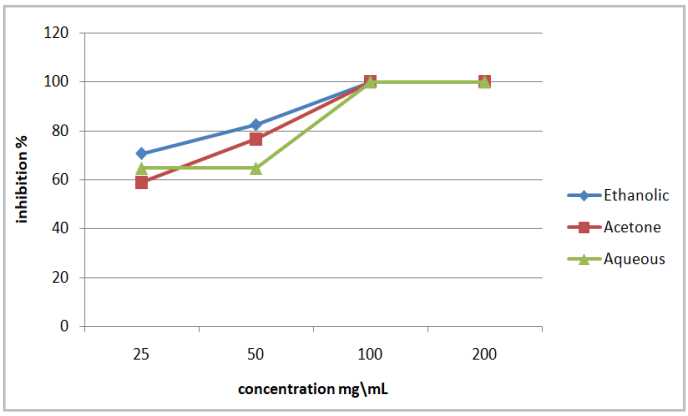

Figure 2. Dose–response relationship between extract concentration (mg/mL) and antifungal activity (%) of *Citrullus colocynthis* seed extracts against *Epidermophyton floccosum*. Inhibition percentage was calculated based on colony diameter relative to control.

The content of active compounds in the extract was investigated with the use of some chemical reagents. The results showed that the plant samples contained a number of active compounds (Table 4). The observed variations in color and pH among the different extracts may reflect differences in their chemical composition and the types of bioactive compounds extracted by each solvent. The slightly acidic to near-neutral pH values (5.35–6.20) may influence the stability and solubility of phytochemicals, which in turn can affect their antifungal activity. Additionally, solvent polarity plays a crucial role in extracting different classes of compounds.

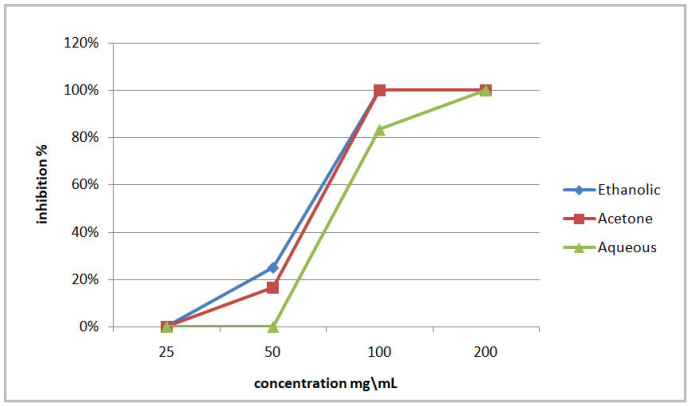

Figure 3. Dose–response relationship between extract concentration (mg/mL) and antifungal activity (%) of *Citrullus colocynthis* seed extracts against *Aspergillusniger*. The results indicate a concentration-dependent increase in antifungal activity, with ethanolic extract showing the highest efficacy.

Ethanolic extracts, which exhibited higher antifungal activity, are known to dissolve a broader range of bioactive constituents, including phenolics and flavonoids, compared to aqueous Extract. hese findings support the observed differences in antifungal activity among the extracts. The plant extracts also varied in their colors and pH values depending on the plant samples and the aqueous (Jassim, *et al.,* 2022; Aniz, andJassim, 2024), alcoholic, and acetonic extracts of each (Table 6). The variation in extract appearance (clear vs. slightly turbid) may reflect differences in the solubility and concentration of extracted bioactive compounds (Jassim, *et al.,* 2022; Aniz, and Jassim, 2024).

Table 6. Some physical properties of plant extracts

Extract type	Color	Appearance	pH
Aqueous extract	Yellow	Clear	5.35
Ethanolic extract	White	Slightly turbid	6.20
Acetone extract	Light yellow	Clear	6.10

Values represent measurements obtained under standard laboratory conditions.

