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Study the Effect of some Medical Plants in Biofilm Formation and Antibiotic Sensitivity for *Klebsiella Pneumoniae*

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Abstract

Twenty clinical and fecal samples (ten clinical samples from patients attending to Imam Ali Hospital and ten chicken faeces samples collected from local poultry farm in Baghdad city) collected during December 2015, for isolated *Klebsiella pneumoniae*. All *K. pneumoniae* isolates were extended-spectrum β- lactamase producers and biofilm formation. The activities of two selected *K. pneumoniae* isolates for their biofilm formation and susceptibility to antibiotics after treatment with several plants extracts were investigated. The results of water and 60% ethanol extracts for *Matricaria chamomile* flowers, *Alhagi maurorum* leafs, *Syzygium aromaticum* buds (clove) and *Arctium minus* leafs were showed reduction of biofilm formation and change the ability of antibiotic susceptibility for these two tested *K. pneumoniae* isolates. The indirect effect of plants extracts on the bacterial growth may be causing indirect effect on the metabolic activity and DNA (chromosome or plasmid) of cells. All tested plant extract contain many antimicrobial and antioxidant component by using GC-mass.

Keywords: *Klebsiella pneumoniae*, medical plants extracts, antibiotic susceptibility, biofilm formation**.**

دراسة تأثير بعض النباتات الطبية على انتاج الغشاء الحيوي وحساسية المضادات الحيوية للكلبسيلا الرئوية

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الخلاصة

اخذت عشرون عينة سريرية و برازية (عشر عينات سريرية معزولة من مرضى مستشفى الامام علي وعشر عينات براز الدجاج جمعت من مزرعة دواجن محلية في مدينة بغداد) خلال كانون الاول 2015 ، لعزل الكلبسيلا الرئوية. كل عزلات الكلبسيلا الرئوية كانت منتجة للبيتالاكتميز وللغشاء الحيوي. انتخبت فعالية عزلتين للكلبسيلا الرئوية لانتاج البايوفلم وحساسيتها للمضادات الحيوية بعد المعاملة بالمستخلصات النباتية. اظهرت نتائج المستخلصات المائية والكحولية وبتركيز 60% لازهار البابونج، واوراق العاقول، وبراعم القرنفل، واوراق الارقطيون اختزال لانتاج البايوفلم وتغيير قدرة الحساسية للمضادات الحياتية لعزلات الكلبسيلا الرئوية المختبرة. التأثير غير المباشر للمستخلصات النباتية على نمو البكتريا ربما يسبب تأثير غير مباشر على

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الفعاليات الايضية والدنا الكرموسومي والبلازميدي للخلايا. اظهر استعمال كروماتوغرافيا الغاز (GC–mass) للمستخلصات النباتية وجود عدد من من المواد مضادة للمايكروبات ومضادة للاكسدة.

Introduction

Serious infections caused by bacteria become resistant to common used antibiotics [1]. The most common mechanism of resistance to number of antibiotics were acquired by pathogenic bacteria from a pool of resistance genes in other microbial genera [2]. Plasmids, transposons, and other molecular mechanisms are responsible for the emergence of resistance to multiple antibiotics [3]. Biofilm formation is one of the virulence factors. Review by Flemming *et al*, (2016) revealed bacterial biofilms are formed by communities that are embedded in a self-produced matrix of extracellular polymeric substances [4].

Klebsiella pneumoniae is an important bacterial pathogen in humans that is commonly associated with opportunistic and hospital-acquired infections [5]. Many studies revealed the relation between biofilm production and antibiotic susceptibility [6- 8].

For centuries, plants have been used as remedies and treatments of diseases [9]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [10, 11]. *Matricaria chamomile* flowers mostly contain phenolic compound and glycosides [12]. Alcoholic extract of Chamomilla was showed potentials for antioxidant and antimicrobial properties [13]. *Arctium lappa* and *Arctium minus* had high levels of flavonoid and hydroxycinnamic acid [14]. Many researcher, recorded high level effect of *Arctium lappa* and *Arctium minus* against many gram negative and gram positive bacteria [15, 16]. Cloves (*Syzygium aromaticum*) are the aromatic dried flower buds, acts as antimutagenic, antioxidant, antifungal, antimicrobial [17- 19]. The major components of cloves were 71.56 % eugenol [20]. Previous study showed that *Alhagi maurorum* with wide range of Pharmacological activities [21]. Phytochemical screening of crude extracts and its subsequent fractions demonstrated the presence of fats, alkaloids, flavonoids, anthraquinones, cardiac glycosides, coumarins, saponins, phlobatannins, tannins and terpenoids in leaves and roots [22]. Antibacterial and antifungal activities of *A. maurorum* were reported by Sulaiman (2013) [23].

Our present study is focused towards different methods to determine the control of the biofilm formation and determine the interference effect between plant extract and antibiotic susceptibility.

