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Detection of Phytoconstituents: Therapeutic, Nutritional and Industrial of Cuscuta Australis Seeds Parasitizing on Basil

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Abstract

Cuscuta australis is a weed parasitic plants and its seeds are rich of many mineral elements, organic and inorganic acids. The current study was conducted with the aim of detecting the beneficial chemical compounds in the seeds of C. australis parasitizing on basil plants. Plant parasite samples were collected from one of the basil growers' fields in Al-Mishkhab region (44.5 E°, 31.89 N°, 177 m elevation from sea level), then they were separated from the host and its seeds were purified. Hexane was used to separate and isolate compounds by GC-MS as well as micro- and macro-mineral elements, dyes, plant hormones, protein, fats, carbohydrates, fibers, sugars, total phenols, and amino acids were estimated. Results of seed analysis by Gas chromatography/mass spectrometry showed the presence of ten common compounds of medical and nutritional importance. There were differences in the concentrations of the estimated micro- and macro elements. Detected hormones, plant dyes, food compounds, and amino acids had significant concentrations. The process of detecting chemical compounds in the seeds of C. australis parasitized on the basil plant was full of many compounds. The methodological importance of this work will provide a great opportunity to find alternatives from the agricultural environment which can be used in therapeutic, nutritional, and industrial aspects in the future.

Keywords:

C. australis, basil, nutrition, plant parasite, seeds.

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Introduction

Cuscuta australis (Dodder) belonging to the Convolvulaceae family, the presence of this weed was recently recorded in 2019 to be with fifteen other different species distributed in all agricultural fields in Iraq that can invade broad- and thin-leaved plants according to their nutritional preference (Romeilah et al., 2021). It can be described as a yellow-orange plant consisting of fibers-like threads with a primitive root, leafless, possesses a special organ called haustoria to invade the host plant to absorb nutrients and water due to its weak photosynthesis (Nayak & Raghatate, 2024). Therefore, it depends mainly on the host plant because it is holoparasitism (Bernal-Galeano et al., 2022). Dodder contains many compounds used as antibacterial, anti-oxidant, and anti-severe diseases (Al-Gburi, 2021; Abu-Izneid et al., 2021). These compounds are very important as the World Health Organization indicated that approximately 80-90% of the world's population uses herbal medicines or plant extracts (Muhammad et al., 2020; Bošković et al., 2018). Many ancient Chinese and Japanese as well as modern medicine studies, have confirmed the effectiveness of dodder seed powder in treating arthritis and osteoporosis, or as antimicrobial especially bacterial, diuretic, antioxidant, reduces blood pressure, a protective substance for the liver, a reducing agent, an anti-tumor cancer, anthelmintic, and antidepressant (Jakovljević et al., 2018; Thirunavukkarasu et al., 2024). The sustainability of dodder growth depends mainly on the type of host plant, the absorbing organic and inorganic materials, so it can be found that there are differences in the number and quantity of active compounds in its filament, flower and seed (Richhariya et al., 2012; Karimi & Bahrami, 2016). The trend based on detecting compounds in dodder seeds was become very important because they are considered the physiological end of growth and the completion of the maturity stage, as they retain many chemical compounds surrounded by a hard shell that enables them to last for more than 10 years (Savitha et al., 2021). Many analytical medical studies on dodder seeds did not specify the type of host plant, but they were rich in information. In contrast, they were directed in a unilateral direction by focusing on estimating polar compounds on the one hand and not estimating nutritional compounds on the other hand for the same sample of dodder (Noureen et al., 2018; Alborji, 2014). Therefore, the current study aimed to reach a key to developing an alternative approach by detecting various therapeutic and nutritional compounds in the seeds of *C. australis* parasitized on basil (Toraman et al., 2020).

Materials and Methods

C. australi s Samples

Infected basil plants by *C. australis* were collected from Al-Mishikhab region (44.5 E°, 31.89 N°, 177 m elevation from sea level) as a fresh sample. The samples were at the mature stage (contains seed and flowers of dodder) washed individually by tap water to get rid of any traces of dust. After that, it was morphologically diagnosed (Spaulding, 2013), and molecularly identified then deposited into National Center for Biotechnology Information (NCBI) and recorded with PP082458 accession number. *C. australis* seeds were purified, then dried in the oven at 70 °C for 2 days. Seed were finally grinded to powder form by using mixer grinder.

The Preparation of C. australis Samples for GC–MS Analysis

30 g of the *C. australis* seeds powder was added to 100 ml of hexane at room temperature to get static maceration samples three times in 7 days with replacing the solvent every 2 days (Marín et al., 2018).

