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The effect of *Cuscuta babylonica* Aucher (*Cuscuta*) parasitism on the phenolic contents of *Carthamus glaucus* Bieb.subsp. glaucus

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ABSTRACT: Cuscuta species are holoparazit plants which obtained all need water and organic material from host plants. The host plants are generally synthesized a variety of phenolic compounds in response to attack of parasitic plants. In this study, the plant *Carthamus glaucus* Bieb. subsp. *glaucus* (Compositae) used as host plant is an important plant that contains several compounds inhibit the STAT-3 gene is directly related to prostate cancer. In the study it was investigated that the effect of Cuscuta babylonica parasitism phenolic compounds of *C. glaucus*. The phenolic compounds of infected and uninfected C.glaucus plants analysed by LC/MS-MS. The results indicated a rise in phenolic contents that known as defense chemicals quinic acid, gallic acid, tr-caffeic acid, hyperoside, quercetin, and naringenin with dodder infestation. Besides in the content of tr-aconitic acid, vanillin, hesperidin, 4-OH-benzoic acid, salicylic acid and kaempferol decreased after dodder infestation.

Keywords: *Cuscuta babylonica* Aucher, *Carthamus glaucus* bieb. subsp. *glaucus* (Compositae), phenolic compound, LC/MS-MS

Cuscuta babylonica Aucher (küsküt) parazitliğinin *Carthamus glaucus* Bieb.subsp. glaucus'un Fenolik İçeriği Üzerine Etkisi

ÖZET: Küsküt türleri, ihtiyaçları olan tüm su ve organik maddeleri konak bitkiden alan holoparazit bitkilerdir. Konak bitkiler genellikle bu bitkilerin saldırılarına karşı çeşitli fenolik bileşikler sentezlerler. Bu çalışmada konak bitki olarak kullanılan*Carthamus glaucus* Bieb. subsp. *glaucus* (Compositae) C.babylonica parazitliliğinin prostat kanseri ile direkt ilişkili olan STAT-3 genini inhibe eden çeşitli bileşikleri içeren çeşitli bir bitkidir. Çalışmada, *C. Glaucus'*un fenolik bileşen içeriği üzerine olan etkisi araştırılmıştır. Parazit bitki ile enfekte olan ve olmayan C.glaucus bitkilerinin fenolik bileşen içerikleri LC/MS-MS ile analiz edilmiştir. Sonuçlar, savunma kimyasalları olarak bilinen kuinik asit, gallik asit, tr-kaffeik asit, hiperosid, kuersetinve naringenin içeriklerinde bir artış olduğunu göstermiştir.Bunun yanında tr-akonitik asit, vanillin, hesperidin, 4-OH-benzoik asit, salisilik asit ve kaemferol içerikleri küsküt bulaşması sonrası azalmıştır.

Anahtar Kelimeler: *Cuscuta babylonica* Aucher, *carthamus glaucus* bieb. subsp. *glaucus* (Compositae), fenolik bileşikler, LC/MS-MS

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INTRODUCTION

Parasitic plants are restrict the lives of earth plants. They are divided into two groups: hemiparasites are still able to make photosynthesis therefore rely only partially on a host plant and holoparasites that are completely dependent on solutes, metabolites, and photo assimilates, from their host crops. *Cuscuta*can be take into account an obligate holoparasite (Kaiser et al., 2015) because all of *Cuscuta*species depend (obviously) on a host plant to survive their life cycle. There are approximately 3.900 recognized parasitic plant species among the flowering plants, and *Cuscuta* spp. is one of the well-known and agriculturally most important genera (Westwood et al., 2010). Secondary metabolites that derived by host plants play an principal role in the interaction between parasitic weeds and their hosts (Bouwmeester et al., 2003).Water and nutrients are transferred uni-directionally through the haustorium from the vascular system of the host into the parasite. Besides, this organ also facilitates hormonal interactions between the two organisms (Kuijt and Toth 1976; Visser and Dorr, 1986; Stewart and Press 1990). Parasitic plants and hosts interactions usually parallel those between herbivores and plants (Pennings and Callaway, 2002).