DISCUSSION

The present study demonstrated that increasing extract concentration significantly enhances antifungal activity, indicating a clear dose-dependent effect. This suggests that the antifungal efficacy of *Citrullus colocynthis* seed extracts is directly related to the concentration of bioactive constituents. The preliminary phytochemical screening revealed the presence of several bioactive groups such as flavonoids, saponins, alkaloids, resins, and coumarins. However, due to the qualitative nature of this analysis, no direct correlation can be established between specific compounds and antifungal activity (Naji *et al.,* 2025). Among the tested solvents, the ethanolic extract exhibited the highest antifungal activity, followed by the acetone and aqueous extracts. This superior performance may be attributed to the ability of ethanol to extract a broader range of phytochemicals due to its intermediate polarity. Ethanol efficiently dissolves both polar and moderately non-polar compounds, including phenolics and flavonoids, which are widely reported to disrupt fungal cell membranes and inhibit enzymatic systems essential for fungal growth (Al-Sultany and Jassim, 2016; Jassim *et al.,* 2026). The statistical analysis revealed significant differences ($p \leq 0.01$) between the different extracts of *Citrullus colocynthis* seeds.

The ethanolic extract showed the strongest antifungal effect, followed by the acetone extract, while the aqueous extract demonstrated the lowest activity. Additionally, significant differences in fungal sensitivity were observed, where *Epidermophyton floccosum* exhibited higher susceptibility compared to *Aspergillus niger*, likely due to structural differences in their cell walls and resistance mechanisms. When compared with the standard antifungal drug Clotrimazole, the ethanolic extract showed comparable activity at higher concentrations (10% and 20%), indicating its potential as a natural antifungal agent (Hassan *et al.,* 2026; Hassan, *et al.,* 2025) .The observed superiority of the ethanolic extract may also be attributed to the solubility of bioactive compounds such as fixed oils, resins, phenolic compounds, and saponins in ethanol. *Citrullus colocynthis* seeds are known to contain a high content of fixed oils, which may contribute to their biological activity. Similar findings were reported by Sanguinetti et al. (2017) and Naji et al. (2024), who demonstrated the antifungal potential of plant-derived oils against dermatophytes such as *E. floccosum*. Furthermore, these results are consistent with previous studies reporting the effectiveness of alcoholic plant extracts against dermatophytes and other pathogenic fungi (Sadeghi-Nejad & Deokule, 2019; Mohammed *et al.,* 2024; Mohi Al-Kahfaji *et al.,* 2023). These studies confirm that solvent polarity plays a crucial role in extracting active antifungal constituents.

The minimum inhibitory concentration (MIC) values varied among the extracts. The ethanolic extract showed the lowest MIC (5%) against both tested fungi, while the acetone and aqueous extracts showed higher MIC values (10%). The low MIC values observed for the ethanolic extract may be attributed to a higher yield of active compounds during extraction or a higher natural abundance of bioactive constituents in the plant material. In contrast, higher MIC values in other extracts may be due to lower concentrations of active compounds or the presence of less efficient phytochemical profiles. These findings are consistent with previous reports by Cox and Balick (1994) and Jassim *et al.* (2024), who highlighted that MIC values are influenced by both extraction efficiency and phytochemical concentration. Similar observations were also reported by Gadhi *et al.* (1999) and Jassim & Ridah (2018).

Conclusion

Citrullus colocynthis seeds extracts have contained many active compounds and carbohydrates that have high inhibitory effectiveness against some fungi. that are pathogenic to humans such as on *a Epidermophyton floccosum* and *Aspergillus niger*

REFERENCES

Abdulazeem, L., Jassim, Y. The effect of Yolk immunoglobulin and heat killed Sallmonellatyphi on Rabbits. *Research Journal of Pharmacy and Technology Open source preview,* 2018, 11(6), pp. 1–4.

Adewale, A.O., David, A.A., Abiodun, O.O. and Craig, O.A. (2017). Studies on antimicrobial, antioxidant and phytochemical analysis of Urenalobata leave extract. *J. Physical & Natural Sci.* 1(2) : 2-9.

Ahmed, I., Mehmood, Z. and Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.* 62 : 183-193.

