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The inhibitory Effect of Crude Extract of *Arctium lappa* on some pathogenic bacteria

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Abstract

This study was intended to evaluate the antimicrobial activity of crude extract of *Arctium lappa* on some pathogenic bacterial species in vitro. The study was included different types of gram-positive and gram-negative bacteria such as (*Pseudomonous aerogenosa, Staphylococcus aureus, Salmonella typhimurium, E.coli, Listeria monocytogene, Streptococcus pyogenes, Brucella abortus* and *Bacillus anthracis*). The results of this study revealed either classified resistant or sensitive according to *Arctium lappa* concentration. A significant difference at ($P<0.05$) was appeared, at 50 mg of the extract, which indicated that gram positive bacteria had more resistant than gram negative bacteria. Whereas, sensitivity were counted more gram negative bacteria than gram positive bacteria. Gram positive bacteria had accounted 5 times in contrast of Gram negative bacteria. The results of this study were also revealed a difference inhibitory effect of *Arctium lappa* crude extract at 100 mg against different pathogenic bacteria. Non-significant difference at ($P>0.05$) were appeared, which indicated that gram positive bacteria had more resistant than gram negative bacteria, whereas sensitivity were counted more gram negative bacteria than gram positive bacteria. In conclusion, the results of this study revealed that *Arctium lappa* crude extract has inhibitory effects against different types of pathogenic bacteria with some variability in its activity depending on the concentration of extract.

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Introduction

Plants have been a valuable source of natural products for maintaining human health and the use of these products can be of great significance in therapeutic treatments (Seenivasan *et al.*, 2006). The root and leaves of *Arctium lappa* are listed by the Council of Europe as a natural source of food flavouring. It is widely used in folk medicine (Gentil, 2006). *Arctium lappa*, is commonly known as burdock, and is being recommended as a healthy and nutritive food in

Chinese societies. Burdock has been used therapeutically in Europe, North America and Asia for hundreds of years (Lou *et al.,* 2010). The roots of edible burdock *Arctium lappa* is contain a higher amount of polysaccharides and residues than other vegetables. It is used as a food in Asia and it can easily obtained all year round (Kato and Watanabe, 1993). A previous studies evaluated the antibacterial activity of *Arctium lappa* (Holetz *et al.,* 2002), and its antioxidant activity (Lin *et al.,* 1996). Previous studies also approved its anti-bacterial and antifungal activities (Teske *et al.,* 1991;Holetz *et al.,* 2002). Other studies referred to the gastro-protective activity of *Arctium lappa* (Oseni and Yussif, 2012) and exhibits antimicrobial activity against oral microorganisms (Perin *et al.,* 2002). *Arctium lappa* extracts showed microbial inhibitory effects against endodontic pathogens as *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *C. albican* (Pereira *et al.,* 2005). *Arctium lappa* is considered as an important natural source and has many health benefits. It have been reported that *Arctium lappa* contain different classes of bioactive secondary metabolites, include among others, flavonoids and lignans (Ferracane *et al.,* 2010). The extract of burdock root has shown activity against Gram negative microorganisms (*Escherichia coli, Shigella flexneri and Shigella sonnei*) (Alonso, 2004). The inhibitory effects of *Arctium lappa* have been appeared from extracts of the root, flowers and the leaves .The flowers and root extracts are active against gram negative bacteria, while the leaves extract is active against gram positive and gram negative bacteria (Chan *et al.,* 2011).
There are scarce studies regarding the activity and action of *Arctium lappa* against pathogenic bacteria in Iraq. So, this study was designed to evaluate the inhibitory effect of *Arctium lappa* crude extract against some pathogenic bacteria isolated from Iraq.

Materials and Methods

Bacterial culture

The following bacterial isolates were used in this study: Staphylococcus *aureus, Salmonella typhimurium, E. coli, Listeria monocytogenes, Streptococcus pyogenes, Brucella abortus and Bacillus anthraces*. These isolates were kindly provide by the Zoonosis Unit/ College of Veterinary Medicine/ Baghdad University. The bacterial isolates were confirmed by its biochemical properties (Quinn *et al.,* 1998). Later on, the pathogenic bacterial suspension (1×10^8) CFU was prepared according to method described previously (Quinn *et al.,* 1998).

Plant material and Extraction

Arctium lappa herbs were purchased from the local market of Baghdad. The plant extract was prepared according to previous method (Ahmed *et al.,* 2006) as follow:
Ethanolic extract of plant: A fifty gram (50g) of plant powder was dissolved in 500 ml of 70% ethanolic alcohol and extracted in Erlyn Myer flask. The

extraction was freezing in deep freeze for 9 days then on magnetic stirrer for 2 hours at room temperature.
Then, the sediments were filtered by gauze and filtered by filter paper (Wattman No.1).
The supernatant was evaporated to dryness (45^0C) under reduced pressure in a rotary evaporator.
The resulted powdered or the crude extract from the plant was weighted and kept at -20 ^{0}C until the time of use.