Phenolics are usually produced and collected in the subepidermal sheets of plant tissues againist to stress factors and pathogens (Schmitz-Hoerner and Weissenbock, 2003; Clé et al., 2008). Parasitic plant affect and are affected by host plant physiology because of similar hormonal pathways between parasite and host plants (Pennings and Callaway, 2002). Because the host and parasite plants share the same primary physiology. Phenolic compounds one of the most prevalent groups of secondary metabolites found in the plants (Harborne, 1980; Boudet, 2007). They move as inhibitors, protective agents, pesticides and natural animal toxicants against phytophagous insects,herbivores, bacterial and fungal pathogens (Dakora and Phillips 1996; Ravin et al., 1989; Lattanzio et al., 2006). Plants requirement to phenolic compounds for growth, pigmentation, reproduction, resistance to pathogens and for several functions (Lattanzio et al., 2006).

Cuscuta babylonica Aucher parasitic plant used in this study, belong to the family Convolvulaceae and about 200 species have been described so far (McNeal et al., 2007). Most of them can cause serious problems for crops. *Cuscuta* has not leaves or roots, develops a haustorium and absorbs nutrients from host plants (Furuhashi et al., 2011). It has known as Bostanbozan, Canavarotu, Bağbozan, Cinsacı, Eftimon, Gelinsacı, Kızıl sarmasık, Küsüt and Seytansacı in Anatolia (Baytop, 1997). Some of *Cuscuta* species are effective in the treatment of headache, itching, migraine, chronic catarrh, amnesia, epilepsy, expectorates, prolonged fever and constipation (Furuhashi et al., 2011).

The effect of parasitization does not always negatively on the host plant. For instance, infection of tomatoes by *Cuscuta* modified some plant hormones (e.g. salicylic acid) and can affect their defense system against insect herbivores (Runyon et al., 2008). Plants can perceive to pathogen attacks and respond by activating defence system (Karban and Baldwin, 1997; Dangl and Jones, 2001). Similarly, in a great number of study were revealed that the *Cuscuta* parasitzm caused to changes on host plants (Mishra and Sanwal, 1995; Runyon et al., 2008; Vurro et al., 2011; Furuhashi et al., 2012).

In this study, *Carthamus glaucus* Bieb. subsp. *glaucus* (Compositae) belong to family Asteraceae has been used as host plant. It is a winter plant anddevelops in the wheat fields (Meshram et al., 2011; Zadeh et al., 2011) and it is reported that this plant contains compounds inhibit the STAT-3 gene is directly related with prostate cancer (Taglialatela-Scafati et. al., 2012).

The aim of this study was to research phenolic compounds differences of *C. Glaucus* plants which infected with *C. babylonica.* The results were compared with their respective controls for obtain and increase of desirable some phenolics that may use as medicinal compounds.

MATERIALS and METHODS

Plant Material

This work was carried out in Dicle University garden where *C. glaucus* grows naturally.

Germination of dodder seeds: Holoparasite *C. babylonica* seeds first submerged concentrated sulfuric acid for 30 minutes for scarification of hard seed coat. Than the seeds washed with tap water and put a cup with moist filter paper and deposited at 4^{o}C refrigerator for 15 days. The stratified seeds were taken at 16 ^{o}C for germination.

After germination, naturally grown flowering *C. glaucus* has been infected with dodder for 15 days, and

than infected *C. glacus* plants were collected and dried at room temperature. Non infected *C. glaucus* plants grown the same area were used as control group.

Extraction

The plant materials of *C. Glaucus* were dried at room temperature and than powdered. Ground samples (0.2 g) were extracted with chloroform (10 mL), and sonicated (Sanyo MSE-Soniprep 150, U.K.) for 5 min. This treatment was repeated twice. The solvent was removed in vacuum, after removing chloroform; the samples were re-extracted with methanol (10 mL) in the sonicator for 5 min. The methanol extraction was repeated three times. And the filtrates were collected and concentrated using a rotary vacuum evaporator (IKA, RV 10 DS 99).

The final samples were resolved in methanol and injected to LC-MS/MS (Shimadzu marka LC-MS 8040 triple quadrupole mass spectrometry) after an appropriate dilution process. Phenolic compound analysis carried out according to the guidelines defined by Ertas et al.(2014) (Table.1).

