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Full Length Article

Inhibitory Activity of *Cuscuta monogyna* Bioactive Compounds against *Pseudomonas aeruginosa*

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Abstract

Four active compounds *i.e*., saponins, alkaloids, glycosides and tannins were extracted from cuscuta (*Cuscuta monogyna* vahl.). Six concentrations (5, 10, 25, 50, 75 and 100 g/L) of these extracted were prepared, and their inhibitory activity was tested against *Pseudomonas aeruginosa,* a Gram-negative bacterium isolated from burns and respiratory infections. All concentrations of the extracts demonstrated inhibitory activity against the tested bacteria. The highest inhibitory activity was observed at a concentration of 5 g/L for the saponin extract with an average of 22.4 mm. Additionally, the bacteria isolated from burns were found to be more susceptible to the inhibitory effects of the extracts showing average inhibition zones of 23.55 mm for saponin 19.75 mm for alkaloid 21.83 mm for glycoside and 25.81 mm for tannin respectively. In bacteria isolated from burns the concentration of 5 g/L was the most effective in inhibiting bacteria growth across all active extracts studied with average inhibition zone diameters of 30.3, 24.3, 28.3 and 38 mm for saponins, alkaloids, glycosides and tannins, respectively. For bacteria isolated from respiratory infections, the most effective concentrations varied by extract. In the saponin extract the concentration of 10 and 25 g/L were most effective showing average inhibition zone diameters of 21 and 21.3 mm, respectively. For the alkaloid extract the concentration of 25 g/L was the most effective with an average inhibition zone diameter of 21 mm. In the glycoside extract the concentrations 75 and 100 g/L were the most effective with average inhibition zone diameters of 21.7 and 21.3 mm, respectively. Finally, in the tannin extract the concentration of 100 g/L was the most effective producing an average inhibition zone diameter of 18.7 mm.

Keywords: Inhibitory; Bioactive; *Cuscuta monogyna*; Compounds; *Pseudomonas aeruginosa*

Introduction

The dodder plant *Cuscuta* spp. is classified within the family Convolvulaceae (Costea *et al.* 2009, 2015). It is a fully parasitic flowering plant and depends entirely on other green plants for water and nutrients. It is characterized by thin cylindrical, yellow or orange stems that are connected to the host plant through specialized organs known as haustoria. These organs penetrate the host's tissues to absorb water and nutrients (Fahmy 2008; Noureen *et al.* 2019). The damage due to this genus lies in its ability to parasitize a wide range of plant families, including Solanaceae and Fabaceae. Additionally, it is capable of producing a large number of seeds that are resistant to unfavorable conditions as well as exhibiting rapid growth (Parker and Wilso 1985; Nickrent and Musselman 2004).

Dodder is considered one of the most dangerous agricultural pests with no effective, selective, economical, easy-to-use, or practical method currently available to mitigate its damage (Parker and Riches 1993). Due to the difficulty of controlling species of the genus *Cuscuta,* there has been a growing interest in exploiting their medicinal and pharmaceutical for treating various diseases (Sandler 2010). This interest is attributed to the active chemical components found in certain species, such as *C. reflexa* from which various compounds have been isolated such as cuscutin, cuscutaline, bergenine, kaempferol, amarbelin, sterols and glycosides. These compounds give *C. reflexa,* a range of medicinal applications, such as anti-inflammatory, antipyretic, hepatoprotective, anticonvulsant, anthelmintic, antifungal, Androgenic, cholesterol-lowering, anti-androgen, blood thinner, diuretic, immune stimulant, anti-arthritic, anti-asthmatic and anti-cancer (Kamede *et al*. 2024). Several species of the genus *Cuscuta* (*e.g*., *C. campestris*, *C. kotschyana*, *C. reflexa*, *C. palaestina* and *C. chinensis*) have demonstrated activity in anti-breast cancer, liver cancer and prostate cancer and their effectiveness is attributed to their active chemical constituents including flavonoids, saponins,

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sterols/triterpenes and tannins (Dermani *et al*. 2020). The hydroalcoholic extract of *C. chinensis* has specifically been found to exhibit anti-breast and anti-prostate cancer effects (Bleischwitz *et al*. 2010).

