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ALKALOID CONTENT AND EFFECTIVENESS OF ANTI-BACTERIAL AND OXIDATION EXTRACTS OF THE SPECIES *Cuscuta chinensis* Lam. (CONVOLVULACEAE)

Salih, S. H. [1] ; T. O. Alkhazraji [2]and A. A. Ali[2]
1- Biology Department - College of Education - Garmian University
2- Biology Department - College of Education - Tikret University

ABSTRACT

Alkaloid content and its components type of the species *Cuscuta chinensis* was determined by using high-performance liquid chromatography (HPLC). The effectiveness of the aqueous extract of the studied species at concentration of 100 mg /ml was evaluated on the growth of four species of pathogenic bacteria positive and negative gram (*Staphylococcus aurens*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Kocuria rosea*). The anti-oxidant effectiveness of the *C. chinensis* was studied by 2,2-Diphenyl-1-picrylhydrazyl (DPPH). Six alkaloids (Isolupanine, Retmine, Cuscutine, Isoquinoline, Aloperine and Lupanine) were diagnosed and the aqueous extract showed its effective on growth inhibition of the studied species of bacteria. The inhibition proportion reached to 92.49% as compared to the standard ascorbic acid (94.09%).

Keywords: Phytochemistry, Antibacterial Antioxidant Effectiveness, Alkaloids, *Cuscuta chinensis*

INTRODUCTION

The studies on the plants chemical content and antimicrobial effectiveness were increased in the recent years due to their antioxidant effective. Drugs derived from medical plants are daunting, harmless and free of side effects compared with antibiotics which increases the resistance with the time, in addition is inexpensive economically as well as other reasons, including the natural appearance of microorganisms' antimicrobial resistance (Aqil and Ahmad, 2003, Randhir, 2007). There are many identified plant species are not studies chemically or medically (Hassan, 2012), *Cuscuta* species (Convolvulaceae) is one of these important plants (Costa et al, 2009), which are widespread use as medicinal plants in India and Southeast Asia and China. The name of *Cuscuta* was reported in the Chinese Constitution of medicinal plants that was used in the treatment of liver disease, kidney and sexual vulnerability through direct eating of plant. However nowadays the *Cuscuta* spp. use as anti bacterial and fungal or inhibitor of cancer cells or antioxidant and against muscle stiffness and anti-viruses with the several other applications. The high effectiveness of genus *Cuscuta* on the microbes growth with other medicinal uses belonging to acquire a group of influential secondary metabolites and to its containing the important alkaloid (*Cuscutine*) (Sharma *et al.,* 2012, Raza *et al.,* 2015 and Nabiabad *et al.,* 2015). Therefore, the aim of this study is to find out the alkaloid content and their effectiveness against bacteria and microbes of the studied species.

MATERIALS AND METHODS

Plant Collection and Identification

The plant specimens in flowering period (March – May) were collected in field trips in plain areas, the fresh specimens was preserved in plastic bags with labeling (collection place, date, collector name, ...)(The date of experiment in January 2016) and then transferred to the laboratory as it was pressed and dried, others of them have been cleaned and then rinsed with water and dried at room temperature away from light (to avoid oxidation). After drying the plants has been grinding by using electric mill and then put them in plastic containers and stored refrigerated until use. Plant identification based on the Flora of Turkey (Davis *et al.*, 1982). Experiments were carried out in the laboratories of the Biology Department of the College.

Extraction and Chemical Analysis:

Extraction with cold water method is used, according to (Al- Joboory and Al-Rawi , 1994) by mixing 40 gm of plant sample with 160 ml of distilled water (ratio of 1:4 Weight: size), move the mixture to break down plant cell walls and leave it in the refrigerator for 24 hours for the soaking. It was extracted through several layers of medical gauze, and extracted once again by Buchner funnel using whatman papers No.1 to get rid of the uncrushed parts and fibers to obtain plant extract raw liquid which put them in a Rotary vacuum evaporator at the temperature of not more than 40^{o} as it works on the basis evaporation under rarefied pressure. Extracted output was placed in Shaker incubator at a temperature of 30-35^{o}. Dried extract was saved freeze in airtight containers and labeled while in use.

