Ibn Al-Haitham Journal for Pure and Applied science https://doi.org/ 10.30526/2017.IHSCICONF. 1774

Antimicrobial activity of some plants extracts on bacteria isolated from acne vulgaris patients

Khetam H. Rasool
khetamhabeeb112@yahoo.com
Dept. of Biology / College of Science / AL-Mustansiriyah University

Abstract

Acne is a cutaneous pleomorphic disorder skin disease most frequently occurring during the adolescent in ages of 12-24, with estimated percentage (85%). There are different ways to treat acne such as using of antibiotics, herpes, and mixing treatments.

Antibacterial activity of four concentrations (100,50,25,12.5)mg /ml of alcoholic and cold aqueous crude extracts of Cinnamon(*Cinnamomum verum*), Henna *(Lawsonia inermis*) , Lupine *(Lupinus luteus*) were studied against aerobic and an aerobic bacteria isolated from inflamed and discharging pus from thirty Iraqi acne vulgaris patients refer to dermatology unit at AL-Kindy Hospital from December 2016 to March 2017.All information (age, sex ,diseases and using topical treatments) were recorded .The bacterial isolates were identify using morphological characteristics, biochemical tests and the Vitek-2 compact system. Among (30) Acne samples taken, 8 (26.7%) samples were from males age range (19-33) years and 22(73.3%) were from females within age (17-29) years. twenty-five (83%) samples were culture positive, and only (17%) of samples revealed no growth. Most frequent bacteria which isolated (aerobically) from acne patients were Staphylococcus *aureus* (60%), *Staphylococcus epidermidis* (20%), *Escherichia coli* (8%), *Pseudomonas aeruginosa* (4%), and an aerobic bacterial isolates were *Propionibacterium acnes* (8%) isolates.

Antibiotic sensitivity tests were performed against, Ampicillin, Clindamycin, Gentamicin, Cotrimoxazole, Erythromycin, Vancomicine, Tetracycline, Doxycycline, and Azithromycin.All bacterial isolates were resistance to Ampicillin. *Staphylococcus aureus* and *S. epidermidis* were sensitive (100%) to Doxycycline and Azithromycin, *P. acne* were also highly sensitive to these two antibiotics (95.5%,97.1%) respectively, while *E. coli* and *P. aeruginosa* were (100%) resistance to these antibiotics. Gentamicin and tetracycline were susceptible by most of the study isolates except for *P. aeruginosa* which was very resistance to CN and TE (100%&94.8%) respectively. The antimicrobial potential of cold water and alcoholic crude extracts of Cinnamon (*Cinnamomum verum*), Hinna *(Lawsonia inermis*), and Lupine *(Lupinus luteus*), in concentrations (100,50,25,12.5) mg/ml against the gram positive and gram negative isolates were tested through a well-diffusion method.

Alcoholic extract of Henna leaves in concentration (100mg/ml) showed high inhibitory activity to all isolates compared with the aqueous extract and the all concentration of cinnamon aqueous and alcoholic extracts, while lupine extracts had no effect on all bacterial isolates. Gram-positive bacteria were the most common microorganisms involved in acne infection. There are variations in the incidence of acne infection in relation to sex, age; Alcoholic extract of the Henna leaves could be used to treat acne.

Key words: Acne vulgaris, Bacterial isolation, antimicrobial effects, Henna, Cinnamon, Lupine.

1. Introduction

Acne vulgaris disease effect the pilosebaceous follicle in the face and neck,it appears like non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules, and nodules)[1]. Commonly most frequent appearance in age (12-25) year, which estimates of 85% of adolescents and young adults [2]. Its pathogenesis is multifactorial factors like hormonal [3] bacterial colonization by *Propionibacterium acnes* (*P. acnes*), and immunological (inflammatory) [4], that results in the formation of acne lesions but the key factor is genetics [5].

Acne may affect the psychological and social quality of individual's life, due to acne scars formation (6). Although it can be treated with wide-range of therapies like (antibiotics, Hormonal Therapies, Corticosteroids, Surgery**,** **Herps**), which used to reduce the inflammatory lesions and scar formation [6]

Many plants have been used in treatment of several pathological conditions, due to its antioxidant compounds which play a major role in human health worldwide [7]. While using of antibiotics for treatment acne vulgaris for long time can lead to bacterial resistance and many side effects problems [8]. Medical plants with anti-inflammatory and antibacterial activities are used in different ways in the treatment of acne alone or in combination with drugs safely [9], where bacterial cells could be killed by the rupture of cell walls and membranes or by the disruption of the inner when treated with these plants or its extracts [10] organelle

Aims*:* to determine the antimicrobial values of some traditional herpes (Cinnamon, Hinna and lupine) alcoholic and water extracts on acne bacteria.