Farhadi *et al.* (2011) reported the effectiveness of flavonoid extracts from *C. lehmanniana* in managing diabetes. Additionally, Al-Smail (2011) revealed that aqueous and ethanolic extracts of various parts of *C. lehmanniana* (stem, flowers, fruits, seeds, and fruit coats) were effective against the fungi *Alternaria alternate* and *Fusarium solani* as well as the bacteria *Staphylococcus aureus* and *Proteus vulgaris.* The study by Al-Smail *et al*. (2020) demonstrated that extracts of active chemical compounds isolated from *C. lehmanniana* (alkaloids, tannins, saponins and glycosides) exhibited inhibitory activity against *S. epidermidis* and *S. aureus* (gram-positive bacteria), as well as *Escherichia coli, Shigella* sp.*, Klebsiella pneumonia* and *Salmonella paratyphi* (gram-negative bacteria). Mikhlef (2020) study on the traditional Alkaloids and flavonoids of *C. monogyna* demonstrated that it contained the alkaloids cuscutin and the flavonoids myricetin, dulcitrol, astragalin, quercetin, arabelin, hyperoside, luteolin, kaempferol and apigenin.

Given the widespread presence of C*uscuta* species in Iraq and the lack of information regarding the effect of extracts from its active compounds on microorganisms particularly in the context of the increasing prevalence and antibiotic resistance of *Pseudomonas aeruginosa* the current research was aimed to isolate certain active compounds from *C. monogyna* and evaluate their inhibitory effectiveness against this bacterium.

Materials and Methods

Plant samples collection and identification

Samples of the *C. monogyna* were collected during the flowering stag in June/July 2018–2019 from the Shirqat Distract Salah Al-Din Governorate, Iraq. The samples were transported to the laboratory, where they were identified and classified. The collection process included all parts of the flowering plant. The samples were then dried at room temperature at 37°C, ground using an electric grinder and stored in tightly sealed shaded plastic containers until use.

Extraction of the active compounds

Saponins: The separation of saponins was carried out following the method described by Harborne (1973) A 10 g of powdered plant sample was added to 50 *M* 20% ethanol and heated the solution in a water bath at 55°C for 3 h with continuous stirring to ensure proper extraction, filtration, re-treatment and filtered. The extract was then treated with the remaining plant material with 100 mL of 20% aqueous ethanol. Heated the resulting mixture in a water bath at 90°C until the volume was reduced to approximately 40 mL, then transferred the concentrated solution to a separating funnel and added 20 mL of diethyl ether. Separated the aqueous layer and discarded the ether layer.

Alkaloid: The separation of alkaloids was carried out following the method described by Harborne (1984). Weighed 10 g of plant powder placed in thimble extraction vessels and extracted using 200 mL of 95% ethyl alcohol for 24 h in a Soxhlet apparatus. The resulting substance was concentrated using a rotary evaporator and then dissolved in 5 mL of ethanol. Then, added 30 mL of 2% sulfuric acid to the ethanol solution. The ethanol was evaporated using the rotary evaporator leaving behind the acid solution. Then, conducted the Mayer test to confirm the presence of alkaloids. For this purpose, added a sufficient amount of 10% ammonium hydroxide to adjust pH to 9. Transferred the basic solution to a separating funnel, added 10 mL of chloroform and shaken the mixture several times. Allowed the mixture to settle until it separated into two layers. Collected the lower chloroform layer, which contained the dissolved alkaloids. This step was repeated three times, combining the lower layers each, resulting in a total volume of approximately 40 mL of chloroform extract. Filtered the chloroform extract and concentrated it using a rotary evaporator to obtain purified alkaloids.

Glycosides: The separation of glycosides was carried out following the method described by Ukiya *et al.* (2006). Added 10 g of plant sample powder to 100 mL of 80% ethyl alcohol and left the mixture in the refrigerator at 4°C for 24 h, then filtered to obtain ethanolic extract. Concentrated the ethanolic extract to one-third of its original volume using a rotary evaporator and added 50 mL of ether and 5 mL of 3.0 *M* lead acetate solution to the extract in a separating funnel while shaking. Transferred the extract to a separating funnel and added 5 mL of 3.0 Mollary lead acetate and 50 mL ether and shaken well. Separated and collect the aqueous layer while discarding the ether layer. Repeated this process three times, collecting the aqueous layer each time. Dried the combined aqueous layer in an oven at 30°C until completely dried.

Tannins: Tannins were separated following the method described by Ahmad and Nazil (1989). Prepared the aqueous extract by boiling 0.5 g of the sample in 50 mL of distilled water for 30 min. Filtered the extract and centrifuged in at 2000 rpm for 20 min. Transfer the filtrate to a 100 mL volumetric flask and add 20 mL of 4% lead acetate solution. Made up the volume using distilled water. Transferred the supernatant to another 100 mL volumetric flask, added 20 *M* of 4% lead acetate solution and made up the volume with distilled water. Filter the extract and dry it in an oven at 60°C until completely dried.

