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Antibacterial Activity for Acne Treatment through Medicinal Plants Extracts: Novel Alternative Therapies for Acne

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Abstract

Acne vulgaris is a chronic skin infection affecting the majority of adults. There are several internal and external factors responsible for this infection. The present study emphasizes the screening and combinations of medicinal plants extracts against acne-causing bacteria and antibacterial activity of these plant extracts. Antibacterial activities of three solvents extracts of *Camellia sinensis*, *Azadirachta indica*, and *Cassia acutifolia* was carried out using disc diffusion method against *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. The results revealed that different plant extracts showed noticeable activity against different test organisms. The acetone extract of combination A (C/A) showed the higher mean of antibacterial susceptibility against six bacterial strain with synergistic effect by 20.33mm than other combinations when combination A (C/A) was added. The antimicrobial susceptibility of combination A (C/A) was higher than combination group at concentration of 10^{-2} with 95% confidence interval. The present study concluded that the acetonic extract of C/A was the best antibacterial agent/candidate to treat acne vulgaris disease. Further trials might confirm its best possible doses for prescription to the dermatologists, physicians and clinicians in the field.

Keywords: Antibacterial activity, plant extracts, acne vulgaris, bacterial strains.

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INTRODUCTION

Acne is one of the commonest skin unrest, for which dermatologists are still struggling since years to treat it successfully. It fundamentally affects teenagers, although it may present at any age of life. It is almost a cosmopolitan disease occurring in all races and affecting 94% of 16-year-old boys and 82% of 16-year-old girls to some degree(Vora *et al.*, 2017). The incidence of severity of acne, peaks at 41% in 13-18-year-old girls and 35% in boys aged 17-20 year (Kim *et al.*, 2017). Acne by definition is a multi-factorial chronic inflammatory disease of pilosebaceous units. It affects the skin of the face, neck and upper trunk. These special sebaceous follicles have capacious follicular channels and voluminous, multi-acne sebaceous glands. Acne develops when these specialized follicles undergo pathogenic change that results in the formation of non-inflammatory lesions and inflammatory lesions (Choi *et al.*, 2011).

In this regard, the *Staphylococcus epidermidis* considered as the major skin bacteria that causes the formation of acne. Propioni-bacterium acnes, a gram positive and an anaerobic pathogen, plays an essential role in the pathogenesis of acne. It is involved in the development of inflammatory acne by its might to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils (Vora *et al.*, 2017). In addition, *Staphylococcus epidermidis*, anaerobic organism, is usually involved in super facial infections within the sebaceous unit (Vora *et al.*, 2017).

On the other hand, the medicinal plants have attracted increasing interest because of their antimicrobial susceptibility against pathogenic oral microorganisms. Plants that are used for traditional medicine contain a wide range of substances that can be used to treat chronic and infectious diseases (Ali *et al.*, 2016). Medicinal plants exploration has become important in the discovery of bioactive natural substances capable of inhibiting the mechanisms of different microbials. According to the World Health Organization (Who) report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major issue to the WHO for the next millennium. Screening of plants for antimicrobial agents has gained much importance because WHO is encouraging and promoting in the development and utilization of medicinal plants resources in the traditional system of medicine. Accordingly, the last decade witnessed an increase in the investigation of plants as a source of human infectious disease management (Mickymaray *et al.*, 2016). According to the WHO about 71%–94% of the world's population in developing countries relies mainly on indigenous medicinal plants for their primary health care. Traditional medicine has not only gained in popularity and approval, but it is sometimes the only system available in many rural areas. Furthermore, the use of medicinal plants to treat skin infections is very common in many rural areas all over the world (Gupta *et al.*, 2017).

Therefore, the present study was designed to determine the antibacterial activity of different medicinal plants extracts used for the treatment of acne.

METHODOLOGY

Assay for antibacterial activity by disc diffusion method

The test organisms used in this study were procured from Faculty of Industrial science and technology (FIST), Pahang. Malaysia, viz., *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* was used.

Medicinal plants extraction and investigation

Medicinal plants used

Preparation of extracts: The selected three commercial leaves of plants used in the study, namely, *Camellia sinensis*(Cs), *Azadirachta indica* (Ai), and *Cassia acutifolia* (Sa), were purchased from the market at kirkuk area, Iraq.

