Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Evaluation of anticandidiasis efficacy of secondary metabolites extracted from *Dianthus caryophyllus* L. flower buds

Zainab Mohammed Karim, Hussein J. Hussein*, Abeer Fauzi Al-Rubaye

Department of Biology, College of Science for Women, University of Babylon, Iraq

** Corresponding author's E-mail: huss2010feb@yahoo.com*

ABSTRACT

The present study was conducted to investigate the effect of crude terpenoid, flavonoid, and alkaloid extracts from flower buds of *Dianthus caryophyllus* L. against *Candida* species isolated from different clinical samples such as mouth and vagina in Babil Province during 2021 in Iraq. Antifungal activity was achieved *in vitro* using agar well diffusion method against *Candida* species by preparing three concentrations for each crude compound (25, 50 and 100 mg mL^{-1}) and compared with positive control represented by Fluconazole (50 mg mL^{-1}) and negative control represented by 10% dimethyl sulfoxide. The aim of this study was to control *Candida* species isolated from different clinical samples such as mouth and vagina using secondary metabolites extracted from flower buds of *Dianthus caryophyllus* L. According to the data collected from the study, the crude terpenoids and flavonoids extracted from flower buds of *Dianthus caryophyllus* L. exhibited significant superiority at $p < 0.05$ over the fluconazole when applied to *Candida* species especially at 100 mg mL^{-1}. Finally, it can be concluded that flower buds of *Dianthus caryophyllus* L. is most effective in controlling *Candida* species, especially its terpenoid and flavonoid compounds.

Keywords: *Dianthus caryophyllus* L, Anticandidiasis, Alkaloids, Flavonoids, Terpenoids.
Article type: Research Article.

INTRODUCTION

Invasive candidiasis is an important health-care-associated fungal infection that can be caused by several *Candida* spp.; the most common species is *Candida albicans*, but the prevalence of these organisms varies considerably depending on geographical location. The spectrum of disease of invasive candidiasis ranges from minimally symptomatic candidaemia to fulminant sepsis with an associated mortality exceeding 70%. *Candida* spp. are common commensal organisms in the skin and gut microbiota, and disruptions in the cutaneous and gastrointestinal barriers promote invasive disease (Pappas *et al.* 2018). Carnation, *Dianthus caryophyllus* L. belongs to *Caryophyllacece* family and the probable origin of the plant is Mediterranean region. The plant has many synonyms such as *D. acinifolius* Schur, *D. arbuscula* Lindl., *D. arrectus* Dumort., *D. binatus* Schur , *D. caryophyllus* var. *coronarius* L. , *D. coronarius* (L.) Burm.f., *D. corsicus* Link ex Spreng., *D. kayserianus* Schur, *D. longicaulis* Costa, *D. miniatus* A.Huet ex Nyman, *D. moschatus* J.F.Gmel., *D. multinervis* Vis. and *Tunica morrisii* (Hance) Walp (Lim 2012).

The plant is annual or perennial, 15-60 cm long, branched and glabrous herb. Its leaves are linear lanceolate, apex acute, margin smooth or ciliate at base. Its flowers are solitary or in clusters at tips of branches. Epicalyx scales are 4-6 in number, broad-ovate, abruptly mucronate at apex, herbaceous, appressed to calyx, covering one fifth to one quarter the length of calyx tube. Calyx tube is cylindrical, 20-30 mm long. Petals are 5, limb exerted, triangular

Caspian Journal of Environmental Sciences, Vol. 21 No. 1 pp. 143-149 Received: March 08, 2022 Revised: June 19, 2022 Accepted: Aug. 28, 2022
DOI: 10.22124/CJES.2023.6205

Publisher: University of Guilan, Iran

obovate, toothed at apex, auricle absent, pink-red or white, sometimes spotted with darker centres, claw cuneate, glabrous (Chandra *et al.* 2016). Antifungal resistance is an emerging problem worldwide, and this further complicates the selection of appropriate antifungal therapy. *Candida* spp. strains that are resistant to first-line antifungals (such as echinocandins and fluconazole) are increasingly being recognized, and their appearance usually correlates with high azole and/or echinocandin background usage in hospitals or specific hospital units (Vallabhaneni *et al.* 2015; Castanheira *et al.* 2016).