Materials and Methods

Sampling

Ten clinical (Cl) samples were obtained from Imam Ali Hospital and ten chicken feces (F) samples were collected from local poultry farm.

Identification tests

Colony morphology, Gram stain and biochemical test were used to identification the *Klebsiella pneumoniae* isolates and confirmed by Automatic Identification System (Vitek 2 with GN card).

Plants

The plants *Arctium minus* L. leaves (R.), *Matricaria chamomilla* flowers (H) and *Syzygium aromaticum* buds (clove: C) were purchased from local market and fresh plant *Alhagi maurorum* leaves (a) were collected from Baghdad city.

Preparation of plant extract

20 g of each dry plant extracted with 180ml of distilled water or 60% ethanol (Alcohol: A) by using Soxhlet apparatus at 60-80 ^{o}C for 3 hr., and then extractions were filtered and dry in 40^{o}C. The residue stored at 4°C for further analysis [24].

Selected isolates

Two isolates had been selected from clinical and chicken feces samples produce beta lactamases and biofilm production. Clinical isolates No.4 and feces isolates No.15 were under test investigation.

Antibiotic sensitivity test

Antibiotic susceptibility profiles of *Klebsiella pneumoniae* isolates was done by the Kirby-Bauer disk diffusion method [25]. These antibiotics with their respective disk concentrations are as follows: β-lactam group [Cephalexin (30µg)]; Aminoglycosides group [amikacin(10µg), Streptomycin (10µg)]; quinolones group [ciprofloxacin(10µg)] and others, such as Chloramphenicol (10µg).

Bacterial cultures suspensions equivalent to 0.5 tube McFarland turbidity standard were spread on Muller-Hinton agar plates using sterile cotton swabs and applying antibiotic disc, then incubated aerobically at 37^0 C for 24 hours. The inhibition zones diameter around antibiotic disks were measured according to the CLSI [26]. Antimicrobial susceptibility test was done before and after reacts with plant extract.

Extended spectrum β- Lactamase production

CHROMagar™ ESBL medium (the Chromogenic Media Pioneer-France) was used to detect the ability of *Klebsiella pneumoniae* isolates to produce extended spectrum beta-lactamase (ESBLs)**.** ESBLs are enzymes that mediate resistance to penicillins, third generation cephalosporins and monobactams.

Biofilm production

Glass tubes method was used for detection biofilm formation visually, according to Sager *et al* 2015 with some modifications. 5mL of brain heart infusion broth was inoculated with 100µL of bacterial suspension of 10^7 CFU/mL of each bacterial isolates, except one tube contain only brain heart infusion broth as control. Tubes were incubated at 36°C for 24h.The culture was discarded and the tube washed with sterile distilled water. Tubes were stained with 1% crystal violet for 15min. Crystal violet was then discarded and then washed with distilled water until runoff was clear. Biofilm formation was assessed visually then compared with a negative control tube and classified as absent (0), weak (+), moderate (++), and strong (+++) [27]. Biofilm formation test was done before and after reacts with plant extract.

The agar well diffusion method

100 µl (18 h cultures) of the pathogenic *K. pneumoniae* species were spread on Nutrient agar plates (All tests were applied as duplicate). The wells were punched in plates using sterile cork borer (6mm diameter).Then the wells were filled with the plant extract under aseptic conditions. The plates were incubated aerobically at 37ºC for 24h. The results were recorded and analyzed in terms of the zones of inhibition formed around each well [28].

Preparation of standard stock solution of crude plant extracts and bacterial culture

It was prepared the final concentrations of aqueous and ethanol extract of raw plants under study by using the sterile distilled water at 500 mg / mL. Two plants extract concentrations was prepared (1/2, 1/4) with peptone water medium and then 10µl of the test bacteria previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. Inoculated tubes were incubated at 37°C for 24 h. After a period of incubation was subculture in a nutrient agar plate and incubated at 37°C for 24 h, then visually determine the density of growth.

GC/MS analysis

The ethanol extracts were analyzed using the GC/MS analysis (Shimadzu GCMS-QP2010Ultra).

Result and Discussion

Medicinal plants are an important source for the therapeutic remedies of various diseases. It is used common medical plants in this study, some used for respiratory and intestinal infections such as Chamomile and Arctium, others which used as a spice in foods such as clove and other little use in medical treatments such as *Alhagi*.

Two isolates had been adopted from 20 isolates produce beta lactamases and biofilm production, selected from clinical (No.4) and chicken faeces (No.15) samples. Yang and Zhang (2008) reported the among 150 clinical sample of *K. pneumoniae* , the 44.7% biofilm formers, 45.3% of them produced ESBLs [29]. Other study indicated all *K. pneumoniae* isolated from sputum and surgical-wound swabs produced biofilms [30].

The results showed in the Table-1 the high effective of clove water and alcohol extract on isolates under study ranged from 8 to 10 mm in diameter, and then Arctium water extract and chamomilla at 7mm in diameter.

Table 1- Antimicrobial activity of medicinal plants by used agar well diffusion method.