Al-Ghanimi, A.A., Al-Ethari, A.Y. and Abdulhusain, H.K. (2017). Partral purification of tannins from Quercusinfectoria galls and study of its effect on some isolated skin pathogenic microorganisms. *J.of Karbala University.* 5(4): 1-14.

Al-Khazragi, S.M. (1991). Biopharmacological study of Artemisia herba Alba. M. Sc. Thesis. Baghdad University.

Al-Sultany, S.J., Jassim, Y.A. Cryoglobulin induction in rabbits by endotoxin injection experimentally. *International Journal of Pharmtech Research Open source preview,* 2016, 9(4), pp. 394–398

Aniz, E.H.S. Jassim Y.A. (2024). Anticancer activity of pyoverdine (PVD) producing by antibiotic-resistant Pseudomonas aeruginosa isolated from burn and wound infections. *Journal of Applied and Natural Science,* 16(2), 777 - 785. https://doi.org/10.31018/ jans.v16i2.5506

Cox, P.A. and Balick, P.J. (1994). The ethnobotonical approach to drug discovery. *J. Sci. Am.* 270: 60-65.

Emmons, C., Binford, C., Utz, J. and Kown-Chung, K. (1977). Medical mycology. 3ed. ed. Lea and Febiger. Philadelphia, U.S.A. p: 147-167.

Gadhi, C.A., Weber, M., Mory, F. and Jana, M. (1999). Antibacterial activity of Aristolochiapaucinerris Pomel. *J. Ethnopharmacol.* 67: 87-92.

Ghafarokhi, M., Razafsha, M., Allameh, A. and Abyaneh, M.R. (2023). Inhibitory effects of aqueous Onion & Garlic extracts on growth of keratinase activity on Trichophytonmentagrophytes. *Iran. Biomed. J.* 7(3) : 113-118.

Ghannoum, M., Isham, N., Hajjeh, R., Cano, M., ALHasawi, F., Yearach, D., Warner, J., Lon, L., Jessup, C. and Elewski, B. (2023). Tineacapitis in Cleveland : Survey of elementary school students. Am*. Acad. of Dermatol. Inc.* 48 (2) : 190-193.

Harborne, J.B. (1984). Phytochemical methods . Aguide to modern techniques of plants analysis. 2nd. ed. Champan& Hall , London, New York.

Hassan, I.A., Jassim, Y.A., Al-Khafaji, N.S.K. . Purification and Identification of Extracellular Secondary Metabolites from Actinomycetes and Study Its Antibacterial Activity Against Pathogenic Bacteria. *Egyptian Journal of Medical Microbiology Egypt Open source preview,* 2025, 34(4), pp. 455–460

Hassan, I.A., Jassim, Y.A., Al-Khafaji, N.S.K. Cytotoxic Activity of Secondary Metabioltic Agent Produce by Actinomycetes. 2026, 29(1), pp. 61–79

Jassim, R.M., Alsultany, S.J., Jassim, Y.A. Evaluation of NGAL Levels in Recurrent Urinary Tract Infections .*Egyptian Journal of Medical Microbiology Egypt Open source preview,* 2026, 35(1), pp. 291–296

Jassim, Y. A. Al-Yassiry Z.A., Awadh E.F.A. (2023). Study of antibacterial activity and cytotoxicity of the bioactive compound of Bacillus megaterium L2 strains isolated from the oral cavity of hospital workers and visitors at Dental Health Centre, Babylon, Iraq. *Journal of Applied and Natural Science,* 15(1), 371 - 378. https://doi.org/10.31018/ jans.v15i1.4313

Jassim, Y.A., Jassim, Z.M., Radhi, S.H., Abed, A.S. Isolation and Diagnosis of Acinetobacterbaumannii THAT produce protease and antimicrobial agents from the milk factory. (2024). Nativa, 12(2), 397-402. https://doi.org/10.31413/ nat.v12i2.17392

Jassim, Y.A., Khudhair, M.K., Radhi, S.H. Genetic Identification of Bacteria Producing Antibacterial Agent Isolation from Soil and Study of their Effectiveness as

Antioxidants. *Annals of Agri Bio Research Open source preview,* 2022A, 27(1), pp. 42–49.