Sensitivity test

The sensitivity test was done according to the following procedures:
Twenty four nutrient agar Petri dishes were used for culture of the bacteria. Bacterial suspension from each isolate was prepared and the number of CFU was calculated according to McFarland method. Then, each four drops were streaked on three petri dishes. The Petr dishes were dried and 3 holes were made in each petri dishes.
Arctium lappa crud extract was added at 50 and 100 mg concentration in the hole of each Petri dish, which were cultured with one type of the pathogenic bacteria. The third hole were filled with ethanol alcohol 70% and act as control.
Then, all the Petri dishes were incubated at 37 ^{0}C for 24 hours. After incubation, the diameter of the inhibition zones were measured to the nearest millimetre (mm) and the results were recorded.

Statistical Methods

All data collected from the experiments were analysed according to different statistical methods. The inferential statistical Cohort indicator (related rates), contingency coefficient (C.C) for probability value and its comparing significant (C.S) were calculated.

Results

The results of the inhibitory effect of *Arctium lappa* crude extract on the growth of different gram-positive and gram-negative pathogenic bacteria species is summarized in Table (1). The results revealed variation in the antimicrobial properties of *Arctium lappa* crude extract at 50mg and 100 mg concentration (Tables. 2, 3). The results revealed strong inhibitory effects against *E.coli* and *Salmonella typhimurium*, with inhibition zone about 25- 27mm, 24- 28 mm in diameter respectively (Figures. 3, 4). In addition, moderate activity against *pseudomonas aeruginosa* was seen, with inhibition zone about 15-20 mm diameter (Figure .3). Moreover, no antimicrobial activity was seen on *Bacillus anthraces* with confined inhibition zone about 1-3mm diameter (Figure .4). *Arctium lappa* crude extract revealed no or limited activity on *Staphylococcus*

aureus, Brucella abortus and *listeria monocytogenes* with inhibition zone about 3- 7 mm, 7-9 mm, 9-11 mm diameter respectively (Figures 5,6).

The distribution of different types of tested gram-positive and gram-negative bacteria (*pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium, E.coli, listeria monocytogenes, Streptococcus pyogenes, Brucella abortus, Bacillus anthracis)* was classified to sensitive or resistant bacteria according to the concentration of *Arctium lappa* crude extract. The results of this study revealed significant difference at ($P<0.05$) at 50 mg concentration. It also express more resistant on gram positive than gram negative bacteria, whereas, the sensitivity was more affected on gram negative bacteria than gram positive bacteria (Table. 2) . In addition, the cohort of gram positive bacteria was 5 times affected, in contrast to gram negative bacteria (Figure .1). The results of this study revealed a non-significant difference at ($P>0.05$) at 100 mg, whereas gram positive bacteria had more resistant than gram negative bacteria. The sensitivity was also more affected gram negative bacteria than gram positive bacteria (Table .3). In addition, the cohort of gram positive bacteria was 3 times affected, in contrast to gram negative bacteria (Figure. 2).

Table 1. Shows the inhibitory properties (inhibition zone diameter in mm) of plant extracts on some pathogenic bacteria :(-) the inhibition zone diameter less than (6) millimeter (Resistant) ;(+) the inhibition zone diameter equal or more than (6) millimeter (Sensitive).

Type of bacteria	Sensitivity Test	Mean zone inhibition in (mm)	
		Dose 50 mg	Dose 100 mg
Gram positive Bacteria			
Staphylococcus aureus	-	3	7
Streptococcus pyogenes	-	3	4
Listeria monocytogene	+	9	11
Bacillus anthracis	-	1	3
Gram negative Bacteria			
E.coli	+	25	27
Salmonella typhimurium	+	24	28
Brucella abortus	+	7	9
Pseudomonous aerogenosa	+	15	20

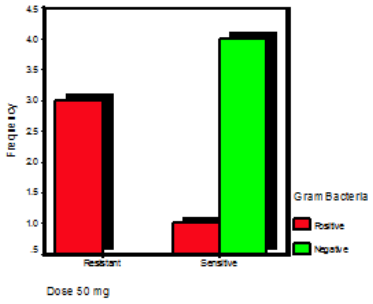


Figure 1. Shows cluster bar chart of different types of tested bacteria among Gram positive and Gram negative bacteria distributed in resistant or sensitive bacteria in 50 mg concentration

Table 2. Represents the distribution of different types of tested bacteria among Gram positive and Gram negative bacteria distributed in resistant or sensitive bacteria in 50 mg concentration

Result	No. and Percent	Gram Bacteria		Total	C.S. (*) P-value
		Positive	Negative		
Resistant	No.	3	0	3	C.C.=0.612 P=0.028 S Cohort G. Bact. Pos. 1 : 5
	% concen. 50 mg	100%	0.0%	100%	
Sensitive	No.	1	4	5	
	% concen. 50 mg	20%	80%	100%	
Total	No.	4	4	8	
	% concen. 50 mg	50%	50%	100%	

(*) S: Sig. at P<0.05

Table 3. Represents the distribution of different types of tested bacteria among Gram positive and Gram negative bacteria distributed in resistant or sensitive bacteria in 100 mg concentration