	Arctium minusL. leaves *(R)*		*Alhagi camelorum* leaves *(a)*		*Matricaria chamomilla* flowers *(H)*		*Syzygium aromaticum buds (clove: C)*	
	Ethanol RA	Water RW	Ethanol aA	Water aW	Ethanol HA	Water HW	Ethanol CA	Water CW
	Zone of inhibition (mm)							
Klebsiella pneumoniae (Cl)	0	7	0	0	0	7	10	10
	Arctium minusL. leaves (R)		*Alhagi camelorum leaves (a)*		*Matricaria chamomilla* flowers (H)		*Syzygium aromaticum buds (cloveC)*	
	Ethanol RA	water RW	Ethanol aA	Water aW	Ethanol HA	Water HW	Ethanol CA	Water CW
	Zone of inhibition (mm)							
Klebsiella pneumoniae (F)	0	0	0	0	0	7	8	8

clinical= Cl chicken feces =F Alcohol=A Water=W

Table- 2 indicate the activity of the antibiotics amikacin, ciprofloxacin and chloramphenicol before mix with plant extract, in the other hand the inhibition zone of growth diameter was change the ability of antibiotic susceptibility after growth in the peptone water medium with concentration 250mg/ml plant extract. Clove alcohol extract showed, enhance the activity of the antibiotic for chloramphenicol and streptomycin, and Arctium alcohol enhance streptomycin activity for clinical isolate (No.4). In the other hand other plant extract interfere with the antibiotic susceptibility Figures- 1, 2, 3 and 4.

Table 2- Antimicrobial susceptibility of five antibiotics before and after reacts with plant extract.

Isolates	No.4 (clinical isolates)					No.15 (chicken faeces isolates)				
AB	amikacin	cipro-floxacin	cephal-exin	chloram-phenicol	strepto-mycin	amikacin	cipro-floxacin	cephal-exin	Chloram-phenicol	strepto-mycin
Zone of inhibition (mm)										
B*	22	40	-	10	-	25	30	-	12	-
aW	10	22	-	-	-	12	20	-	-	-
aA	12	25	-	-	-	8	25	-	-	-
CW	15	25	-	-	-	12	20	-	-	-
CA	22	25	-	15	10	8	22	-	-	-
HW	8	22	-	-	-	8	22	-	-	-
HA	8	25	-	-	-	12	20	-	-	-
RW	20	30	-	-	-	10	22	-	-	-
RA	11	20	-	-	10	8	25	-	-	-

*****Zone of inhibition (mm) before react with plant extract.

Previous study shows that *K. pneumoniae* antimicrobial drug resistance increased for every antimicrobial class studied except tetracyclines [31]. *Klebsiella spp*. is intrinsically resistant to penicillin's and third- and fourth-generation cephalosporins due to the production of plasmid-mediated extended-spectrum beta-lactamases (ESBLs) [32].