Table 1. Analytical LC-MS/MS method parameters

Analytes	**RT[a]**	**Parent ion (m/z)[b]**	Ioniza tion Mode	**R^{2c}**	**RSD%[d]**	**Linearity Range (mg/L)**	**LOD/LOQ (µg/L)[e]**	**Reco-very (%)**	**U[f]**
Quinic acid	3.32	190.95	Neg	0.9927	0.0388	250-10000	22.3 / 74. 5	103.3	4.8
Malic acid	3.54	133.05	Neg	0.9975	0.1214	250-10000	19.2 / 64. 1	101.4	5.3
tr-Aconitic acid	4.13	172.85	Neg	0.9933	0.3908	250-10000	15.6 / 51. 9	102.8	4.9
Gallic acid	4.29	169.05	Neg	0.9901	0.4734	25-1000	4.8 / 15. 9	102.3	5.1
Chloroge nic acid	5.43	353	Neg	0.9932	0.1882	250-10000	7.3 / 24. 3	99.7	4.9
Protocate chuic acid	5.63	152.95	Neg	0.9991	0.5958	100-4000	25.8 / 85. 9	100.2	5.1
tr- caffeic acid	7.37	178.95	Neg	0.9942	1.0080	25-1000	4.4 / 14. 7	98.6	5.2
Vanillin	8.77	151.05	Neg	0.9995	0.4094	250-10000	10.1 / 33. 7	99.2	4.9
p-Coumaric acid	9.53	162.95	Neg	0.9909	1.1358	100-4000	15.2 / 50. 8	98.4	5.1
Rutin	10.18	609.1	Neg	0.9971	0.8146	250-10000	17.0 / 56. 6	102.2	5.0
Hesp eridin	9.69	611.1	Poz	0.9973	0.1363	250-10000	21.6 / 71. 9	100.2	4.9
Hyperoside	10.43	463.1	Neg	0.9549	0.2135	100-4000	12.4 / 41. 4	98.5	4.9
4-OH Benzoic acid	11.72	136.95	Neg	0.9925	1.4013	25-1000	3.0 / 10. 0	106.2	5.2
Salicylic acid	11.72	136.95	Neg	0.9904	0.6619	25-1000	4 / 13. 3	106.2	5.0
Quercetin	14.48	300.9	Neg	0.9995	4.3149	25-1000	2.0 / 6. 8	98.9	7.1
Naringenin	14.66	270.95	Neg	0.9956	2.0200	25-1000	2.6 / 8. 8	97.0	5.5
Luteolin	15.43	284.95	Neg	0.9992	3.9487	25-1000	5.8 / 19. 4	105.4	6.9
Kaempferol	15.43	284.95	Neg	0.9917	0.5885	25-1000	2.0 / 6. 6	99.1	5.2
Apigenin	17.31	268.95	Neg	0.9954	0.6782	25-1000	0.1 / 0. 3	98.9	5.3
Chrysin	21.18	253	Neg	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	5.3

[a]RT: Retention time
[b]Parent ion (*m/z):* Molecular ions of the standard compounds (mass to charge ratio)
[c]R^2: coefficient of determination
[d]RSD: relative standard deviation
[e]LOD/LOQ (µg/L): Limit of deteection/Limit of quantification
[f] U (%): Percent relative uncertainty at 95% confidence level (k=2).

RESULTS and DISCUSSION

Cuscuta is an obligate parasite attacking the shoot system of numerous species of dicotylednous plants, specially legume crops (Farah, 2010). Phenolic acids and lignin comprised a second type of defence mechanism against *Cuscuta* parasitism. They are chemical barriers used by certain incompatible hosts to resist the attack of parasitic weeds (Farah, 2007). Farah, (2010) indicated a rise in both phenolic acids and lignin contents with *Cuscuta* infestation in hyacinth bean and kidney bean. The increase in the levels of both chemicals in the infected kidney bean may be attributed to the fact that these chemicals were stimulated as part of the defence reactions of this crop against the penetration of haustorium into it is tissues. These results are in line with those of Arnaud et al.(1996) in *Cusuta reflexa* and *Phaseolus vulgaris;* Antonove and terBorg (1996) in *Orobanche cumaua* and *Helianthus annuus*, and Goldwasser et al. (1999) in *Orobanche aegyptiaca* and *Viciaatropurpurea*, who attributed the resistance of the host plants to a number of factors including phenolic compounds and lignin. Plant phenolic compounds are maintained in the nontoxic reduced state by antioxidants and stored in the cell vacuoles (Miles, 1999).