There are also some reports about fungal diseases in other organisms around the world (Al- Abbasi *et al.* 2021; AL- Ethawi & AL-Taae 2022). However, the aim of this study was to investigate the anticandidiasis activity of secondary metabolites such as terpenoids, flavonoids, and alkaloids extracted from flower buds of *Dianthus caryophyllus* L. against to *Candida* spp. isolated from different clinical samples.

MATERIALS AND METHODS

Plant material: The carnation, *D. caryophyllus* L. flower buds were purchased from the local markets, identified based on the taxonomic features by a botanist, then were cleaned, dried, and kept according to Harborne (1975).

Extraction of the crude terpenoid compounds: Crude terpenoid compounds were extracted according to Harborne (1984).

Extraction of the crude flavonoid compounds: Crude Flavonoid compounds were extracted according to Boham & Kocipai Abyazan (1974).

Extraction of the crude alkaloid compounds: Crude Alkaloid compounds were extracted according to (Harborne 1973) Stock solution of 100 mg mL^{-1} for alkaloid, flavonoid and terpenoid were prepared in 10% Dimethyl Sulfoxide (DMSO) then sterilized by Millipore filter (0.22 µm) and stored at -20 ºC until use (Al Jassani 2017).

Anti-candidiasis Efficacy: The anti-candidiasis activity of the secondary metabolites extracted from the flower buds of *D. caryophyllus* L. was tested against the isolates of *Candida* species using agar-well diffusion method (Perez *et al.* 1990). Wells were made using cork porer (6 mm in diameter). Dimethyl sulfoxide 10% (DMSO) was used as a negative control and Fluconazole antibiotic as a positive control.

Candida Isolates: All isolates used in this study were isolated from hospitals located at Hillah City, Iraq (Table 1).

Table 1. Types of Candida isolates and their sources.

No	Fungal isolates	Type of specimen
1	Candida Isolates	Mouth
		Vagina

Statistical analysis

All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance using SPSS 16.0 program, a completely randomized design was used and least significant difference (LSD) was performed at $p \leq 0.05$.

RESULTS

The results of antifungal activity of the crude terpenoid compounds extracted from the flower buds of *D. caryophyllus* L. against *Candida* species isolated from different clinical samples such as mouth and vagina are presented in Table 2. The antifungal activity of terpenoid secondary metabolites with three concentrations (25, 50, and 100 mg mL^{-1}) was screened by agar well diffusion methods. According to results, the crude terpenoid compounds extracted from the flower buds showed significant reduction ($p < 0.05$) in the growth of *Candida*

species. Growth inhibition represented by zone of inhibition ranging from 24 ± 1.00 mm in 25 mg mL^{-1}, 32 ± 1.00 mm in 50 mg mL^{-1}, and 38 ± 1.00 mm in 100 mg mL^{-1} (Fig. 1), compared to negative control representative by 10% DMSO and positive control representative by Fluconazole (50 mg mL^{-1}) where inhibition zones were 0.00 mm and 35 ± 1.00 mm for negative and positive controls respectively. On the other hand, the crude flavonoid compounds showed zone of inhibition of 19 ± 1.00 mm, 28 ± 1.00 mm and 37 ± 1.00 mm at 25 mg mL^{-1}, 50 mg mL^{-1}, and 100 mg mL^{-1} respectively (Table 3), Thus, it differed significantly compared to the negative and positive controls (Fig. 2).

In the same context, the crude Alkaloid compounds showed significant activity at three concentrations (25, 50 and 100 mg mL^{-1}) compared to negative control against *Candida* species isolated from different clinical samples (Table 3). The highest zone of inhibition 25 ± 1.00 mm was recorded at 100 mg mL^{-1}, while 21 ± 1.00 mm at 50 mg mL^{-1} (Fig. 3). In the case of the highest zone of inhibition in the crude terpenoid compounds, it was reached up to 38 ± 1.00 mm at 100 mg mL^{-1}, while the highest zone of inhibition in the crude flavonoid compounds was 37 ± 1.00 at 100 mg mL^{-1}.