Chemical Study:

The alkaloid content of complete flowering plant (flowers and stem) was estimated in species *Cuscuta chinensis* by using a technique (HPLC) High-Performance Liquid Chromatography as follows:

Extraction:

The method used to extract alkaloids was recommended by Kamada *et al.* (1986). Where this method provides a good amount of alkaloids derived from the use of a small amount of the sample (about 20 gm) compared to the other methods used for complete extraction of alkaloids for analysis by HPLC technique. The steps of extraction were accorded follows:

Powdered plant material
↓
OH-NH4OH (25%) (15:5:1)-Extraction with CHCl3 Me
ml per 100 mg of sample) sonicated for 10 min 10)
↓
Leave at room temperature for 1h
↓
٣CHCl paper and wash 2× with 1 ml filter Filter through
↓
Evaporate to dryness
↓
and 2ml 1 N H2SO4and mix thoroughly ٣CHCl ml Add 5
↓
on ice NH4OH fraction and adjust to Ph. 10 with 28% ٣CHCl Remove
↓
CHCl3 Extract alkaloids once with 2 ml CHCl3and twice with 1 ml
↓
CHCl3 and wash the residue with 1-2 ml Filter after the addition of anhydrous Na2SO4
↓
Evaporate and dissolve the sample in 1ml Me OH

Separation conditions of alkaloid compounds were:
column of HPLC (I.D. 50X2.0mm) C18
mobile phase: [(70:30, V/V) 0.05M phosphate buffer pH 6.2: acetonitrile].
wavelength 210 nm
flow ratio 1.0 ml/min
temperature 30^{o}
standard concentration 25 mg/ml

Alkaloids concentrations of *Cuscuta chinensis* estimated compared confined area under the peaks of such standard and adopted alkaloids appeared at the same detention time calculated in minutes

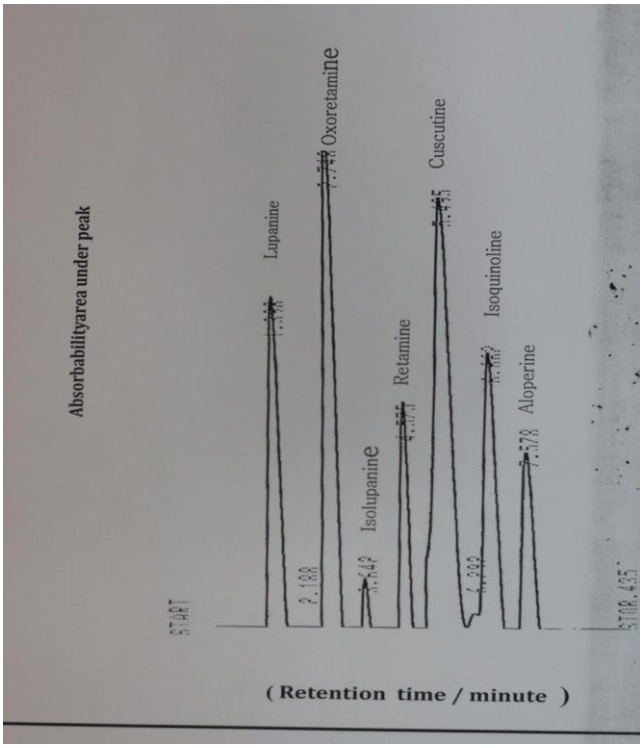

Figure1. The standard alkaloid peaks by HPLC

To calculate the concentrations of unknown diagnosed alkaloids in the three species as well as the percentage of the diagnosed compound, the following equations was used, (Sawhney and Singh, 2011)

= x x

Concentration percentage = 100 x

Table1. The standard analysis of alkaloids with retention time, area and concentration.

Seq.	Alkaloids	Retention time minute	Area	Concentration ٢٥µgm \ml
١	Lupanine	١.٣٩	١٠٦٩٦٨	٢٥
٢	Oxoretamine	٢.٧٤	١٤٣٦٨١	٢٥
٣	Isolupanine	٣.٦٤	٥٩١٦٨	٢٥
٤	Retamine	٤.٥٧	١٠٤٣٩٠	٢٥
٥	Cuscutine	٥.٤٩	٢٦٢٦٠٣	٢٥
٦	Isoquinoline	٦.٦٦	١٣٦٣٥٢	٢٥
٧	Aloperine	٧.٥٧	٨٦٥٥٩	٢٥

Antibacterial Activity:

This study was conducted by the following steps:

Sterilization of used extracts and concentrations :

The method of Mukhtar (2009) was adopted in preparation of the aqueous extract and sterilization by putting 1 gm of dry plant extract powder in 10 ml of distilled water, the extract concentration became 100 mg/ml. the solution sterilized by whatman paper No.1 to get rid of microbial contaminants existing

Culture Media Preparation:

The culture media (Mueller Hinton Agar) and (Nutrient Broth) was prepared according to the manufacturer's instructions and by the methods of Atlas (1995). The media sterilized at a temperature of 121 C^o and pressure of 15 pounds/inch for 15 min. The media were distributed in Petri dishes (9 cm diameter) and left the dishes in laboratory temperature for hardening.

Isolates of tested Microorganisms:

Two species of gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and two species of gram positive bacteria (*Staphylococcus aurens* and *Kocuria rosea*) where used. Agar diffusion method was used by welling (Egorove, 1985), to note microorganisms sensitivity of the aqueous extracts of *Cuscuta chinensis* at the higher concentrations (100 mg/ml).