Using GC–MS to Analysis the Samples

Hexane was used to extract seeds samples by Gas chromatography/mass spectrometry (Al-Gburi et al., 2019), Compounds were identified using molecular formula according to (Tripathi et al., 2013; Tassakka et al., 2021).

Estimation the Mineral Nutrients of Seeds

C. australis seeds content of macro and micro elements was estimated following procedures (Biel et al., 2018; Dutta & Khaled, 2021; Radha et al., 2021).

The Determination of Pigments (mg. g-1)

Pigments content in *C. australis* seeds was estimated using UV–Visible spectrophotometer (Sumanta et al., 2014).

Free Hormones Determination (µM)

GA3, ABA IAA and Zeatin content in the seeds of *C. australis* was determined following the procedure of (Al-Gburi et al., 2024).

Amino Acid Estimation (mg. g-1)

The method described (Dahl-Lassen et al., 2018) was used to extract amino acids using UHPLC.

The Estimation of Protein%

Protein was determined using Kjeldahl method (Chavez-Murillo et al., 2011).

Total Phenolic Compound Determination (mg. g-1)

Folin–Ciocalteu reagent was used to determine the total phenol, according to (Asaduzzaman et al., 2013).

The Estimation of Total Soluble Carbohydrates (mg. g-1)

Total soluble carbohydrates percentage in the seeds of *C. australis* was estimated following (Himani & Madan, 2018) method.

Nutritional Content of Seeds of C. australis

The percentage of fat %, fibre%, total sugars (mg. g^{-1}) and Amylose/Amylopectin ratio in the seeds of *C. australis* were determined (Shanita et al., 2011; Milala et al., 2018; Al-Gburi & Al-Gburi, 2024).

Results

GC–MS Analysis

GC–MS analysis of *C. australis* seeds showed that there were ten known compounds with its peak area% (Table 1) and these compounds were differed in its beneficial use to humans as $C_{32}H_{62}O_2$ and $C_{36}H_{74}$ are useful in the manufacture of sanitary detergents, while, $C_{29}H_{44}O_2$, $C_{28}H_{48}O_2$ and $C_{28}H_{48}O$ are used as antioxidants, $C_{24}H_{41}F_7O_2$ is active compound against microbes and insects, $C_{29}H_{48}O$ is used to prepare progesterone, $C_{19}H_{34}O_2$ considered an bio-active compound against SARS-CoV-2, $C_{29}H_{52}O_2$ is active compound against cholesterol, triglycerides and sugar, finally, $C_{30}H_{50}O$ is essential for membrane integrity as well as it used in the synthesis of steroid hormone.

Table 1. GC–MS analysis of C. australis seeds

Peak	Retention time (min)	Name of Compound	Molecular Formula	Molecular weight (g/mol)	Peak Area (%)
1	23.493	9-Octadecenoic acid (Z)-, tetradecyl ester	$C_{32}H_{62}O_2$	478.8	27.32
2	24.068	Hexatriacontane	$C_{36}H_{74}$	507.0	7.14
3	24.416	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R- [2	$C_{29}H_{44}O_2$	424.7	6.03
4	25.121	gamma. - Tocopherol	$C_{28}H_{48}O_2$	416.7	10.16
5	26.722	5-Cholestene-3-ol, 24-methyl-	$C_{28}H_{48}O$	400.7	5.61
6	26.892	Eicosyl heptafluorobutyrate	$C_{24}H_{41}F_7O_2$	494.6	3.42
7	26.956	Stigmasterol	$C_{29}H_{48}O$	412.7	3.24
8	27.288	E, E, Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294.5	14.52
9	27.593	gamma. - Sitosterol	$C_{29}H_{52}O_2$	432.7	19.26
10	27.756	Cholest-5-en-3-ol, 24-propylidene-, (3. beta)	$C_{30}H_{50}O$	426.7	3.30
100					

Macro and Micro Mineral Elements

There are differences in the percentage of mineral elements concentrations that estimated in *C. australis* seeds when the highest concentration was recorded in sodium reached 0.468 in comparison with the lowest concentration recorded 0.00086 in boron (Table 2).

Table 2. C. australis seeds content of macro and micro mineral elements

%Nitrogen	**% Potassium**	**%Calcium**	**% Magnesium**	**%Sodium**	**% Iron**
0.125	0.432	0.392	0.306	0.468	0.144
%Manganese	**% Zinc**	**% Copper**	**% Phosphorus**	**% Silicon**	**% Boron**
0.018	0.0079	0.364	0.113	0.027	0.00086

Pigments and Plant Hormones

Table 3 showed that the concentration of total chlorophyll amounted 0.0249 mg. $g^{-1}$and Carotene concentration reached 0.0168 mg. g^{-1} in *C. australis* seeds. The concentration of plant growth regulators amounted 0.24 µM for IAA, 0.36 µM for GA_3, 98.15 µM for ABA and 1.108 µM for Zeatin.