Table 2. Anti-candidiasis activity of the crude Terpenoid compounds extracted from *Dianthus caryophyllus* L. flower buds.

Concentrations (mg mL^{-1})	**Terpenoid compounds**
	Inhibition zone* (mm)
Negative Control	0 ± 0.00
25 mg mL^{-1}	24 ± 1.00
50 mg mL^{-1}	32 ± 1.00
100 mg mL^{-1}	38 ± 1.00
Positive Control	35 ± 1.00
LSD	1.62

*Mean ± Standard deviation.

Table 3. Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Dianthus caryophyllus* L. flower buds.

Concentrations (mg mL^{-1})	**Flavonoid compounds**
	Inhibition zone* (mm)
Negative Control	0 ± 0.00
25 mg mL^{-1}	19 ± 1.00
50 mg mL^{-1}	28 ± 1.00
100 mg mL^{-1}	37 ± 1.00
Positive Control	35 ± 1.00
L.S. D	1.60

* Mean ± Standard deviation

Table 3. Anti-candidiasis activity of the crude alkaloid compounds extracted from *Dianthus caryophyllus* L. flower buds.

Concentrations (mg mL^{-1})	**Alkaloids compounds**
	Inhibition zone* (mm)
Negative Control	0 ± 0.00
25 mg mL^{-1}	14 ± 1.00
50 mg mL^{-1}	21 ± 1.00
100 mg mL^{-1}	25 ± 1.00
Positive Control	35 ± 1.00
L.S. D	1.62

*Mean ± standard deviation

Terpenoids and flavonoids were the most effective compared to alkaloids (Fig. 4), exhibiting significant superiority ($p < 0.05$) over the Fluconazole antibiotic (35 ± 1.00 mm).

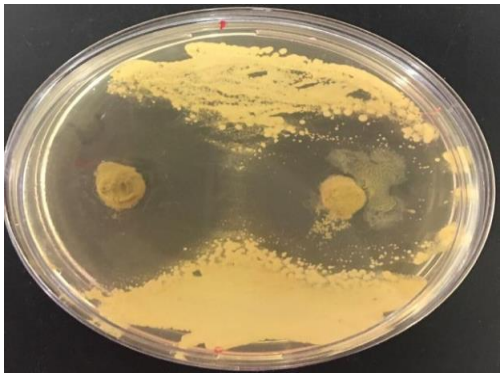

Figure 1: Anti-candidiasis activity of the crude terpenoid compounds extracted from *D. caryophyllus* L. flower buds at 50, and 100 mg mL^{-1}.

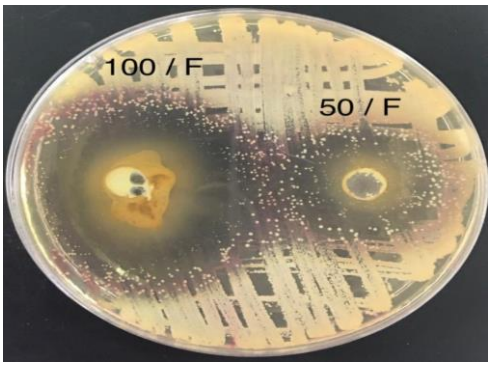

Figure 2: Anti-candidiasis activity of the crude flavonoid compounds extracted from *D. caryophyllus* L. flower buds at 50, and 100 mg mL^{-1}.