Host plant secretes various phenolic compounds, as a response to parasitic plant. The main ones are rosmarinic acid, caffeic acid, chlorogenic acid, tannic acid and quercetin (Lindroth and Batzli, 1984; Par and Tumlinson, 1999; Petersen et al., 2009; Serghini, 2001). In this study it was revealed that the *Cuscuta* parasitism caused an increase on amount of quinic acid (2.8 fold), gallic acid (2.4 fold), malic acid (1.01 fold), tr-caffeic acid (1.7 fold), hyperoside (2.4 fold),protocatechuic acid (PCA) (2.1 fold), quercetin (8.1 fold) and naringenin (3.1 fold) used as defence compounds. Likewise the amount of flavonoids luteolin (1.4 fold), apigenin (1.7 fold) and chrysin (1.05 fold) also improved(Table.2).

Our results very clearly show that the amount of PCA (2.1 fold) increased in infected plants. PCA is a natural phenolic acid and exist in several plants such as mushrooms and microorganisms (Williams et al., 2012; Nguyen et al., 2013; Delsignore 1997 et al.). It is known that the PCA has antiinflammatory and antioxidant (Liu et al., 2002; Syafni et al., 2012) and antibiotic activities (Nguyen et al., 2015). Besides antioxidative, nematicidal, and resistant effects, antibiotic and antibacterial activity of the PCA have been reported (Link et al., 1929; Syafni et al., 2012; Nguyen et al., 2013). Quinic acid is a metabolite related to metabolic response (inducible defense) to biotic stress (Murthy et al., 2009). It is reported that quinic acid and quercitol are present in high concentrations in wounded leaves of genus *Quercus* plants (Gargallo-Garriga et al., 2010). In this study it is revealed that the amount of quinic acid (2.8 fold) was increased in infected plants.Gallic acid is known to play an important role in insect-plant and plant-pathogen interactions (Arrantlrakrislmanet al.,1997). As our data, the amount of gallic acid (2.4 fold) was increased in infected plants. Similarly, it is reported that gallic acid showed chronic effects on growth, ingestion and utilization of food in *Helicoverpa armigera,* in relation to cotton (Ananthakrishnan et al., 1994). Flavonoids are the largest group of phenolics and they have antimicrobial and antioxidant properties (Lorenc-Kukula et al., 2005).Chlorogenic acid and hyperoside are present in several plants and have antioxidant capacity in plant defense system (Korkina, 2007; Leiss et al., 2009; Ngadze et al., 2012). The amount of chlorogenic acid and hyperoside increased (1.7 fold and 2.4 fold) after *Cuscuta* attack as our results. Quercetin is a flavonoid that has allelopathic feature (Inderjit and Gross, 2002; Weir et al., 2004). The rise of quercetin in the infected plants could be attributed primarily to it is toxicity and caused to reduction of plant growth (Lindroth and Batzli 1984). Lindroth and Batzli (1984) reported that the quercetin caused reduced growth rates. Our data showed that the quercetin increased 8.1 fold in infected plants compared to uninfected ones. It is reported that the concentration of the flavonol glycoside rutinusually remained unaffected after *Cuscuta* attack (Sham, 1993) our result also supported this finding.It was found that caffeic acid and other plant-derived phenolics had been transformed to active compounds against *Streptococcus faecalis* in the gut of *Bombyx mori* (Iizuka et al., 1974; Koike et al., 1979). In this study the amount of tr-caffeic acid (1.7 fold) and p-coumaric acid(1.1 fold) were increased. Likewise, detection of phenolic acids in stems of broomrape, parasitizing faba bean, indicated the occurrence of chlorogenic acid, m-coumaric acid and caffeic acid at different stages of growth of the