Isolation and diagnosis of bacteria

P. aeruginosa Isolates were collected from Shirqat General Hospital between 1 to 30 January 2020, from burns and respiratory infections. The samples were transferred to the laboratory where the bacteria were identified through

morphological examination (Public Health England 2015), microscopic analysis (Betsy and Keogh 2005) and biochemical testing (Stehling *et al.* 2010). The biochemical tests included catalase, oxidase, IMViC, motility, TSI and gelatinase assays (Procop *et al*. 2016). Confirmatory identification was performed using the Vitek 2 compact system technology.

Antibacterial activity of active compounds

The stock solution was prepared and sterilized by dissolvent 4 g of dry plant extract powder and in 10 mL of sterile distilled water. The solution was kept refrigerated until used to prepare concentrations of 5, 10, 25, 50, 75 and 100 g/L (Mitscher *et al.* 1972). Bacterial suspensions were prepared from new colonies (24 h after culture) using physiological solution saline and the suspension was adjusted to match McFarland's solution of 1.5×10^8 cells/mL (Baron *et al.* 1994). The bacterial suspension was prepared as described in Baron *et al.* (1994) and 200 μL of the suspension was spread onto the surface of Mueller-Hinton agar using a flame-sterilized L-shaped glass applicator. The plates were left at room temperature for about 30 min until solidified. Using a sterile cork borer, three holes of 10 mm diameter were made in each dish, and 200 μL each of the six concentrations (5, 10, 25, 50, 75 and 100 g/L) of the active compounds were placed into each hole. Two control groups were included: one with three replicates the other with three replicates of petri dishes containing a single hole filled with sterile distilled water (negative control) and the other with three replicates of dishes containing ciprofloxacin (positive control). The dishes were then incubated at 37°C for 24 h, and the diameter of the inhibition zones was measured (Egorove 1985; Jethinlakhosh and Lathika 2012).

Statistical analysis

The effectiveness of the extracts of the active compounds (saponins, alkaloids, glycosides and tannins at concentrations of 5, 10, 25, 50, 75 and 100 g/L) was compared with three replicates for each concentration, alongside control set. The results were analyzed using an analysis of variance (ANOVA) test based on factorial arrangement. The arithmetic means of the coefficients were compared using Duncan's multiple range test (Al-Rawi and Aziz 2000) at a significance level of 5%. The analysis was performed using Minitab software.

Results

The results showed the effectiveness of the active compounds (saponin, alkaloid, glycoside and tannins) isolated from *C. monogyna* at concentrations of 5, 10, 25, 50, 75 and 100 g/L in inhibited Gram-negative *P. aeruginosa* bacteria isolated from burns and respiratory infections (Table 1–4). Bacteria isolated from burns were most affected by extracts of active compounds, with average inhibition zones of 23.55, 19.75, 21.83 and 25.81 mm for saponins, alkaloids, glycosides and tannins, respectively (Tables 1–4). The current results showed that the saponin extract was particularly effective in inhibiting the studied bacteria (Table 1).

Notably, 5 g/L concentration with an average inhibition zone of 24.8 mm was the most effective in inhibiting the bacterial infection. Additionally, 5 g/L concentration exhibited the most significant inhibiting of *P. aeruginosa* bacteria with an average inhibition zone of 30.3 mm compared to the two control treatments. In the case of *P. aeruginosa* isolated from respiratory infections, the ciprofloxacin control treatment with an average inhibition zone of 26 mm was the most effective. However, the 5 and 10 g/L concentrations were the most effective in inhibiting the bacteria with an average inhibition zone of 21 and 21.3 mm, respectively. The results showed that alkaloid extract concentrations were effective in inhibiting *P. aeruginosa* bacteria (Table 2).

The ciprofloxacin control treatment with an average inhibition zone of 26 mm was the most effective in inhibiting bacteria isolated from both burns and isolated from respiratory infections. However, 5 g/L concentration was the most effective in inhibiting *P. aeruginosa* bacteria isolated from burns, with an average inhibition zone of 24.3 mm compared to the rest of the concentrations and the negative control (distilled water). For bacteria isolated from respiratory infections, 25 g/L concentration was the most effective with an average inhibition zone of 21 mm, compared to the other concentrations. Additionally, 5 and 10 g/L concentrations were the most effective in inhibiting bacteria with an average inhibition zone of 21.5 and 20 mm, respectively. The results showed that the concentrations of glycoside extracts were effective in inhibiting *P. aeruginosa* (Table 3).