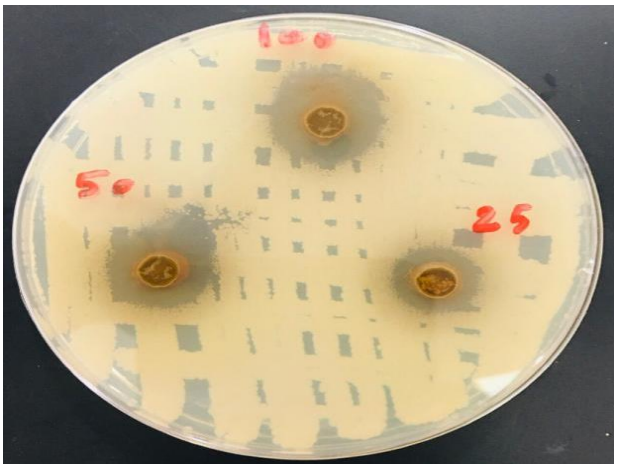

Fig. 3. Anti-candidiasis activity of the crude alkaloid compounds extracted from *D. caryophyllus* L. flower buds at 25, 50, and 100 mg mL^{-1}.

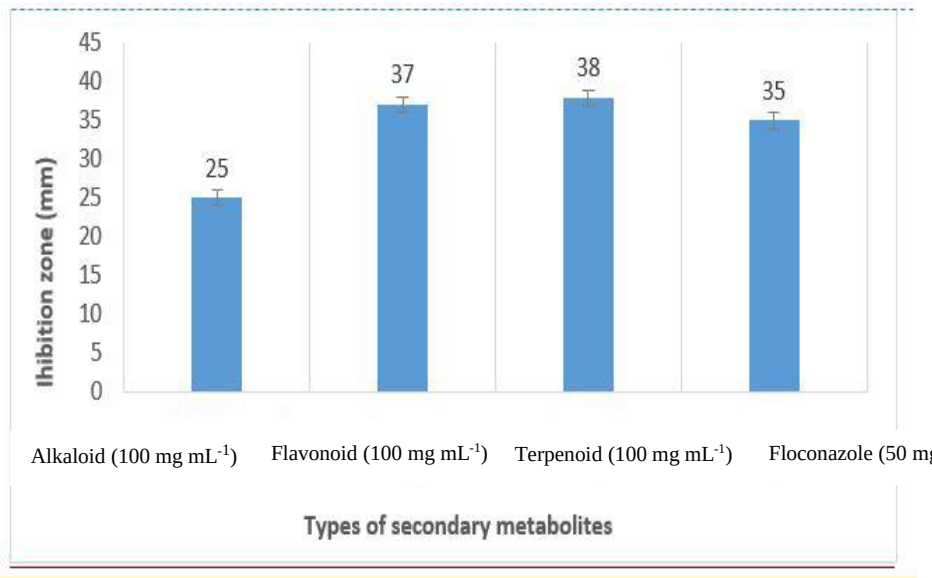

Fig. 4. Inhibition zones of alkaloids, terpenoids and flavonoids at 100 mg mL^{-1} against *Candida* species, LSD = 1.82.