Isolates Bacteria planted in Petri dishes prepared in advance by diffusing 0.2 ml the Bacteria commentator on the Petri dishes using sterilized glass rod L-type then left at room temperature for half an hour in order to get absorption. After drying the surface of agar layer, make holes (drill) by sterilized hand drill with diameter of 5 mm^2, agar discs lifted and neglected, 50 ml of the abstract be taken by micropipette (use one-time), and placed in holes. Dishes incubated at temperature 37^o (±2) for 18-24 hours and the inhibition area was measured (mm) if found according repeaters. The control treatment was prepared as the previous method except putting sterile distilled water instead of plant extraction in the grill (Adiguzel et al, 2009)

Anti-oxidant Effectiveness:

The anti-oxidant bioactivity was examined by measurement of plant specimens ability to inhibit free radicals and using a spectrophotometer and checking style 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and using ascorbic acid as a control to compare the ascorbic acid ability with plant samples ability to free radicals inhibition depending on the ability of antioxidants to the donation electron and convert free radicals into stable compounds that unable to interact with bimolecules in the body thus it act to remove harmful activity of free radicals. The following steps according Stojicevic *et al*, (2013) with some modification as needed

Preparation of standard curve of ascorbic acid:

The solution was prepared by weighting of 10 mg of ascorbic acid and dissolved in 1 ml of absolute ethanol, the final concentration is 10 mg/ml, five concentrations was used are 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml, they were examined with the preparator by dissolving 0.002 mg of DPPH in 100 ml of ethanol, samples kept in the dark at room temperature for 30 minutes and then measured by spectrophotometer at wavelength 517 nm at the inhibition ratio of using aqueous extract of plant specimens in the same concentrations however the plant extract has been used instead of ascorbic acid. 0.5 ml of the sample was added to 2.5 ml of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), the mix was incubated for 30 minutes in a dark place, and Absorbance readings were taken at a wavelength of 517 nm by spectrophotometer.

The ratio of inhibition of free radicals measured by using the following equation:

The Inhibition % = x100

The results shown in figure 2. Inhibition ratio has reached to 94.09% at the concentrate of 10 mg/ml

Figure 2. Percentage of free radicals inhibition by ascorbic acid at different concentrations between 2 and 10 mg / ml.

RESULTS AND DISCUSTION

Alkaloid Contents of *C. chinensis:*

Chemical analysis by HPLC showed that the *C. chinensis* contained six types of alkaloids they were lupanine, Isolupanine, Retamine, Cuscutine, Isoquinoline and Aloperine (Fig. 3). The concentration of alkaloids was identified as follows, lupanine 29.59%, Isolupanine 50.50%, Retamine 16.75%, Cuscutine 23.66%, Isoquinoline 38.08% and Aloperine 24.48%,. It was seemed existence of a significant difference in the concentration of alkaloids in the same species, where the largest proportion is the alkaloid Isoquinoline 38.08% and the lowest rate is for the Retamine 16.75%. The alkaloids are secondary metabolites products, produced by the plant in response to external conditions that like herbivores, bacteria, fungi and insects. The existence of alkaloids in studied species was concurrent with the studies of Wink (1987, 1993 and 1998), which pointed out that the alkaloids (Quinolizidine alkaloids group) of the host plant *Genista acanthoclada* (Fabaceae) transferred to the species *Cuscuta palaestina* by the parasite plant nutrition. According to the literatures reviewing this is the first that in Iraq. The identified alkaloids in this study have medical importance as toxins, anti-microbial and treatments, Erdemoglu et all (2009) which indicated to significance of Lupanine as anti-microbial.

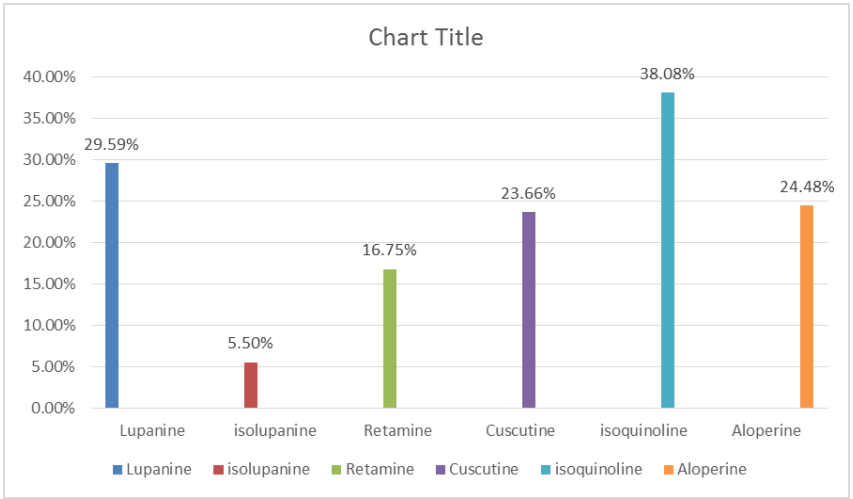

Figure 3. Identified alkaloids concentration in *Cuscuta chinensis*

Impact of the aqueous extract of *C. chinensis* on the bacteria growth:

The results showed that the high ability of cold aqueous extracts of *C. chinensis* to the bacteria inhibition at concentration 100 mg /ml, inhibition area diameter (mm) as compared to control factor (Table 3). Where the average diameter of inhibition area in the species *Staphylococcus aurens*, *Kocuria rosea* and *Pseudomonas aeruginosa* 15.6 mm and 7.6 mm and 6 mm respectively at the concentration of 100 mg/ml and in comparison with control. However the aqueous extracts of *C. chinensis* did not show any effectiveness against species *E. coli* gram negative, it may be due to possession resistance gene, or for non-aqueous extract efficiency due to the fact that some of the few active substances soluble in water. However the gram positive bacteria *Staphylococcus aureus* was more species of bacteria affected aqueous extract of *C. chinensis* while bacteria *E. coli* more resistant species to aqueous extracts of the whole flowering plant of the *C. chinensis* at concentration 100 mg /ml. conform to the current study, with many of the previous studies on the effect of aqueous extract species of *Cuscuta* against bacterial species differ with them at the same time of diameter of inhibition area where the aqueous extract at 100 mg/ml is highly effective due to the high percentage of concentration as compared to the concentrations used in other studies, also the results of the current study are consistent with the results of Anjum and Khan (2003), who found that there are differences in the inhibitory effectiveness of extracts of *Cuscuta reflexa* to microorganisms inhibition in bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* and *Bacillus licheniformis*.

The study of Summit *et al.* (2010) on the species *C. reflexa* parasite on *Acacia arabica* and *Zizyphus jujuba* appeared that there are important differences in the use of extract of *C. reflexa* to inhibition studied microorganisms depending on the species of host plant, as well as this study are consistent with the Nabiabad et al, (2015) on the effect of aqueous extract of the *C. chinensis* on the four species of bacteria (*Clavibacter michiganensi, Pectobaterium carotovorum, Ralstonia solanaceara* and *Streptomyces scabies*)

Anti-oxidants Effectiveness of *C. chinensis:*

The results (Fig. 2) showed that the aqueous extract of the species *C. chinensis* has a good effective in the free radicals inhibition despite the non-superiority over the standard ascorbic acid, but it was close to it which defined with high effective in curbing, the activity of free radicals also results showed the species *C. chinensis* had inhibited the free roots by the inhibition reached 92.49% compared to standard ascorbic acid 94.09%. The results came close with results Nabiabad *et al*. (2015) about the effectiveness of anti-oxidant of the *C. chinensis* in Iran by using aqueous extract and methanol extract and ethanol extract. The anti-oxidation effectiveness of *C. chinensis* consistent with the medical use of species as an antidote for many diseases in the body.

CONCLUSION

This study concluded that the studied species of *Cuscuta* contain secondary metabolites with high medical importance to inhibition harmful microbes so need to extensive studies in future.

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المحتوى القلويدي والفعاليه المضادة للبكتريا والأكسدة لخلاصة نوع جنس الحامول *Cuscuta chinensis* Lam. (Convolvulaceae)

سيروان حسن صالح*, طالب عويد الخزرجي و علي احمد علي****

*** قسم علوم الحياة - كلية التربية - جامعة كرميان**

****قسم علوم الحياة - كلية التربية - جامعة تكريت**

تم دراسة المحتوى الكيمائي (القلويدات) للنوع *Cuscuta chinensis* باستخدام تقنية High-performance liquid chromatography (HPLC) وتحديد انواع المركبات القلويدية ونسبة تركيزها اضافة الى معرفة مدى فعالية المستخلص المائي للنوع المدروس عند التركيز ١٠٠ملغم/مل على نمو اربعة انواع من البكتريا المرضية الموجبة والسالبة لصبغة غرام (*Staphylococcus aurens, Escherichia coli , Pseudomonas aeruginosa* and *Kocuria rosea*) ودراسة الفعالية المضادة للأكسدة في النوع *Cuscuta chinensis* بإعتماد طريقة , 2,2-Diphenyl-1-picrylhydrazyl (DPPH) و قد تم تشخيص ستة قلويدات (Isolupanine, Retmine, Cuscutine, Isoquinoline, Aloperine and Lupanine) وكما اظهر المستخلص المائي فعاليته في تثبيط النمو لانواع البكتريا المدروسة بنسبة تثبيط وصلت الى ٩٢.٤٩% مقارنة بدون المعيار حامض الاسكوربك ٩٤.٠٩%.