Bacteria isolated from burns, the 5 g/L concentration was the most effective in inhibiting bacteria, with an average inhibition zone of 28.3 mm compared to the control. For bacteria isolated from respiratory infections, the ciprofloxacin control treatment was the most effective with an average inhibiting zone of 26 mm. Extract concentrations of 75 and 100 g/L were also effective with average inhibition zone of 21.7 and 21.3 mm. respectively compared to the other concentrations and the negative control. As shown in Table 3, the 5 g/L concentration was the most effective in inhibiting bacteria, with an average inhibition zone of 23.1 mm.

The results showed that the concentrations of tannin extracts were effective in inhibiting *P. aeruginosa* (Table 4). For bacteria isolated from burns, the 5 g/L concentration was the most effective with an average inhibition zone of 38 mm compared to the control. For bacteria isolated from respiratory infections, the ciprofloxacin control treatment was the most effective with an average inhibiting zone of 26 mm. Additionally, 100 g/L concentration was the most effective with an average inhibition zone of 18.7 mm compared to the control. For the remaining concentrations and the negative

Table 1: The inhibitory activity of saponins extract from *C. monogyna* against *P. aeruginosa*

Concentrations (g/L)	Burns isolates	Respiratory tract infections isolates	Average concentration
5	30.3 a	19.3 b	24.8 a
10	19.0 b	21.0 ab	20.0 ab
25	20.0 b	21.3 ab	20.6 ab
50	27.8 a	18.3 b	23.0 ab
75	25.0 ab	19.3 b	22.1 ab
100	20.0 b	19.3 b	19.6 b
Distilled water	0 c	0 c	0 c
Ciprofloxacin	26 ab	26 a	26 a
Average concentrations	23.55	19.75	

Values sharing same letters differ non-significantly ($P > 0.05$)

Table 2: The inhibitory activity of alkaloid extracts of *C. monogyna* against *P. aeruginosa*

Concentrations (g/L)	Burns isolates	Respiratory tract infections isolates	Average concentration
5	24.3 a	18.7 b	21.5 b
10	21.3 a	20.7 ab	21.0 b
25	19.3 ab	21 ab	20.1 b
50	24.0 a	17.3 b	20.6 b
75	15.3 b	18.3 b	16.8 c
100	14.3 b	16 b	15.1 c
Distilled water	0 c	0 c	0 d
Ciprofloxacin	26 a	26 a	26 a
Average concentrations	19.75	18.66	

Values sharing same letters differ non-significantly ($P > 0.05$)

Table 3: The inhibitory activity of the glycoside extracted from *C. monogyna* on against *P. aeruginosa*

Concentrations (g/L)	Burns isolates	Respiratory tract infections isolates	Average concentration
5	28.3 a	18.0 b	23.1 ab
10	22.7 ab	17.0 ab	19.8 b
25	22 ab	17.7 ab	19.8 b
50	20 b	20.3 b	20.1 b
75	19.7b	21.7 b	20.2 b
100	18.3 b	21.3b	19.8 b
Distilled water	0 c	0 c	0 c
Ciprofloxacin	26 a	26 a	26 a
Average concentrations	21.83	19.33	

Values sharing same letters differ non-significantly ($P > 0.05$)

Table 4: The inhibitory activity of tannin extracted from *C. monogyna* against *P. aeruginos*

Concentrations (g/L)	Burns isolates	Respiratory tract infections isolates	Average concentration
5	38.0 a	12.7 cd	25.3 a
10	25.3 b	14.3 c	19.8 a
25	20.3 b	13.7 cd	17.0 a
50	26.3 b	13.0 cd	19.6 a
75	20.0 bc	15.3 c	17.6 a
100	25.0 b	18.7 b	21.8 a
Distilled water	0 d	0 e	0 b
Ciprofloxacin	26 b	26 a	26 a
Average concentrations	25.81	14.61	

Values sharing same letters differ non-significantly ($P > 0.05$)

control (distilled water), the results showed that the 100 g/L concentration was the most effective in inhibiting the bacteria, with an average inhibition zone of 21.8 mm (Table 4). The current results showed that the 5 g/L concentration was the most effective in inhibiting *P. aeruginosa* bacteria with an average inhibition zone of 22.4 mm (Fig. 1).

Discussion

Al-Smail *et al*. (2020) found that active compounds isolated from *C. lehmaniana* have inhibitory activity against *S. aureus, Shigella* sp. and *K. pneumonia* bacteria. Pizzorno and Murray (2020) reported that saponins inhibit peptidoglycan synthesis and bacterial adhesion reduce peptidoglycan uptake and prevents bacterial endotoxin adhesion. Saponins also interact with lipopolysaccharides and affect plasma membrane permeability which facilitates the entry of antibiotics into bacterial cells (Arabski *et al*. 2012; Dong *et al*. 2020). Additionally, saponins have antimicrobial, anti-inflammatory, anti-cancer, reduce

cholesterol and regulate the body's immunity (Khan *et al.* 2018; Schoenlechner *et al.* 2008). Here we showed that *C. monogyna* extracts were inhibitory to *P. aeruginosa* growth isolated from patients suffering from various ailments, with an average inhibition zone of ~22.4 mm (Fig. 1).