parasite (El-Akkad et al., 2002). Phenolic compounds, such as caffeic acid, and kaempferol-glycoside, are excellent inhibitors of IAA oxidation (Mumford et al., 1961; Krylov et al., 1994; Beckman, 2000; Mathesius, 2001). IAA accumulation is able to induce synthesis of flavonoids as a response to auxin accumulation (Peer and Murphy, 2007). Kaempferol is important part of the auxin dependent defence response (Likić et al.,2014). Howower, as our results, plants infected with *Cuscuta* exhibited lower concentration of kaempferol compared to control, with a 72.4 fold decrease. Antimicrobial effects of flavonoids have been defined to associate in allelopathic interactions between plants (Chou, 1999; Inderjit and Gross, 2000). But the roles of flavonoids and style of action are not yet completely understood. In our study, it is showed that a flavanon naringenin increased 3.1 fold in infected plants. It is found that the flavanone naringenin caused a recession on growth of gramineous plants (maize, rice, and *Echinochloa oryzicola*), and it is joined to the inhibition of 4-coumarate CoA ligase and lignification (Deng et al., 2004). Besides, it is reported that naringenin is produced after a pathogen attack in *Oryza sativa* L. (Jwa et al., 2006). In this study, the concentration of malic acid (MA) (1.01 fold) a slightly increased. Current studies proposed that the metabolic grades of low-MA have a remarkable role in initiating plant defense system (Klessig et al., 2000; Hückelhoven, 2007).

Cuscuta attacks caused to various morphological and physiological changes on the host plants (Serghini et al., 2001; Walters 2011). Salicylic acid (SA) has an foremost function in the defence response of plants (Dmitriev, 2003). SA is frequently synthesized in a response to pathogens (Loake and Grant, 2007). In the flowering phase, various plant hormones and chemical compounds are increase as well as SA and 4-OH benzoic acid (Khurana and Cleland, 1992; Martínez et al., 2004; Daayf et al., 2012).

It was showed that the amount of 4-OH benzoic acid (1.7 fold) and SA (1.7 fold) from *C. glaucus* plants that infected with *Cuscuta* attack and collected in the flowering phase were decreased compared with uninfected plants. These cases may be interpreted as evidence of inhibition reproduction of host by parasitic plants (Serghini et al., 2001; Pennings and Callaway, 2002; Furuhashi et al., 2011). It is known that the *trans*-aconitic acid has antirheumatic and diuretic properties (Schnitzler, 2007) althought the distribition of this compound is rare (Nierhaus and Kinzel, 1971). In infected plants, the trans-aconitic acid decreased about 1.4 fold relatively control plants. As our data, the amount of hesperidin (1.2 fold) decreased in infected plants. Hesperidin (Hsd) and hesperetin (Hst) have several biologycal activity such as antioxidant, antiinflammatory and anticancer effects. These substances play an important role in plant defense systems to combat different pathogens. Similarly, Soares et al., (2015) suggested that hesperidin plays an important role in the plant-pathogen interaction, probably as a phytoanticipin. In this stuy it was found that the amount of vanillin (2.2 fold) decreased in infected plants compared to control plants. Vanillin is the major component of natural vanilla, a well-known food and cosmetic additive and has antioxidant and antimutagenic properties. Their accumulation is highly sensitive to environmental conditions such as light, water and nutrient availability, and pathogen infection (Harvell and Bosland, 1997).

In several studies were revealed that the *Cuscuta* parasitism caused to changes on host plants (Mishra and Sanwal, 1995; Runyon et al., 2008; Vurro et al., 2011; Furuhashi et al., 2012). The effect of parasitic plants sometimes can be positively on the host plant. Plants have develop the ability to sense attacks and respond by activating defense system (Karban and Baldwin, 1997; Dangl and Jones, 2001). For instance it is exposed that *Cuscuta* changed some phytohormones (e.g. SA) and influenced their defense mechanism towards to insect herbivores on tomatoes (Runyon et al., 2008).

So we propose the phenolic changes that induced by parasitic attacks may be effective especially on the production of medicinally important compounds. For instance, our results revealed that the *Cuscuta* attack increased the amount of some phenolics in Carthamus. In addition, it is known that the Carthamus plant contains compounds inhibit the STAT-3 gene is directly related to prostate cancer (Taglialatela-Scafati et al., 2012). Raza et al., (2015) revealed that *Carthamus oxyacantha* has terpenoids, natural phenolics and alcaloidal skeleton and has the DPPH activities.