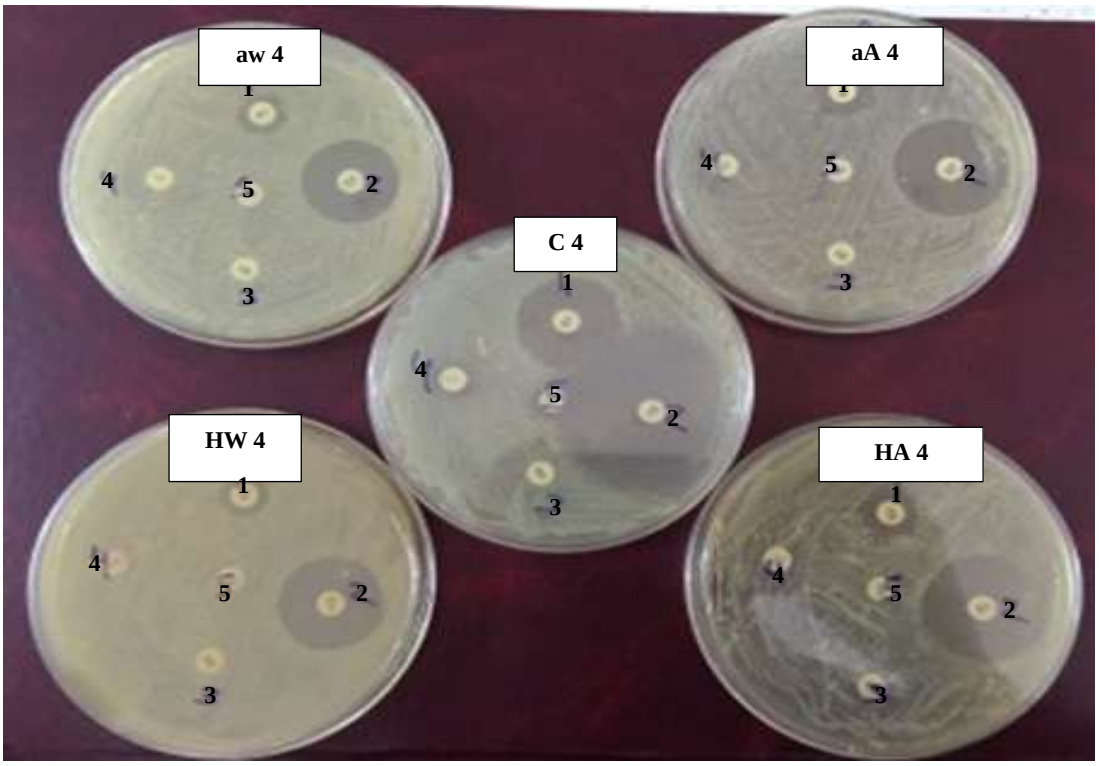

Figure 1- Antimicrobial susceptibility of five antibiotics before and after reacts with plant extract. Clinical isolates (No.4) aW: alhagi Water, aA: alhagi Alcohol, HW: Chamomile Water, HA: Chamomile Alcohol, C (Control: in the middle) without treatment with plant extract.

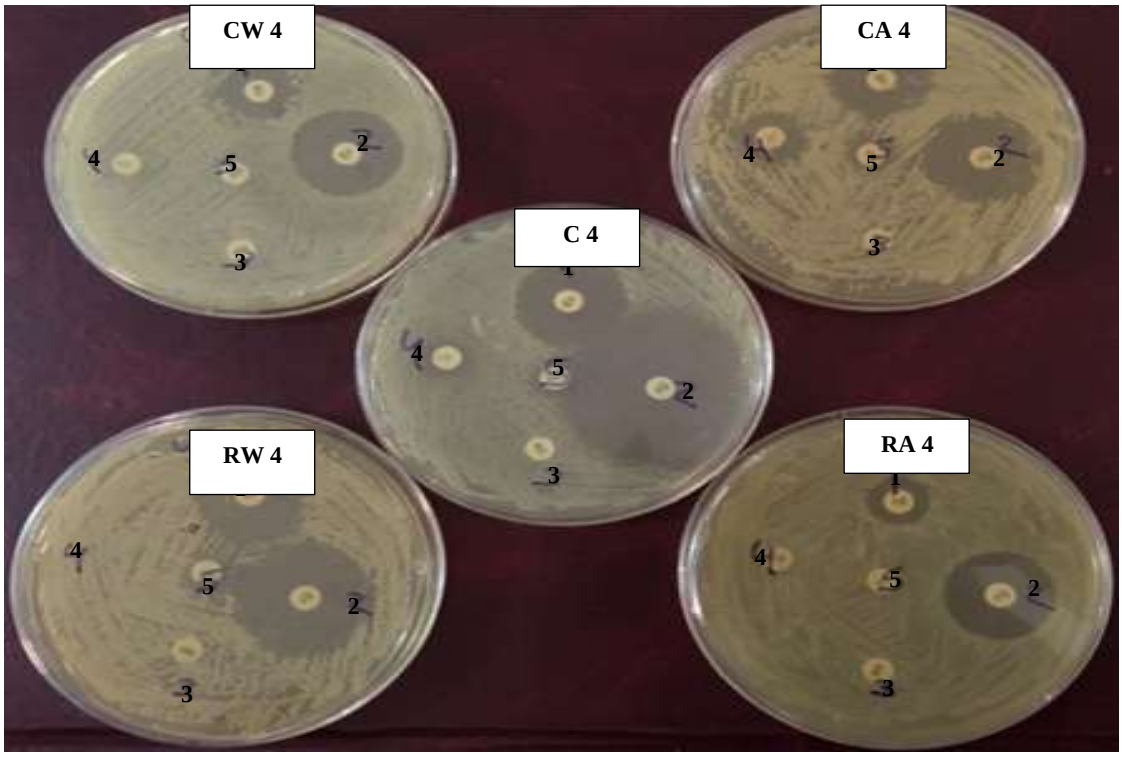

Figure 2- Antimicrobial susceptibility of five antibiotics before and after react with plant extract. Clinical isolates (No. 4), CW: Clove Water, CA: Clove Alcohol, RW: Arctium Water, RA: Arctium Alcohol, C (Control: in the middle) without treatment with plant extract.