DISCUSSION

Emergence of multi-drug resistance in human and animal pathogenic bacteria and fungi like *Candida* species as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Secondary metabolites extracted from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves, *Lepidium sativum* leaves, *Myrtus Communis* leaves, *Cassia senna* leaves, *Ricinus communis* leaves, *Cassia didymobotrya* leaves, *Melia azedarach* leaves, *Dianthus caryophyllus* flower buds and *Salvia hispanica* seeds), possess ability of antibacterial for controlling several pathogenic microorganisms isolated from different clinical samples (Al Marzoqi *et al.* 2015; Al Marzoqi *et al.* 2016; Hussein *et al.* 2017; Hussein *et al.* 2018; Hussein *et al.* 2018; Hussein *et al.* 2019; Hussein & Al Marzoqi 2020; Kamil *et al.* 2020; Hussein *et al.* 2020). Hussein *et al.* (2018) reported that phytochemical compounds extracted from the unicellular primitive plant like *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria. Kamal *et al.* (2019) used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp. (Kamal *et al.* 2020) used phytochemical compounds extracted from *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. AL Masoodi *et al*. (2020) used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* spp. isolated from seeds of maize. (Hussain *et al*. 2021) used terpenoid compounds extracted from *Carthamus tinctorius* seeds and flavonoid compounds extracted from *M. communis* leaves against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by alkaloids and flavonoids extracted from *M. communis* leaves presented a worthy source for controlling pathogenic microorganisms isolated from haemodialysis fluid specimens (Sharara *et al.* 2021). Hasan *et al.* 2022) used the *Callistemon viminalis* leaf extracts for controlling isolates of urinary tract infections. Alkaloids and terpenoids extracted from the roots of *Saussurea costus* have powerful antifungal activity against *Candida* species (Karim *et al.* 2022). Phytochemical studies showed that *D. caryophyllus* contained triterpenes, alkaloids, and coumaruns (Eltayeb 2016). *D. caryophyllus* grown in Egypt contained four chemical groups: monoterpene hydrocarbons 19.59% (tricyclene 0.17%, α-pinene 2.05%, camphene 0.98%, β-pinene 3.11%, phellandrene 3.52 %, P-cymene 3.32%, limonene 4.91, ϒ-terpinene 1.53%); oxygenated monoterpene 26.71% (elemol 5.51%, citronellol 1.11%, bornyl acetate 3.12%, eugenol 15.29%, methyl eugenol 1.68%); sesquterpenes hydrocarbons 12.83% (ϒ-cadinene 4.12%, calamene 8.71%) and various compounds 20.97% (benzyl benzoate 14.12%, benzyl salicylate 6.85%), (Ibrahim 2016). Al Snafi (2017) reported that *D. caryophyllus* contained triterpenes, alkaloids, coumaruns, and volatile oil, and the plant possessed anticancer, antiviral, antibacterial, antifungal, insecticidal, repellent, antioxidant, reno-protective, anaesthetic and analgesic effects. The flavone datiscetin (3, 5, 7, 2'- tetrahydroxyflavone), presence in *D. caryophyllus* exhibited an appreciable fungitoxic activity towards *Fusarium oxysporum* (Curir *et al.* 2003). In contrast, natural bioactive compounds extracted from medicinal plants make their effects by many mechanisms. Terpenoids and flavonoids make their effects by disruption of microbial membranes and polypeptides embarrassment of linkage of bacterial proteins to host polysaccharide receptors and alkaloids complexes make their effect by inhibiting efflux pump (Okusa *et al.* 2009) Finally, anticandidiasis efficacy of *D. caryophyllus* L. might belong to phytochemical compounds such as terpenoids, flavonoids, alkaloids and their effect in proteins and polysaccharides and disruption in membranes permeability or inhibiting of efflux pump or DNA synthesis.

CONCLUSION

Secondary metabolite compounds extracted from the *D. caryophyllus* L. flower buds such as terpenoid and flavonoid represent a good source for controlling *Candida* species isolated from different clinical samples.

REFERENCES

Al- Abbasi, SHA, Al-Majmaei, AA M, Al-Naqib, ATH, Hameed, AM, AL-Samarraie, MQ & Altaef, AH 2021, Isolation and identification of some fungi from rhizospheric soils of some wild plants at Samarra University, Iraq. *Caspian Journal of Environmental Sciences*, 19: 829-839

AL- Ethawi, MB & AL-Taae, HH 2022, First record at molecular level for *Rhizoctonia solani* causing Rot Root on *Aleo vera* plants in Iraq. *Caspian Journal of Environmental Sciences*, 20: 955-965

Al Jassani MJ 2017, *Tropaeolum majus* leaves extract as an antifungal, antiaflatoxigenic and antiaflatoxin agent. *Journal of Global Pharma Technology*, 12: 328-333.

Al Marzoqi, AH, Al Khafaji, NM & Hussein, HJ 2016, In vitro antibacterial activity assessment of the crude phenolic, alkaloid and terpenoid compounds extracts of *Lepidium sativum* L. on Human Pathogenic Bacteria. *International Journal of ChemTech Research*, 4: 529-32.

Al Marzoqi, AH, Hussein, HJ & Al Khafaji, NM 2015, Antibacterial activity of the crude phenolic, alkaloid and terpenoid compounds extracts of *Lactuca serriola* L. on human pathogenic bacteria. *Chemistry and Materials Research*, 7: 8-10.