2. Materials and Methods

Subjects: Thirty Iraqi patients diagnosed by dermatologist as acne vulgaris where enrolled in this study. They refer to AL-Kindy Hospital in Baghdad /Iraq during the period from December (2106) till March 2017. Eight 8 (26.7%) were males age range (19-33) years and 22(73.3%) were females within age (17-29) years.

Bacterial isolation: For isolation of bacteria from patient's skin, the area of the forehead of a volunteer was cleansed with a sterile swab, and the swab was placed in its tube that containing transporting medium and transport immediately to the laboratory of microbiology for culturing. The samples were cultured on blood agar, MacConkey agar and thioglycolate broth. The cultures were then incubated at 37°C under both aerobic and anaerobic conditions for seven days [11]. Thioglycolate broth used as a medium for enhancing the growth of Propionobacter from skin swab. After incubation an aerobically in CO2 incubator for 5-7 days at 35-37C, if growth is detected in the broth, sub culturing was done on 5% human blood agar to categorize and isolate Propionobacter. [12]

The colonies were determined morphologically and identify by biochemical tests, Vitec-2 system was used for final identification.

Antibiotic susceptibility test: The standard Kirby-Bauer test (disc diffusion **test**) for antibiotic susceptibility, has been used for determination the effects nine of standard synthetic antibiotics [13].

Preparation of Plant Extract [14].

The leafs of Hinna, Cinnamon sticks, lupine seeds, samples were obtaining from herbal medicine store in Baghdad /Iraq, washed with water, leave to dry, and grounded to powder by blender. The samples powder was added to Ethanol (>99.5%), and cold distilled water

to make a 20% concentration, in sterile 250mL flask wrapped with aluminum foil to avoid evaporation and light. The flask content was mixed thoroughly by platform shaker at (70 rpm) for 3 days, then the mixtures were centrifuged for 10min at 4,000 rpm at room temperature, get off sediment and collected supernatant dried in oven (40) ∘C, and powder kept till use.

Antibacterial Activity:

Acne bacteria isolates were recultured on nutrient agar plates and incubated at 37∘C for 18 to 24 h. for pure colonies isolation. Single pure colony was transferred by cotton swab to a test tubes contain sterile normal saline (4ml) and mixed well, to get about 1.5 × 108 CFU/ml. compared turbidity to that of the 0.5 McFarland standard solution. Mueller Hinton plates were streak with bacterial isolates, five millimeter diameter wells were punched into the medium using a sterile Cork borer, a well-diffusion method was used for testing antibacterial activity of extracts. 50 μL of extracts solution were employ per well, controls were pure solvents only. The plates were sealed, labeled, and incubate at 37∘C.for 24 hours, inhibition zones in millimeters (mm) were measured by ruler and the average was estimated.

Results and Discussions

samples collection and bacterial identification

Skin swabs were collected from thirty Iraqi acne vulgaris patients. Eight (26.7%) of samples were from males age range (19-33) years and 22 samples (73.3%) were from females within age (17-29) years.

Swabs were grown both aerobically on (blood and MacConkeys agar) and anaerobically (in thioglycolate broth and sub cultured on thioglycolate agar), 25 (83%) of samples were culture positive, and only 5 samples (17%) revealed no bacterial growth **(Table 1).**

Table (1): Number and percentage of bacteria obtained from acne swabs culturing

Cultures	**Samples**	***S. aureus*** ***No (%)***	***S. epidermidis*** ***No (%)***	***E. coli*** ***No (%)***	***P. acne*** ***No (%)***	***P. aeruginosa*** ***No. (%)***	**Total No (%)**
Aerobic	acne lesions swabs n=	15 (60%)	5 (20%)	2 (8%)	-	1 (4%)	23 (92%)
Anaerobic	Acne lesions Swabs n=(*30*)	-	-	-	2(8%)		2(8%)
Total	30	15(60)	5(20%)	2(8%)	2(8%)	1(4%)	25(100%)

n=number of acne swabs

N =number of isolates

The Bacterial hyper colonization to sebaceous follicle, especially with *Propionibacterium acnes*, has been considered as a major causative factor for acne vulgaris [15,16]. This bacterium is an anaerobic bacteria found in acne lesions, lead to stimulates inflammation by producing proinflammatory mediators like interleukins and tumor necrosis factor [17,18], and sebum free fatty acids [19,20]. This bacterium with *Staphylococcus epidermidis* are common pus-forming microbes and formation of different types of acne vulgaris. However, it is by no means clear that either bacteria or their products initiate follicular inflammation. therefore, its role in acne cannot be explained by its presence in the follicle. [21]