Result	No. and Percents	Gram Bacteria		Total	C.S. P-value
		Positive	Negative		
Resistant	No.	2	0	2	C.C.=0.500 P=0.102 NS Cohort G. Bact. Pos. 1 : 3
	% concen. 100 mg	100%	0.0%	100%	
Sensitive	No.	2	4	6	
	% concen. 100 mg	33.3%	66.7%	100%	
Total	No.	4	4	8	
	% concen. 100 mg	50%	50%	100%	

(*) NS: Non Sig. at P>0.05

DISCUSSION

In this study *Arctium lappa* crude extract showed higher activity against gram negative bacteria specially *E.coli, Salmonella typhimurium* and *pseudomonas aeruginosa*. These results are in agreement with previous study (Popescu *et al.*, 2010). Popescu *et al.*, (2010) evaluated the antibacterial activity of a phyto therapeutic agent prepared from an ethyl acetate fraction extracted from *Arctium lappa*, they found that this agent inhibited the growth of all tested microorganisms (*Pseudomonas aeruginosa, Escherichia coli, Lactobacillus acidophilus, Streptococcus mutans* and *Candida albicans*). A similar result was also reported by Gentil *et al.*, (2005). They used *Arctium. lappa* ethyl acetate fraction as intra-canal medication for 5 days in teeth infected with *C. albicans, E. coli, L. acidophylus, P. aeruginosa* and *S. mutans* and they found the inhibition of microbial growth after 14 days.

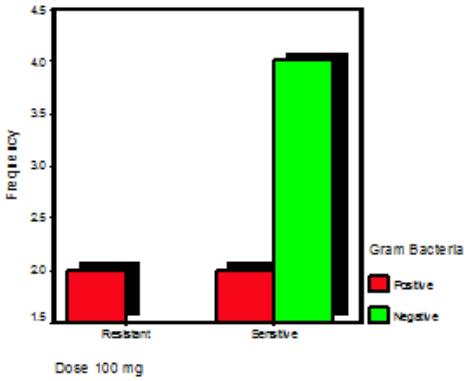


Figure 2. Shows the cluster bar chart of different types of studied Bacteria among Gram Pos., Neg. and the resulted either resistant or sensitive in 100mg concentration.

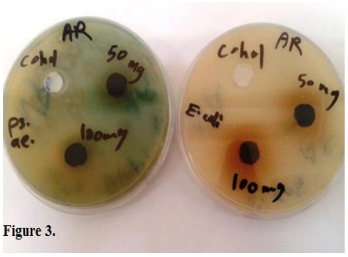
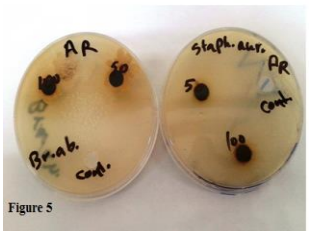




Figure 3. Shows the inhibition zone diameter in millimeter of crude extract against *E.coli* & *pseudomonas aeruginosa*
Figure 4. Shows inhibition zone diameter in millimeter of crude extract against *Salmonella typhimurium* & *Bacillus anthracis*



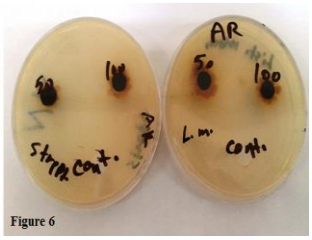


Figure 5. Shows the inhibition zone diameter in millimeter of crude extract against *Staphylococcus aureus* and *Brucella abortus.*
Figure 6. Shows inhibition zone diameter in millimeter of crude extract against *Listeria monocytogenes* and *Streptococcus pyogenes*

Another study were also reported by Ionescug *et al.*, (2013), they showed that the extracts of artichoke, burdock, dandelion, undiluted (100%) and diluted (50%), exhibited antimicrobial activity (bactericidal) against the tested microorganisms (*Eschericha coli*, *Salmonella abonyenteric*). The *Arctiopicrin* is one of the terpenoids components of the leaves, which had been approved its ability to act as antimicrobial agent against gram negative bacteria. The *Arctiopicrin* leaves act against both gram negative and positive bacteria, while

the flowers and roots are only active against negative bacteria (Smith & Boon,1999). The results of the current study showed that *Arctium lappa* crude extract had no activity against *Bacillus anthracis*, *Streptococcus pyogenes* and absence or limited activity against *Staphylococcus aureus*. These results are in agreement with previous study (Ionescu *et al.*, 2013). Ionescu *et al*., (2013) approved that the extracts of burdock, undiluted (100%) and diluted (50%), had no antimicrobial activity (bactericidal) against *Staphylococcus aureus*.
In conclusion, this study revealed that *Arctium lappa* crude extract had higher activity against gram negative bacteria than gram positive bacteria. In addition, the study showed the resistance of gram positive bacteria to this extract. The authors recommend another *in vivo* and *in vitro* future studies to evaluate the activities of *Arctium lappa* crude extract against different types of bacterial and diseases.

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