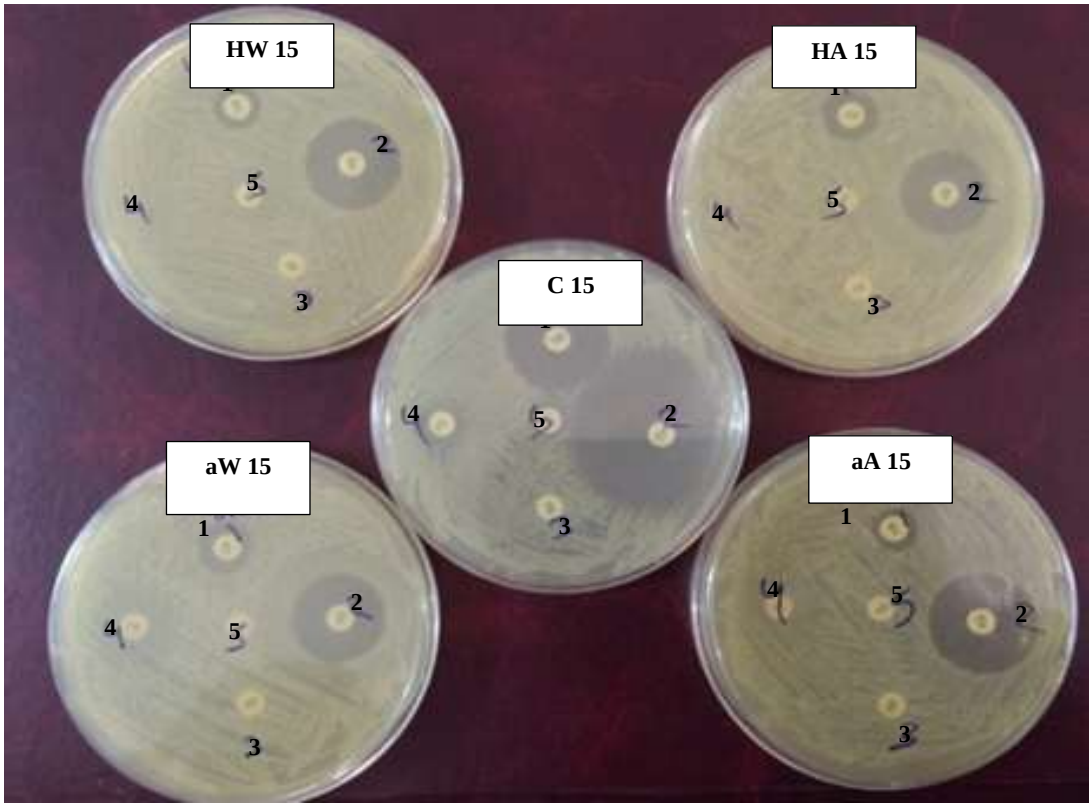

Figure 3- Antimicrobial susceptibility of five antibiotics before and after react with plant extract. Chicken faeces isolates (No. 15), HW: Chamomile water, HA: Chamomile alcohol, aW: Alhagi water, aA: Alhagi alcohol, C (Control: in the middle) without treatment with plant extract.

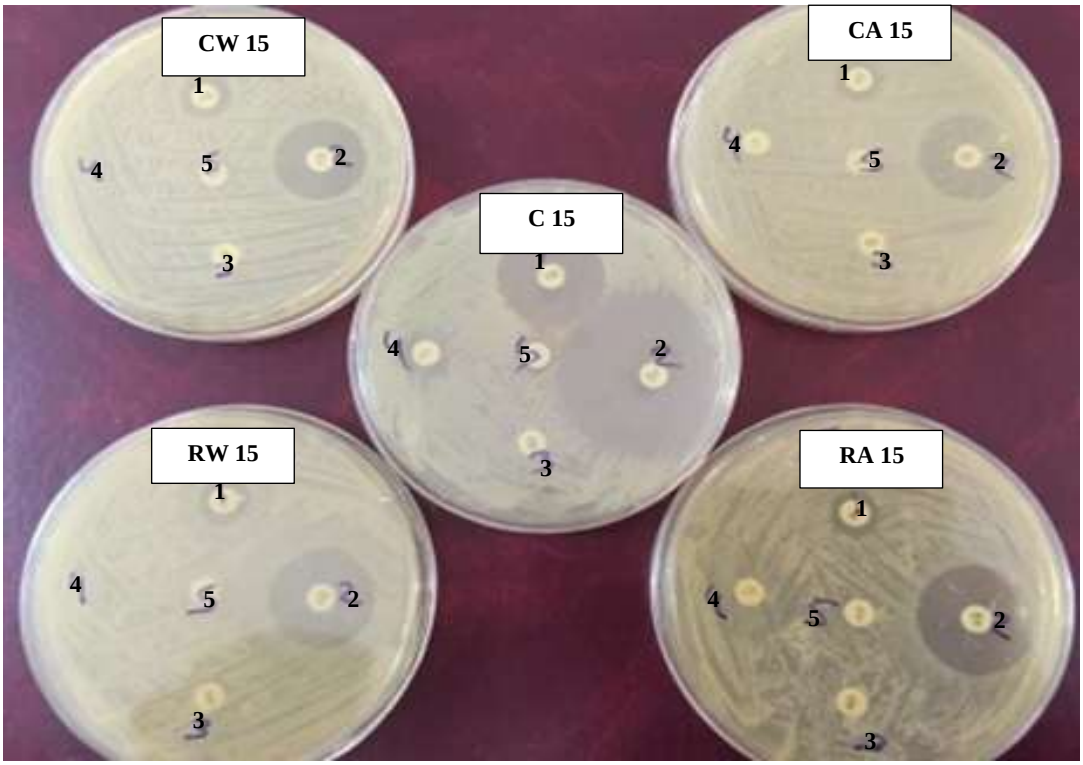

Figure 4- Antimicrobial susceptibility of five antibiotics before and after react with plant extract. Chicken faeces isolates (No. 15), CW: Clove Water, CA: Clove Alcohol, RW: Arctium Water, RA: Arctium Alcohol, C (Control: in the middle) without treatment with plant extract.