CONCLUSION

A higher concentrations of malic acid, quinic acid, chlorogenic acid, tr-aconitic acid, and hyperoside were obtained from *C. glaucus* Bieb. plants. On the other hand, lower concentrations of gallic acid, protocatechuic acid, tr- caffeic acid, vanillin, p-coumaric acid, hesperidin, 4-OH benzoic acid, quercetin,salicylic acid, naringenin, luteolin, kaempferol, apigenin, chrysin were obtained from *C. glaucus* plants that infected with *Cuscuta.*

As result of this study, when the amount of quercetin (8.1 fold), quinic acid (2.8 fold), gallic acid (2.4 fold), protocatechuic acid (2.1 fold), hyperoside (2.4 fold), malic acid (1.01 fold), chlorogenic acid (1.7 fold), tr- caffeic acid (1.7 fold), rutin (1.2 fold), p-coumaric acid (1.1 fold), naringenin (3.1 fold), luteolin (1.4 fold), apigenin (1.7 fold), and chrysin (1.05 fold) increased, the amount of kaempferol (72.4 fold), vanillin (2.2 fold), 4-OH benzoic acid (1.7 fold), salicylic acid (1.7 fold), tr-aconitic acid (1.4 fold), and hesperidin (1.2 fold) compounds decreased (Table 2) in infected *C. glaucus* plants.

Table 2. The phenolic changes in infected plants

Compounds	Parent ion(m/z)[a]	MS^2(CE)[b]	Ionization Mode	Amount(µg analyte/g extract)[c]	
				Control	Infected plant
Quinic acid	190.95	85 (22).93 (22)	Neg	7794.62±37. 4146	21977.30± 10.549
Malic acid	133.05	115 (14).71 (17)	Neg	191553.83±101. 52	194456.14± 103. 06
tr-Aconitic acid	172.85	85 (12).129 (9)	Neg	4222.33±206. 89	2970.00± 145. 53
Gallic acid	169.05	125 (14).79 (25)	Neg	4.165± 0.212	10.12± 0.516
Chlorogenic acid	353	191 (17)	Neg	8199.86± 401. 79	13943.77± 683. 24
Protocatechuic acid	152.95	109(16).108 (26)	Neg	40.389± 2.059	86.721± 4.422
tr- caffeic acid	178.95	135(15).134 (24).89 (31)	Neg	79.844± 4.151	137.603± 7.155
Vanillin	151.05	136 (17).92 (21)	Neg	34.656± 1.698	15.374± 0.753
p-Coumaric acid	162.95	119 (15).93 (31)	Neg	26.338± 1.343	29.683± 1.513
Rutin	609.1	300(37). 271 (51). 301 (38)	Neg	94.649± 4.732	119.947± 5.997
Hesperidin	611.1	303(24).465 (12)	Poz	511.851± 25.08	422.168± 20.68
Hyperoside	463.1	300(27).301 (26)	Neg	2152.131± 105. 45	5276.118± 258. 52
4-OH Benzoic acid	136.95	93 (17).65 (27)	Neg	32.118± 1.670	18.521± 0.963
Salicylic acid	136.95	93(16).65 (31).75 (30)	Neg	33.787± 1.68	19.807± 0.99
Quercetin	300.9	179(19).151 (21).121 (28)	Neg	42.260± 3.00	343.350± 24.37
Naringenin	270.95	151(18).119 (24).107 (26)	Neg	25.839± 1.42	81.893± 4.50
Luteolin	284.95	217(25).199. (28).175(29).151 (25)	Neg	72.630± 5.01	104.701± 7.22
Kaempferol	284.95	217(29).133 (32).151 (23)	Neg	75.834± 3.94	1.047± 0.05
Apigenin	268.95	151(25).117 (35)	Neg	770.217± 40.82	1309.9± 69.42
Chrysin	253	143(29).119 (32).107 (26)	Neg	0.767± 0.04	0.810 ± 0.04

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