AL Masoodi, H, Hussein, HJ & Al Rubaye, AF 2020, Antifungal activity of the two medicinal plants (*Curcuma longa* L. and *Boswellia carteri* Birdwood) against *Fusarium* species isolated from maize seeds. *International Journal of Pharmaceutical Research*, 12: 408-14.

Al Snafi, AE 2017, Chemical contents and medical importance of *Dianthus caryophyllus*: A review. *IOSR Journal of Pharmacy*, 7: 61-71.

Boham, BA & Kocipai Abyazan, R 1974, Flavonoids and condensed tannins from leaves of Hawaiian V*accinium vaticulatum* and *V. calycinium*. *Pacific Science*, 48:458-463.

Castanheira, M, Messer, SA, Rhomberg, PR & Pfaller, MA 2016, Antifungal susceptibility patterns of a global collection of fungal isolates: Results of the SENTRY antifungal surveillance program. *Diagnostic Microbiology and Infectious Disease*, 85: 200-204.

Chandra, S, Rawat, DS, Chandra, D & Rastogi, J 2016, Nativity, Phytochemistry, ethnobotany and pharmacology of *Dianthus caryophyllus*. *Research Journal of Medicinal Plant*, 10: 1-9.

Curir, P, Dolci, M, Dolci, P, Lanzotti, V & De Cooman, L 2003, Fungitoxic phenols from carnation (Dianthus caryophyllus) effective against *Fusarium oxysporum* f. sp. *dianthi*. *Phytochemical Analysis*, 14: 8-12.

Eltayeb, RA 2016, Study of some chemical constituents of *Dianthus caryophyllus* and *Elettaria cardamomum*. PhD Dissertation, University of Khartoum, Sudan.

Harborne, JB, Mabray, TY & Marby H 1975, Physiology and function of flavonoids. Academic Press, New York, 970 p.

Harborne, JB 1973, Phytochemical methods, London. Chapman and Hall, Ltd. pp. 49-188.

Harborne, JB 1984, Phytochemical methods. Chapman and Hall, New York. 2nd ed., 288 p.

Hasan Radhi, S, Kamal SA, Mohammed Sahi, N & Hussein HJ 2022, Assessment of antibacterial efficacy of *Callistemon viminalis* (Sol. ex Gaertn.) G. Don against some isolates obtained from urinary tract infections. *Archives of Razi Institute*, 77: 891-789.

Hussain, AY, Hussein, HJ & Al Rubaye, AF 2021, Antifungal efficacy of the crude flavonoid, terpenoid, and alkaloid extracted from *Myrtus communis* L. against *Aspergillus* species isolated from stored medicinal plants seeds in the Iraqi markets. *Journal of Biotechnology Research Center*, 15: 73-80.

Hussein, HJ, Al Khafaji, NM, Al Mamoori, AH & Al Marzoqi, AH 2018, Evaluation of in vitro antibacterial properties of the crude Phenolic, Alkaloid and Terpenoid extracts of *Cassia senna* L. against human gram-negative pathogenic bacteria. *Plant Archives*, 18: 354-6.

Hussein, HJ, Al Khafaji, NM, Al Mamoori, AH, Juaifer, WA, Al Marzoqi, AH & Al Zobiady, RA 2017, Antimicrobial effect of the crude phenolic, alkaloid and terpenoid compounds extracts of *Myrtus communis* L. against human gram-negative pathogenic bacteria. *Journal of Global Pharma Technology*, 8: 130-3.

Hussein, HJ & Al Marzoqi, AH 2020, The Antibacterial efficacy of the secondary metabolites extracted from (*Melia azedarach* L.) leaves against pathogenic microorganisms isolated from burns and gingivitis infections. *EurAsian Journal of Biosciences*, 14: 561-5.

Hussein, HJ, Kaizal, AF, Al Khafaji, NM, Sadiq, ZF & Shahad, AS 2018, Evaluation of antibacterial potential of the crude Phenolic, Alkaloid and Terpenoid extracts of *Ricinus communis* L. against gram-negative pathogenic bacteria. *Journal of Global Pharma Technology*, 05: 384-388.