Table- 3 revealed the intensity of growth on nutrient agar after growth in peptone water mix with plant extract at 1/2 and 1/4 dilution for both isolates. High effect of clove alcoholic extract, then clove water extract, Arctium alcoholic extract, and Chamomile alcoholic extract at 1/2 on isolates No.15. The highest influence on intensity growth focus at 1/2 dilution for clove water and alcoholic extract, Arctium alcoholic extract, and Chamomile alcoholic extract for isolates No.4 (Figure-5). Many studies reported the extract of *Arctium minus L. leaves and Matricaria chamomilla* flowers showed successful treatment to the burned or damage areas of the skin [33, 34]. Clove oil showed strong antibacterial activity against all bacterial isolates tested [35] and crude extracts of *Alhagi maurorum* showed antibacterial and antioxidant activities [36]. This method showed the effect of plant extracts on the ability of cell division and growth density.

Table 3- Bacterial growth in Nutrient agar after mix with plant extract.

Isolates No.	4 (clinical isolates)		15 (chicken faeces isolates)	
Plant code	1/2	1/4	1/2	1/4
CW	++	+++	+	+++
CA	++	+++	few	++
RW	+++	+++	++	+++
RA	++	+++	+	++
aW	++	+++	+++	+++
aA	+++	+++	++	+++
HW	+++	+++	+++	+++
HA	++	++	+	+++

+=weak growth ++= moderate growth +++=strong growth

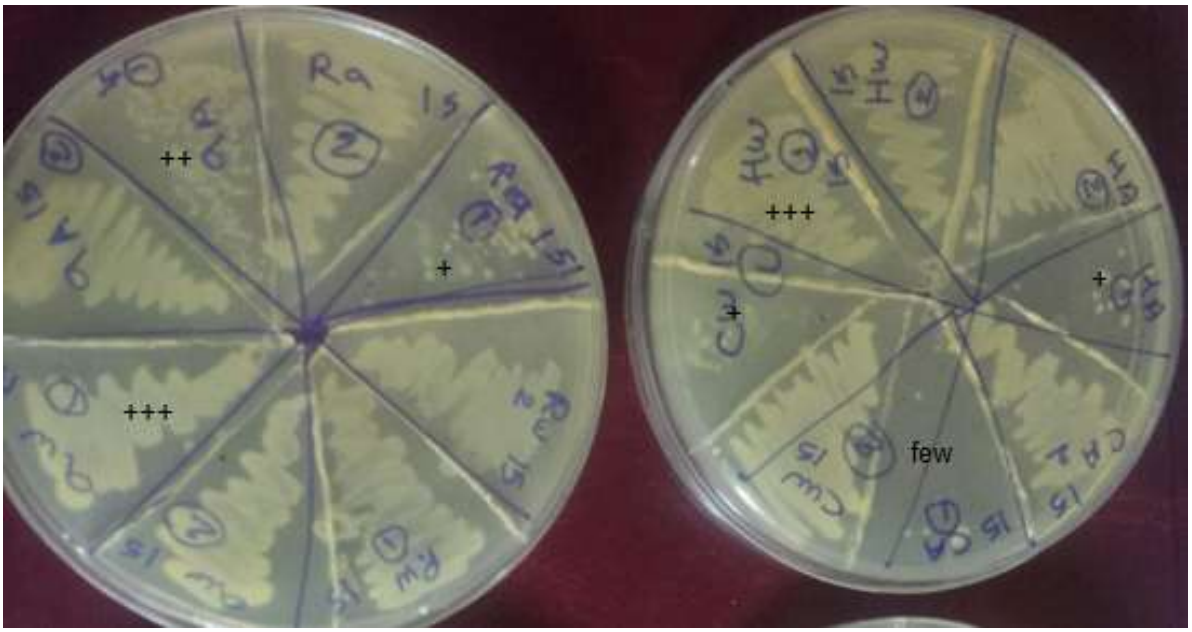

Figure 5-Bacterial growth in Nutrient agar after mix with plant extract.

*K. pneumonia*e of clinical isolates (No.4) and chicken faeces isolates (No.15) have the ability to produce biofilm more than the strong degree. The results showed a considerable reduction in biofilm formation when treated with different plant extract. The highest effect of Arctium alcoholic extract on isolation 4 and chamomile alcoholic extract and Alhagi alcoholic extract on isolates 15 and then followed by other plant extracts in different degree at both isolates Table- 4. The ethanolic extract of *Zingiber officinale* demonstrated best result anti-biofilm activity on *Proteus mirabilis* [37] *Rosmarinus officinelis L*. essential oil had antibacterial activity and considerable antibiofilm activity against *Klebsiella pneumoniae* [38].