In our study a higher percentage of isolation was for *Staphylococcus aureus* (60%) followed by, *Staphylococcus epidermidis* (20%), *Escherichia coli* (8%), *Propionibacterium acne* (8%) and *Pseudomonas aeruginosa* (4%). This indicate that Staphylococcus *aureus* isolates, have major role in acne vulgaris formation instead of *Propionibacterium cane* bacteria [22,23]. Our results

is in contrast to the results of [24,25] which implicated that both *Staphylococcus epidermidis* and *Propionibacterium acnes* as causing acne vulgaris bacteria .

Antibiotic susceptibility patterns of bacterial isolates:

Antibiotic sensitivity tests were performed against, Ampicillin, Clindamycin, Gentamicin, Cotrimoxazole, Erythromycin, Vancomicine, Tetracycline, Doxycycline, Azithromycin, Interpretation of the results was done according to CLSI (13). Table (2) & Fig (1).

Table (2): Percentage of antibiotics susceptibility pattern of bacterial isolates in the study

Antibiotics	Bacterial isolates									
	S. aureus		***S. epidermidis***		***E. coli***		***P. acne***		***P. aeruginosa***	
	S*	R**	S	R	S	R	S	R	S	R
Ampicillin	0	100	0	100	0	100	0	100	0	100
Clindamycin	67	33	61.3	39.7	54.7	45.3	17.6	82.4	6.9	93.1
Gentamicin	93	7	96.1	3.9	90	10	93.4	6.6	0	100
Cotrimoxazole	66.4	33.6	70.7	29.3	60.4	39.6	9.4	90 .6	96.9	4.1
Erythromycin	80	20	85.4	14.6	86.4	13.6	11.8	88.2	3.4	69.6
Vancomicine	87	1	40	60	0	100	0	100	0	100
Tetracycline	91.2	8.8	95.1	4.9	90.2	9.8	93.1	6.9	5.2	94.8
Doxycycline	100	0	100	0	0	100	95.5	4	0	100
Azithromycin	100	0	100	0	0	100	97.1	2.9	0	100

S* =Sensitive
R**= Resistance

Ibn Al-Haitham Journal for Pure and Applied science https://doi.org/ 10.30526/2017.IHSCICONF. 1774

Figure (1): antibiotic susceptibility test to *S. aureus* isolated from acne patients.

Our results showed that all bacterial isolates were resistance to Ampicillin. *Staphylococcus aureus* and *S. epidermis* were sensitive (100%) to Doxycycline and Azithromycin, *P. acne* were also highly sensitive to these two antibiotics (95.5%,97.1%) respectively, while *E. coli* and *P. aeruginosa* were (100%) resistance to these antibiotics. Gentamicin and tetracycline were susceptible by most of the study isolates except for *P. aeruginosa* which was very resistance to Gentamicin and tetracycline (100%&94.8%) respectively. On the basis of these results, we suggest Gentamicin and tetracycline are suitable antibiotics for acne patients. Table (2)

Resistance to antibiotics in bacteria that may cause acne vulgaris has been increased globally, with geographical regions differences [26].

Antibacterial activity of plants extracts

Results of this study indicated that only the crude solvent extracts prepared from the leaves of *Henna* and callus of Cinnamon cold water and alcohol, showed inhibitory activity on isolated acne vulgaris bacteria (Table 3), (Table 4), (Table 5), (Table 6). While all isolates where resistance to cold water and alcohol lupine extracts in different concentrations (100,50,25,12.5) mg/ml.

Table (3:) Mean zone diameter of inhibition in millimeter (mm) of cold water henna leaves extracts.

Bacterial isolates	Mean water extract concentration of Henna (mg/ml) 100	50	25	12.5
S. aureus	24.9	19.5	17.2	13.5
S. epideridis	28.8	22.1	18.4	13.6
E. coli	24	17.6	14	11.3
P. acne	22.5	14.5	10.5	8.5
P. aeruginosa	23	15	12	10

Table (4): Mean zone diameter of inhibition in millimeter (mm) of cold alcoholic Henna leaves extracts.