Hussein, HJ, Kamal, SA & Sahi, NM 2020, Antibacterial efficacy of the seed extract of *Saliva hispanica* L. against pathogenic bacteria isolated from diarrhoea cases. *Biochemical and cellular archives*, 20: 3491-94.

Hussein, HJ, Naji, SS & Al Khafaji, NM 2018, Antibacterial properties of the *Chlorella vulgaris* isolated from polluted water in Iraq. *Journal of Pharmaceutical Sciences and Research*, 10: 2457-2460.

Hussein, HJ, Sahi, NM, Saad, AM & Altameme, HJ 2019, The antibacterial effect of bioactive compounds extracted from *Cassia didymobotrya* (Fresenius) irwin & barneby against some pathogenic bacteria. *Annals of Tropical Medicine and Public Health*, 22: SPe 116.

Ibrahim, ME 2016, Agrochemical studies on *Dianthus caryophyllus* L. grown in Egypt. *International Journal of PharmTech Research*, 9: 113-117.

Kamal, AS, Hussein, HJ & Tolaifeh, ZA 2019, Antibacterial potential of *Hibiscus sabdarifa* L. against some Enterobacteriaceae: in vitro. *Biochemical and Cellular Archives*, 19: 4291-4294.

Kamal, SA, Al Kaim, HW & Hussein, HJ 2020, Antibacterial activity of phytochemical compounds extracted from *Ficus carica* Linn. Leaves against human pathogenic bacteria. *EurAsian Journal of BioSciences*, 14:2293-2298.

Kamil, SS, Hussein, HJ & Al Marzoqi, AH 2020, Evolution of antibacterial efficacy of *Dianthus caryophyllus* L. extracts against some hospitals pathogenic bacteria. *International Journal of Pharmaceutical Research*, 12: 1274-1279.

Karim, ZM, Hussein, HJ & Al Rubaye. AF 2022, In vitro anticandidal activity of the secondary metabolites extracted from *Saussurea costus* (Falc.) Lipschitz roots. *International Journal of Health Sciences*, 6: 7461-7470.

Lim, TK 2012, Edible medicinal and non-medicinal plants. Dordrecht, the Netherlands: Springer.

Okusa, PN, Stévigny, C & Duez, P 2009, Medicinal Plants: A Tool to Overcome Antibiotic Resistance? In: A, Varela, & J, Ibañez (Eds.). Medicinal plants: classification, biosynthesis and pharmacology. Nova Science Publishers, Incorporated, 315 p.

Pappas, PG, Lionakis, MS, Arendrup, MC, Ostrosky Zeichner, L & Kullberg, BJ 2018, Invasive candidiasis. *Nature Reviews Disease Primers*. 4:1-20.

Perez, L, Pauli, M & Bazequre, P 1990, Antibiotic assay by the agar well diffusion method. *Journal of Actabiology*, 15: 113-115.

Sharara, DT, Al Marzoqi, AH & Hussein, HJ 2021, In vitro antibacterial efficacy of the secondary metabolites extracted from Myrtus communis L. against some pathogenic bacteria isolated from Hemodialysis Fluid. *Annals of the Romanian Society for Cell Biology*, 25: 9267-9274.

US National Plant Germplasm System, *Dianthus caryophyllus* L. https://npgsweb. ars-grin.gov/gringlobal/taxonomydetail.aspx?13826.

Vallabhaneni, S, Cleveland, AA, Farley, MM, Harrison, LH, Schaffner, W, Beldavs, ZG, Derado, G, Pham, CD, Lockhart, SR 2015, Smith RM. Epidemiology and risk factors for echinocandin non-susceptible *Candida glabrata* bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008–2014. In Open Forum Infectious Diseases, 1 (Vol. 2, No. 4).

Bibliographic information of this paper for citing:

Mohammed Karim, Z, Hussein, HJ & Al-Rubaye, AF 2023. Evaluation of anticandidiasis efficacy of secondary metabolites extracted from *Dianthus caryophyllus* L. flower buds. Caspian Journal of Environmental Sciences, 21: 143-149.