Table 4- Bacterial biofilm after mix with plant extract.

Isolates No.	**4 (clinical isolates)**		**15 (chicken faeces isolates)**	
Plant code	**1/2**	**1/4**	**1/2**	**1/4**
HW	+	++	++	++
HA	+	+	**0**	++
RW	++	+++	++	++
RA	**0**	+	+	++
CW	+	++	+	++
CA	+	++	++	++
aW	++	+++	++	++
aA	++	+++	**0**	++

Note: isolates No.4 and 15, control ++++ biofilm.

The major components and area percentage are summarized in Tables- 5, 6, 7 and 8. Among the identified compounds, some of them are known for their interesting antimicrobial agent, anti-oxidant, antitumor and anti-inflammatory. *Syzygium aromaticum* contain at least 6 out of ten antimicrobial agent and 4 out of ten anti-inflammatory properties. *Matricaria chamomile* contain at least 6 out of eleven anti-microbial agent and 5 out of eleven anti-inflammatory properties. *Alhagi maurorum* contain at least 4 out of eleven anti-microbial agent and 6 out of eleven anti-inflammatory properties. *Arctium minus* contain at least 6 out of ten anti-microbial agent and 3 out of ten anti-inflammatory properties. Various pharmacological activities for plants extracts, including anti-allergy, antibacterial, anti-hepatitis and anti-tubercular have been reported from long paper [39].

Table 5- Percentage of chemical constant for *Syzygium aromaticum* by using GC mass.

No.	Syzygium aromaticum (component)	Area %	Effect	Reference
1	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (**Eugenol acetate**)	53.66	antiseptic, antimutagenic, antigenotoxic, anti-inflammatory properties, prooxidant and antioxidant activities	Toxicology**,** 2002, 177 Issue 1:39-54. Journal of Food Science,1977 42 Issue 4: 1107–1109.
2	Caryophyllene	4.02	anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities.	J Pharem Pharmacol. 2007 59(12):1643-7. .
3	1-(+)-Ascorbic acid 2,6-dihexadecanoate	3.62	antioxidant , Antimutagenic Agents, Antineoplastic Agents	European Chemicals Agency - ECHA
4	Benzaldehyde, 4-(acetyloxy)-3-methoxy- **(Vanillin, acetate**)	3.27	carcinostatic or antitumor properties, Benzaldehyde did not produce mutations in bacterial assays, lifespan extension	Aging Cell. 2007, 6(1): 35–43. Arch Biochem Biophys 2001,391(1):79-89.
5	Diethyl phthalate	2.55	antimicrobial agent	Asian J Pharm Clin Res, 2014 7 Issue 4:141-142.
6	1-Propanol, 2-(2-hydroxypropoxy)	1.35	Antibacterial agent	Romanian Biotechnological Letters, 2011, 16 (2):6034-6041.
7	2-Furancarboxaldehyde, 5-(hydroxymethyl)	2.92	no mutagenic activity or cytotoxicity of HMF in TK6 human lymphoblast cells at doses as high as 75 ug/ml.	Chem. Res. Toxicol. 1994, 7:313-318

8	9,12-Octadecadienoic acid(Z,Z) (**Leinoleic acid**) Fatty Acids*	2.78	Prevention of preeclampsia, reduces body fat, anti-inflammatory, antibacterial	Obstet Gynecol, 1998, 91(4):585-90. Int J Obes Relat Metab Disord. 2001, 25(8):1129-35. Br J Nutr. 2008,100(1):112-119. FEMS Immunology and Medical Microbiology 2003, 36 :9-17.
9	6-Octadecenoic acid	1.21	antiviral activity, antibacterial	In J Sci & Tech Res 2013, 2, Issue 10: 181-184. Asian Journal of Plant Science and Research, 2013, 3(2):47-54
10	Methyl 11,14-eicosadienoate	0.99	anti-inflammatory	Lipids in Health and Disease 2002, 1(5):1-12.

Table 6 -Percentage of chemical constant for *Matricaria chamomile* by using GC mass.