Bacterial isolates	Mean alcoholic extract concentration of Henna (mg/ml) 100	50	25	12.5
S. aureus	32.1	18.1	15.4	11.5
S. epidermidis	25	21.6	18.2	16
E. coli	20	17.5	15.5	12.5
P. acne	18.5	16	12.5	7
P. aeruginosa	20	13	12	9

Table(5): Mean zone diameter of inhibition in millimeter (mm) of cold water Cinnamon extracts

Bacterial isolates	Cold water Cinnamon extract concentration (mg/ml)			
	100	50	25	12.5
S. aureus	10.5	9.4	5.0	0
S. epidermidis	10.6	7.6	4.5	0
E. coli	14	9.5	4.3	0
P. acne	9.5	6.5	3.5	0
P. aeruginosa	10	7	5	0

Table (6): Mean zone diameter of inhibition in millimeter (mm) of cold alcoholic Cinnamon extracts

Bacterial isolates	alcohol Cinnamon extract concentration (mg/ml)			
	100	50	25	12.5
S. aureus	12.5	8.5	6.1	0
S. epidermidis	12.1	12	4.6	0
E. coli	13.5	8.5	7	0
P. acne	13	9	6.5	0
P. aeruginosa	18	10	7	0

Although antimicrobial drugs have greatly reduced the incidence of certain infection, including acne [27], alternative and effective natural compounds replacement to antimicrobial chemical drug are needed [28].

Antibacterial activity of water and alcoholic extracts of leaves of henna against *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from acne vulgaris patients have been studied by [29]. Their results showed that alcoholic extracts were highly effective than water extracts and Staph. *Epidermidis* bacteria was more susceptible to extract activity than *Staph. aureus*, with inhibition zone diameter (22) mm for extract of 1000 µg /ml. (29), this results was in contrast with our result were cold aqueous extract of Henna had the higher effect on all bacterial isolates compared with alcoholic extract. previous studies showed that the extracts of henna and roselle may have promising antibacterial activity [30]

Our results showed that alcoholic extract of *Cinnamomum* in concentration 100mg/ml have the highest inhibition to growth of all isolated acne bacteria, while 12.5 mg/ml of cold alcoholic and water extract had no effects on growth of acne bacteria under the study, this may be due to dilution of the effective material in these extracts.

Identification of the antimicrobial activity in *Cinnamomum zeylanicum (C. zeylanicum)* bark against bacterial skin infection were studied for the potential uses as alternative remedies in the treatment of skin infections. The cinnamon oil extract at 25%, 50% and 100% concentrations were found to inhibit *S. aureus* with clear zone diameters of 22.3mm, 23.3mm and 25.3mm respectively whereas the inhibition zone of Vancomicine (positive control) was 19mm. [31].

In our study Gram negative isolated bacteria were more resistance to all plant extracts than Gram positive one. This resistance may be due to its cell wall structure, which prevent the penetration of the plant extract, and increase resistant to these extracts.

References

[1] K. Bhate; HC.Williams Epidemiology of acne vulgaris. Br J Dermatol. ;168(3):474–485. 2013

[2] S. Hanna; J. Sharma; J. Klotz Acne vulgaris: More than skin deep. Dermatol Online J. ;9:8. 2003

[3] A. Pappas, Epidermal surface lipids. Dermatoendocrinology, 1, 72–76,2009

[4] Y. Olutunmbi; K. Paley; J.C. English, , 3rd. Adolescent female acne: Etiology and management. J. Pediatr. Adolesc. Gynecol. 21, 171–176. 2008