Preparation of plants extracts

The three plant parts were crushed using a blender and then sieved to obtain fine powders. Approximately 10 g of the powdered plants were soaked in 100 ml of four different solvents (95% ethanol, hot water, and acetone) kept at room temperature for 24 h, and the suspension was then filtered through a Whatman No.1 filter paper. The filtrates were evaporated to 5 ml the final concentration was 10% (g/v), using a rotary evaporator (BUCHI, Rotavapor R-3 Vacuum pump V-700), according to the modified method of (Gahlaut et al., 2013). The extracts were stored

in sterile bottles at 4°C until further use. Filter paper discs of 6 mm diameter were prepared using Whatman No. 1, UV sterilized, and loaded with 25µl plant extracts, left to dry, and then used an antibacterial screening test (Ali *et al.*, 2015).

Plants extract against bacterial isolates

Muller–Hinton agar medium was used for the antimicrobial susceptibility. The plants extract discs from previously prepared solvents were allowed to set onto the inoculated agar surface. Inoculum from primary culture plates were prepared, inoculating into a replicated plate, and incubation at 37°C for 24 h. After the incubation period, each plate was observed, and the inhibition zone of all isolates were recorded in millimetre (mm) calculated and represented as previously mentioned (Mukhtar *et al.*, 2012). The density of each bacterial suspension was adjusted using 0.5 McFarland as a standard. Control discs for different solvents were used.

Combination of plant extracts

The combination of plant extracts obtained using the three different solvents was studied at a ratio of 1:1 (v:v). the two combination groups, A and B, were prepared using the combination of two plant extracts together (A) (C/A, C/S, A/S) and all tree extracts (B) (C/A/S), respectively. All combinations were tested for antimicrobial susceptibility against bacterial isolates as previously mentioned.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration of combination prepared of 10 different dilutions from $10^{-1} - 10^{-10}$, the bacterial suspension was adjusted using a 0.5 McFarland standard at 600 nm. The tube dilution assay with slight modification (Donaldson *et al.*, 2005) was selected as the method for determining the minimum inhibitory concentration. Serial dilutions were used to prepare a series of ten tubes. The initial tube was filled with 200µl (v/v) of a highest combination of plant extract were added. The mixture was vortexed and 100µl was removed and placed into a second tube containing 900µl of acetone. This process was repeated to create ten dilutions with concentrations of $10^{-1} - 10^{-10}$. Each concentration was streaked onto Mueller-Hinton agar media; acetone was used as a control. Bacterial plates were incubated for 24 hours at 37°C and the

concentration that inhibited the bacteria was identified and the resultant MIC was recorded in (mm) with MIC being defined as the lowest concentration of combination for inhibiting the growth of the microorganism (Mccutcheon *et al.*, 1994). The acetone disc was used as control and the experimental assays were replicated three times.

Statistical analysis

Statistical analyses were performed using ANOVA for data on disc diffusion assays, to test the antibacterial activity profile of isolates within medicinal plant extracts on the zone of inhibition using software Minitab 17. All antibacterial activity data were determined in diameter (mm), calculated and represented in this paper as antibacterial activity mean of inhibition zone against all the bacterial isolates under study.

RESULTS AND DISCUSSION
Antibacterial susceptibility of medicinal plants against bacterial isolates

The acetone extracts of *Camellia sinensis*, *Azadirachta indica* and *Cassia acutifolia*, showed higher antibacterial susceptibility of the mean of inhibition zone against bacterial isolates, reaching to 34.67, 18.76, and 12.76mm, respectively. By comparison, the antimicrobial susceptibility was exhibited by the 95% ethanolic and the aqueous extracts of medicinal plants did not exhibit an inhibitory effect against the tested microorganisms as shown in Figures 1.a,b,c and

Table 1. Screening of antibacterial susceptibility of *Camellia sinensis* extracts using three different solvents against bacterial isolates