Table 3. C. australis seeds content of pigments and plant hormones

Total chlorophyll (mg. g^{-1})	Carotene (mg. g^{-1})	IAA (µM)	GA_3 (µM)	ABA (µM)	Zeatin (µM)
0.0249	0.0168	0.24	0.36	98.15	1.108

Amino Acids

There were 13 amino acids identified in *C. australis* seeds and these acids were differed in their concentration (Table 4) as proline recorded the highest concentration reached 12.16 mg. g^{-1}in comparison with the lowest concentration recorded in valine amounted 0.19 mg. g^{-1}.

Nutritional Compounds

Nutritional compounds of seeds of *C. australis* were listed in Table 5, the percentage of protein amounted 0.784, fat, 0.372 and fibers 0.549, while, the total sugars amounted 7.543 mg. g^{-1}, the total phenols12.026 mg.

g^{-1}. Total soluble carbohydrates concentration reached 14.854 mg. g^{-1}and Amylose/Amylopectin ratio reached 0.182.

Table 4. C. australis seeds content of amino acids

Phenylalanine (mg. g^{-1})	**Serine (mg. g^{-1})**	**Valine (mg. g^{-1})**	**Tryptophan (mg. g^{-1})**	**Isoleucine (mg. g^{-1})**
5.85	1.74	0.19	2.34	1.67
Alanine (mg. g^{-1})	**Arginine (mg.g^{-1})**	**Aspartic (mg.g^{-1})**	**Methionine (mg. g^{-1})**	**Cysteine (mg.g^{-1})**
4.52	1.43	1.09	1.98	1.33
Proline (mg. g^{-1})		**Lucien (mg. g^{-1})**		**Glycine (mg. g^{-1})**
12.16		8.38		1.79

Table 5. C. australis seeds content of nutritional compounds

% Protein	**% Fat**	**% Fibre**	**Total sugars (mg. g^{-1})**	**Total phenols (mg. g^{-1})**
0.784	0.372	0.549	7.543	12.026
Total soluble carbohydrates (mg. g^{-1})				**Amylose/Amylopectin ratio**
14.854				0.182

Discussion

Previous studies have considered basil a valuable plant resource due to its nutritional and health benefits and its containment of antioxidant compounds, fatty acids, essential oils, phenols, flavonoids, alkaloids, and nutrients (Hariyanti et al. 2019; Shahrajabiana et al., 2020). The soft cuticle and peel of basil stems make it a suitable host for *C. australis* parasitism and complete the penetration of haustoria to absorb natural chemical resources to avoid death (Shimizu et al., 2019; Toman & Al-Gburi, 2023). The explanation of the presence of various mineral elements in the seeds of *C. australis* is that the process of transporting and storing nutrients in the basil stem occurs through a process of transport from the root over long distances through the xylem, then transport through the phloem, then redistribution takes place through membrane transport (Förste et al., 2020; Bais & Kakkar, 2013) indicated that the chemical components (Hikmawanti & Nurhidayah, 2019) of dodder depend mainly on the type of host plant, as the growth stages of *C. australis* require a variety of acids, alkaloids, and oils, which are made by bridging with the vascular bundles of basil to transport water and substances the eventually *C. australis* forms reproductive organs to complete its life cycle (Kokla & Melnyk, 2018; Al-Gburi & Mohammed, 2019). The intimate vascular connections between *C. australis* and basil lead to plant parasite having a set of allelochemicals (Moreno-Robles et al., 2022) to develop mechanisms that enable it to withstand unfavorable conditions, herbicides, and biotic and abiotic stresses to form fully developed seeds that produce active seedlings to attack the host plant (Azimi et al. 2017; Masi et al. 2022). The increase in the concentration of the Abscisic acid hormone has an important role in the cumulative or multiple gene expression in order to maintain the dormancy of *C. australis* seeds. In addition, the nutritional reserves stored in the endosperm of *C. australis* can play a role in increasing the seeds' tolerance to adverse unsuitable environmental or biological conditions and protection of seedlings from death in the event that they are unable to determine a compatible host plant and complete the process of parasitism (Olszewski et al., 2020).

Conclusion

The process of detecting chemical compounds in the seeds of *C. australis* parasitized on the basil plant was full of many compounds that have nutritional, medicinal, and industrial benefits as well. It was found that the difference in the type and concentration of these compounds varies mainly according to the type of host plant, in addition to the suitability of the surrounding conditions for the continued growth and reproduction of *C.*

australis. This work could provide a great opportunity to find alternatives from the agricultural environment which can be used in therapeutic, nutritional, and industrial aspects in the future.

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