Jassim, Y.A., Ridah, H.M. Molecular identification and optimization of cellulose hydrolyzing bacteria isolated from the cow dung. *International Journal of Pharmaceutical Research Open source preview,* 2018, 10(1), pp. 678–682.

Khanzada, Sh.A., Iqbal, Sh.M. and Akram, A. (2016). In vitro efficacy of plant leaf extracts against SclerotiumrolfsiiSaac. Mycopath. 4(1) : 51-53.

Khdhair, O.A., Jassim, Y.A., Alkremy, N.A.A., Al-Ameedy, T.H.Antibacterial activity of the alcoholic extract of berberine against Staphylococcus aureus isolated from burn and wound infections. *Rev. Clin. Pharmacol. Pharmacokinet. Int. Ed.* 38 (Sup2): 137-139 (2024). https://doi.org/10.61873/FMPT5825

Kosalec, I., Pepeljnjak, S. and Kustrak D. (2015). Antifungal activity of fliud extract and essential oil from anise fruits (Pimpinellaanisum L., Apiaceae). *Acta. Pharm.* 55 : 377-385.

Malheiros, A., Filho, V.C., Schmitt, C.B., Yunes, R.A., Escalante, A., Svetaz, L., Zacchino, S. and Monache, F.D. (2005). Antifungal activity of drimanesesquiterpenes from Drimysbrasiliensis using bioassy – guided fractionation. J. Pharm. &Pharmaceut. 8(2) : 335-339.

Meena, M.Ch. and Panti, V. (2008). Isolation and identification of flavonoid "Quercetin" from *Citrullus colocynthis* (Linn.) *Schrad. Asian J. Exp. Sci.* 22 (1) : 137-142.

Memon, U., Brohi A.H., Ahmed, S.W., Azhar, I. and Bano, H. (2003). Antibacterial screening of *Citrullus colocynthis*. *Pakistan J. Pharmacol. Sci.* 16(1) : 1-6.

Mohi AL-kahfaji, M. H. A., AL-Amer S.M. , Jassim Y.A. (2023). Effect of Coffeaarabica L on antibiotic-resistant Pseudomonas aeruginosa . Journal of Applied and Natural Science, 15(2), 748 - 753. https://doi.org/10.31018/jans.v15i2.4525

Naji, N.M., Al-Mamoori, S.O.H., Al-Taee, Z.M., Abu-Serag, N.A., Jassim, Y.A.Silver nitrate and bio-silver nanoparticles on some morphological, physiological, and anatomical variations of tomato plants., 2025, 13(2), pp. 226–233

Naji, N.S., Jassim Y.A., Al-Budairi L.Q. A., Abass Z.M. (2024). Efficiency of Bacillus mucilaginosus isolated from the soil in dissolving potassium in its microenvironment. *Journal of Applied and Natural Science,* 16(1), 196 - 201. https://doi.org/10.31018/jans.v16i1.4728

Sadeghi-Nejad, B. and Deokule, S.S. (2019)b. Antidermatophytic activity of Drynariaquercifolia (L.). J.Smith. Judishapur *J. Microbiol.* 2(1) : 25-30.

Sanguinetti, M., Posteraro, B., Romano, L., Battaglia, F., Lopizzo, T., De Carolis, E. and Fadda, G. (2017). In vitro activity of Citrus bergamia (bergamot) oil against clinical isolets of dermatophytes. *J. Antimicrob.Chemo.* 95(2) : 305-308.

Shihata, I.M. (1951). A pharmacological study of Anagallisarvensis.M.D. vet . Thesis. Cairo University.

Sofowora, A. (1993). Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd. ed. Spectrum Books Ltd. Sunshine House, Ibadan, Nigeria. P: 134- 156.

Souri, E., Amin, Gh., Farsam, H., Jalalizadeh, H. and Barezi, S. (2007). Screening of thirteen medicinal plant extracts for antioxidant activity. *Iranian J. of Pharmaceu. Res.* 7(2) : 149-154.

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