Bacterial strains	Ethanol Mean±SD*	Hot water Mean ±SD	Acetone Mean ±SD
P. acnes	13.67±0.47	8.67±0.47	16.33±0.47
S. aureus	14.33±0.47	8.67±0.47	21.67±0.47
S. epidermidis	15.67±0.47	9.67±0.47	21.33±0.47
P. aeruginosa	8.67±0.47	8.67±0.47	30.33±0.47
E. coli	10.33±0.47	9.33±0.47	30.33±0.47
B. subtilis	14.67±0.47	14.67±0.47	34.67±0.47

Results were expressed as mean ± SD, n =3. SD* standard deviation

Tables 1-3. The acetonic extracts of *Camellia sinensis*, *Azadirachtaindica* exhibited effective antibacterial preparation against bacterial isolates. These extracts were not harmful when consumed in products and used as agents that inhibit the growth of bacteria. The hot water extracts of three medicinal plants showed low antibacterial susceptibility, which may be due to the polarity of compounds; most antibacterial agents are extracted more effectively by organic solvents than by aqueous extraction (Aneja *et al.*, 2010). The results in current study were contrary to those in the study of Al-Emran *et al.* 2011 who reported that the results depict that leaf extracts of *Azadirachtaindica* could be used as a potential source of antimicrobial agents against the bacterial strains tested (Abdullah-Al-Emran *et al.*, 2011). These results were similar to the finding of Gupta and Kumar (2017), who investigated that the antimicrobial susceptibility of *camellia sinensis*

and *Terminalia arjuna* are traditional medicinal plants and represent rich source of compounds possessing antimicrobial properties (Gupta *et al.*, 2017).

Combination of plant extracts

The results of antimicrobial susceptibility profile of four combinations prepared, with the use of acetone plant extracts to obtain medicinal plants extracts, are shown in Table 4. The acetone extract of combination A (C/A) showed the higher mean of antibacterial susceptibility against six bacterial strains with synergistic effect by 20.33mm than other combinations, followed by acetone extract of combination B (C/A/S), with 13.67.

These results were similar to the finding of Naveed et al. (2013), who reported that most of the essential oils of *Cuminum cyminum, Cinnamom umverum, Amomum subulatum* and *Syzygium aromaticum*. Their antibacterial activities were investigated by minimum inhibitory concentrations

Table 2. Screening of antibacterial susceptibility of *Azadirachta indica* extracts using three different solvents against bacterial isolates

Bacterial strains	Ethanol Mean±SD*	Hot water Mean ±SD	Acetone Mean ±SD
P. acnes	12.33±0.47	8.67±0.47	18.67±0.47
S. aureus	10.33±0.47	8.67±0.47	12.33±0.47
S. epidermidis	12.33±0.47	9.67±0.47	11.33±0.47
P. aeruginosa	10.33±0.47	8.67±0.47	13.33±0.47
E. coli	11.33±0.47	9.33±0.47	14.33±0.47
B. subtilis	0.00±0.00	8.67±0.47	13.33±0.47

Table 3. Screening of antibacterial susceptibility of *Cassia acutifolia* extracts using three different solvents against bacterial isolates

Bacterial strains	Ethanol Mean±SD	Hot water Mean ±SD	Acetone Mean ±SD
P. acnes	10.67±0.47	8.67±0.47	12.67±0.47
S. aureus	11.67±0.47	9.33±0.47	9.33±0.47
S. epidermidis	10.67±0.47	8.33±0.47	11.33±0.47
P. aeruginosa	0.00±0.00	0.00±0.00	10.67±0.47
E. coli	9.33±0.47	0.00±0.00	9.33±0.47
B. subtilis	15.67±0.47	0.00±0.00	9.33±0.47

Table 4. Antimicrobial susceptibility of acetonic extract of combinations against the bacterial isolates

Group code Combination group Bacterial strain	(C/A) Mean±SD*	A (C/S) Mean±SD	(A/S) Mean±SD	B (C/A/S) Mean±SD
P. acnes	14.67±0.47	11.67±0.47	10.67±0.47	13.67±0.47
S. aureus	15.67±0.47	10.33±0.47	9.33±0.47	11.33±0.47
S. epidermidis	18.67±0.47	10.33±0.47	11.33±0.47	13.67±0.47
P. aeruginosa	16.67 ±0.00	0.00±0.00	10.67±0.47	8.67±0.47
E. coli	20..33 ±0.47	0.00±0.00	9.33±0.47	10.33±0.47
B. subtilis	15.67±0.47	0.00±0.00	9.33±0.47	12.67±0.47

Combination group: C: *Camellia sinensis*; A: *Azadirachta indica*; S: *Cassia acutifolia*; Results were expressed as mean ± SD

possessed anti-bacterial activities against selected multi-drug resistant clinical and soil bacterial strains (Naveed *et al.*, 2013).