No.	Matricaria chamomile	Area%	Effect	Reference
1	2H-Pyran-3-ol,tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-,[3S-[3.alpha.,6.alpha.(R*)] **(alpha-bisabolol oxide A**)	16.93	anti-inflammatory (Antihyperalgesic and antiedematous	Phytother Res. 2014 , 28(5):759-66.
2	9,12-Octadecadienoic acid(Z,Z)	17.31	Mention above	
3	1-(+)-Ascorbic acid 2,6-dihexadecanoate	15.94	Mention above	
4	2H-1-Benzopyran-2-one,7-methoxy- **(Coumarin)**	6.87	anti-tumor cell, antimicrobial	in vivo 2005,19: 705-712 Biol Pharm Bull. 2004, 27(8):1312-6. Evidence-Based Complementary and Alternative Medicine Volume 2015 (2015), Article ID 919616, 10 pages
5	Hexadecanoic acid,ethyl ester **(Palmitic acid)**	4.29	Antibacterial agent, Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor	Asian Journal of Plant Science and Research, 2013, 3(2):47-54 Asian J Pharm Clin Res, 2012, 5 (Issue 2), 90-94.
6	Diethyl Phthalate	3.44	Mention above	
7	Tetradecanoic acid (**Myristic acid**)	3.37	antitrypanosomal drugs, anti-inflammatory	Proc. Natl. Acad. Sci. USA 1994, 91:9735-9739. Chem Biol Drug Des 2012, 80:434-439.
8	1-Propanol, 2-(2-hydroxypropoxy)	2.94	Mention above	
9	Butyl 9,12-	2.92	antioxidant	Tropical Journal of

	octadecadienoate Fatty Acid*			Pharmaceutical Research, 2013; 12 (5): 735-742
10	2-Furanmethanol,tetrahydro-alpha,alpha,5-trimethyl-5-. (**α-Bisabolol oxide B).**	2.41	Anti-inflammatory	Food Chem Toxicol, 2011, 49(10):2580-5
11	1-Benzoxepin-2(3H)-one,octahydro-	2.25	Antitumor	*J. Med. Chem.*, 2004, *47* (23): 5612–5615

Table 7- Percentage of chemical constant for *Alhagi maurorum* by using GC mass.

No.	Alhagi maurorum	Area%	Effect	Reference
1	Diethyl Phthalate	13.36	Mention above	
2	1-Butanol,3-methyl-,formate (**Isopentyl formate)**	11.04	flavouring agent	Pub Chem / Online
3	1-Propanol, 2-(2-hydroxypropoxy)	8.56	Mention above	
4	2-Propanol, 1,1-oxybis- (**Dipropylene glycol)**	6.80	Antibacterial and antifungal	Acta Derm Venereol. 1991;71(2):148-50. J Int Soc Prev Community Dent. 2015, 5(2): 114–119.
5	Phenylethyl Alcohol	4.59	Antibacterial	J Bacteriol 1976, 127(3): 1359-1369.
6	1-(+)-Ascorbic acid 2,6-dihexadecanoate	4.27	Mention above	
7	2-Furancarboxaldehyde, 5-(hydroxymethyl)	3.39	Mention above	
8	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	2.98	Antioxidant, anticancer	Prev Nutr Food Sci 2013, 18(1): 76–79. Arch Pharm Res 2007, 30(11):1455-63.
9	Cyclopentaneacetic acid ,3-oxo-2-pentyl-, methyl ester (**Methyl dihydrojasmonate)**	2.44	Antitumor	Int J Nanomedicine 2015, 10: 585–594.
10	Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl (**Galaxolide**)	2.85	Antiestrogenic	Environ. Sci. Technol 2004, 38 (4):997–1002.
11	Menthol	0.22	Antitumor	APJCP 2014, 15(4):1551-1556. in vivo 2007, 21: 285-290.

Table 8- Percentage of chemical constant for *Arctium minus* by using GC mass.

No.	Arctium minus	Area%	Effect	
1	1-Propanol, 2-(2-hydroxypropoxy)	15.97	Mention above	
2	Diethyl Phthalate	13.88	Mention above	
3	1-(+)-Ascorbic acid 2,6-dihexadecanoate	9.70	Mention above	
4	2-Propanol, 1,1-oxybis-	9.42	Mention above	
5	Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl (**Galaxolide)**	6.11	Mention above	
6	Cyclopentaneacetic acid ,3-oxo-2-pentyl-, methyl ester	3.90	Mention above	
7	Octanoic acid , 2-hexyl- (**Caprylic acid)**	3.15	Antibacterial	FEMS Immunology and Medical Microbiology 2003, 36 :9-17.
8	Benzoic acid,2-hydroxy-,phenylmethyl ester (**Benzyl salicylate)**	1.53	fragrance ingredient	
9	Tetradecanoic acid	1.88	Mention above	
10	2-Pentadecanone,6,10,14-trimethyl- (**Hexahydrofarnesyl acetone)**	1.62	Antimicrobial , antioxidant	Int J Biol Pharm Res 2014, 5(11): 861-869

In conclusions, a number of studies indicate there is a diverse mix of effective antimicrobial compounds and other component act as anti-inflammatory effect. This diversity in the composition is more effective and safer to use than concentrated antibiotics and chemical treatments. Moreover, we can use different methods to understand the biofilm formation and antimicrobial susceptibility for study the effect of plant extract in vitro. All plants extracts used in our study showed reduction of the biofilm formation in different degree, interfere with antibiotic susceptibility and some antibacterial activity.

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