The results revealed a discrepancy in the inhibitory effect of alkaloid extract concentrations, which did not align with the findings of Al-Smail *et al*. (2020) who reported that alkaloid extracts of *C. lehmanniana* exhibited the highest inhibitory activity against *S. aureus, Shigella* sp. and *K. pneumonia* bacteria. However, the results were consistent with those of Mabhiza *et al*. (2016) who demonstrated the inhibitory effects of alkaloids particularly their impact on bacterial efflux pumps. Othman *et al*. (2019) suggested that the antibacterial effect of alkaloids arises from their interference with cellular metabolic reactions, disruption of plasma membranes or alteration of vital enzymes and inhibition of bacterial growth and reproduction and including inhibitory of biofilm formation. Some alkaloids have been shown to act as detergents against Gram-negative bacteria destroying their outer membranes, while their effect on Gram-positive bacteria is to depolarize their membranes (Alhanout *et al*. 2010). Olszewski (2019) also highlighted the role of alkaloids in targeting bacterial virulence genes.

The results of the current study on the glycosidic compound extract are consistent with Al-Smail *et al.* (2020) who found that glycosidic extracts of *C. lehmanniana* at different concentrations exhibited inhibitory activity against *S. epidermidis* and *S. aureus* and four types of Gram-negative bacteria *E. coli, shigella* sp*., K. pneumonia* and *S. paratyphi*. The antimicrobial activity of glycosidic compounds is attributed to the non-sugar part (glycone) in their chemical structure which varies from one glycosidic compound to another granting them high efficacy and a broad antimicrobial spectrum (Kytidou *et al.* 2020). Tagousop *et al.* (2018) reported that the biological activity of plant glycosides results from their interference with cellular membrane functions particularly their binding to proteins and membranes and inhibiting the activity of certain enzymes.

Glycosides have been reported to possess notable biological effects including antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant and antidiabetic activities (Xiao *et al.* 2016). The results of the current study also showed that the active compounds (saponins, glycosides and tannins) extracted from the *C. monogyna* at a concentration of 5 g/L exhibited high inhibitory activity against *P. aeruginosa* with an average inhibition zone of 30.3, 28.3 and 38 mm, respectively, compared to the control treatment with ciprofloxacin which had an average inhibition zone of 26 mm (Table 1–4). This demonstrated that the active compound tested had higher inhibitory activity with fewer side effects compared to the antibiotic ciprofloxacin.

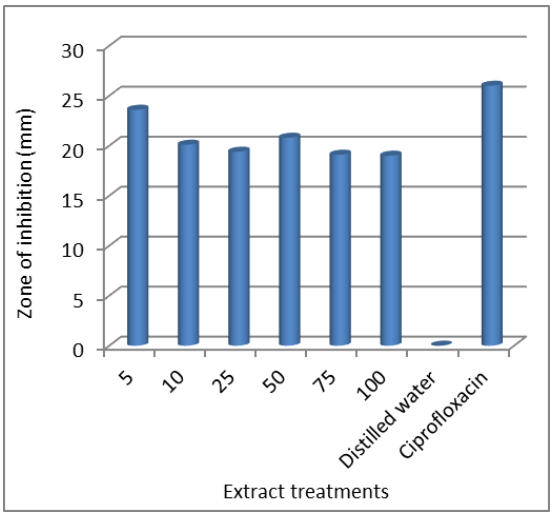

Fig. 1: The inhibitory effect of different concentrations of *C. monogyna* on *P. aeruginosa* bacteria

Conclusion

The active compounds extracted from the *C. monogyna* demonstrated significant inhibitory activity against *P. eruginosa* isolates, with the saponin extract exhibiting the highest efficacy. A concentration of 5 g/L showed higher inhibitory activity across all active compounds tested. Therefore, it is recommended to investigate the molecular-level effects of these active compounds against *P. aeruginosa* bacteria and to study their impact on the tissues of rats infected due to this bacterium.

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Author Contributions

MQ planned the experiments, interpreted the results and performed the statistical analysis of data, while YS and MK were responsible for isolating and identifying *P. aeruginosa* using a typing procedure.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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