[5] V. Goulden; CH McGeown; WJ. Cunliffe The familial risk of adult acne: a comparison between first-degree relatives of affected and unaffected individuals. Br J Dermatol. 141(2):297-300. 1999
[6] B.Capitanio; J. L. Sinagra; V. Bordignon, Fei P. C., M. Picardo, and Zouboulis C. C., "Underestimated clinical features of postadolescent acne," Journal of the American Academy of Dermatology,. 63(5), pp: 782–788. 2010
[7] K. Annan & Houghton P.Antibacterial, antioxidant And fibroblast growth stimulation of aqueous extracts of Ficus asperifolia Miq. and Gossypium arboretum L Wound- Healing plants of Ghana. Journal of Ethnopharmacology.119:141-144. 2008
[8] M.Patel; W.P., Bowe, C.Heughebaert, and A.R Shalita,. The development of antimicrobial resistance due to the antibiotic treatment of acne vulgaris: a review. Journal of Drugs in Dermatology .;9: 655-664. 2010
[9] H. Nasri, M. Bahmani, N. Shahinfard, A.M. Nafchi, S. Saberianpour, M.R. Kopaei ,Medicinal Plants for the Treatment of Acne Vulgaris: A Review of Recent Evidences. Jundishapur J Microbiol.. 8(11): e25580. 2015
[10] F. YuJie,; C. LiYan,; Z. YuanGang; L. ZhiGuo ; L. Xia; L.Ying and E. LiPing Thomas, , The Antibacterial Activity of Clove Essential Oil Against Propionibacterium acnes and Its Mechanism of Action. Arch. Dermatol. 145:86-88, 2009.
[11] P. Hassanzadeh; M. Bahmani, and D. Mehrabani,Bacterial resistance to antibiotics in acne vulgaris: an *in vitro* study.Indian J Dermatol.; 53(3): 122–124. 2008
[12] OA .Alexeyev; AC. Jahns, Sampling and detection of skin Propionibacterium acnes: current status. Anaerobe. ;18(5):479-83. 2012
[13]CLSI (Clinical Laboratory Standard I nstitution). Performance standard for antimicrobial susceptibility testing;Twenty first informational supplement .,31(1). 2011
[14] I E .Al-Saimary; S S Bakr; T. Jaffar; A E. Al-Saimary; H. Salim and Al-Muosawi R. Effects of some plant extracts and antibiotics on *Pseudomonas aeruginosa* isolated from various burn cases, Saudi Med J. , 23(7) : 802-805. 2002
[15] Minegishi K, Aikawa C, Furukawa A, et al. Complete genome sequence of a Propionibacterium acnes isolate from a sarcoidosis patient. Genome A;1:3e4. 2013
[16] H .Brüggemann; A .Henne; F .Hoster, et al. The complete genome sequence of Propionibacterium acnes, a commensal of human skin. Science;305:671e3. 2004
[17] T .Nakatsuji; Tang DCC; L .Zhang, et al. Propionibacterium acnes camp factor and host acid sphingomyelinase contribute to bacterial virulence: Potential targets for inflammatory acne treatment. PLoS ONE;6:e14797. 2011
[18] I.Nagy; A.Pivarcsi; K.Kis, et al. Propionibacterium acnes and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/ chemokines in human sebocytes. Microbes Infect;8:2195e205. 2006
[19] I. Kurokawa; FW. Danby; Ju Q, et al. New developments in our understanding of acne pathogenesis and treatment. Exp Dermatol;18:821e32. 2009
[20] E .Contassot and LE. French, New insights into acne pathogenesis: Propionibacterium acnes activate the inflammasome. J Invest Dermatol;134:310. 2014
[21] B .Kumar; R. Pathak ; P. Bertin Mary ; Diksha Jha; K .Sardana and Hemant K. G .New insights into acne pathogenesis: Exploring the role of acne-associated microbial populations.Dermatologica Sinica . 34:67-73.2016.
[22[M .Toyoda and M. Morohashi An overview of topical antibioticforacne treatment. Dermatology. ;196:130–4. 1998

[23] E .Rodriguez-Cavallini and P. Vargas-Dengo Etiologyia bacteriana y susceptiblidad a antibioticos en pacientes con acne. Rev Biomed. ;15:101–6. 2004
[24] D. Thiboutot New treatments and therapeutic strategies for acne. Arch Fam Med. ;9:179–87. 2000
[25] Leyden JJ. Effect of topical benzoyl peroxide-clindamycin versus topical cindamycin and *Propionibacterium acnes*. Cutis. ;69:475–80. 2002
[26] H .Ashkenazi; Z .Malik; Y .Harth and Y. Nitzan; Eradication of Propionibacterium acnes by its endogenic porphyrins after illumination with high intensity blue light. FEMS Immunol Med Microbiol. ;35:17–24,2003
[27] N.Mendoza,; P. O. Hernandez,; S. K. Tyring,; K. A. Haitz, & A. Motta, Antimicrobial susceptibility of Propionibacterium acnes isolates from acne patients in Colombia. Int J Dermatol. 52, 688–692. 2013.
[28] C.Gupta,; A.P Garg,; R.C. Uniyal and A. Kumari, Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. Afr. J. Microbiol. Res. ; 2(9):247-251. 2008
[29] I.E. Alsaimary, Antistaphylococcal Activity of Henna extracts Lawsonia inermis L.(Lythraceae). Donnish Journal of Microbiology and Biotechnology Research..1(2) 008-011, 2014
[30] KK .Al-Rubiay; NN. Jaber; Al-Mhaawe BH; Alrubaiy LK. Antimicrobial efficacy of henna extracts. Oman Med J .; 23(4): 253-256. 9. 2008
[31] N.R.M. Shabani; Z. Ismail; W.I. Ismail; N. Zainuddin; Rosdan N.H.; Roslan ,M.N.F and Azahar ,N.M. Antimicrobial activity of cinnamon oil against bacteria that cause skin infections. Journal of Scientific Research and Development.. 3 (2): 1-6. 2016