The antibacterial susceptibility of plant extract combination A (C/A) against bacterial strains was higher than other combination groups. To our knowledge, probably this is the first report to try the combination of *Camellia sinensis* and *Azadirachtaindica* experiment. One way ANOVA Tukey test was utilized and results showed in Table 5 illustrates the grouping information using Tukey method with 95% confidence interval. The p-value was < 0.001 highly significant.

Statistical analysis showed the pair-wise comparisons indicate that combinations of medicinal plants are not significantly different as they share the same grouping of "A". While combinations A/S and C/S were not significantly different as well since grouping "B" was shared.

But if the comparison were made, between C/A and A/S, the two combinations are significantly different; because they do not share common a letter as shown in Fig. 1. However; it is very clear that combination C/A was superior in terms of antibacterial susceptibility when compared with other combinations. Therefore; C/A was the best candidate as an antimicrobial agent.

Determination of the Minimum Inhibitory Concentration of the combination A (C/A)

MIC was recorded as the lowest concentration of the extract at which visible bacterial growth was completely inhibited. The experiment was performed in triplicate. MIC of combination A of medicinal plant extract against bacterial strains was determined using disc diffusion method assay. The highest concentration of combination possible to test was 8mg/μl considered as MIC of antibacterial agent.
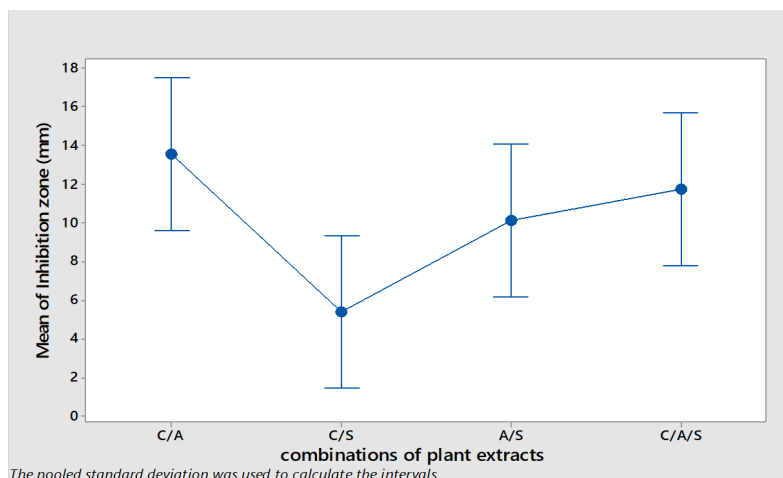

The pooled standard deviation was used to calculate the intervals.

Fig. 1. The antibacterial susceptibility of combination of plant extract combination with 95% confidence intervals (CI) for the mean. C/A: combination of *Camellia sinensis* with *Azadirachta indica*; C/S: combined with *Camellia sinensis* and *Cassia acutifolia*; A/S: combined with *Azadirachta indica* and *Cassia acutifolia*; C/A/S: combinations of all three medicinal plant together *Camellia sinensis*, *Azadirachta indica* and *Cassia acutifolia.*

Table 5. Tukey Pairwise Comparison of antibacteria susceptibility of combined action of plant extracts at p-value 0.001 against bacterial strains

Combination Factor	Mean	Grouping
C/A	13.56	A
C/A/S	11.72	A
A/S	7.11	B
C/S	5.39	B

CONCLUSION

The acetone extracts of the medicinal plant in our study (C, A, and S) showed the higher mean of antibacterial susceptibility against bacterial isolates. The acetonic extract of combinations of medicinal plants A (C/A) showed high antibacterial susceptibility against bacterial isolates. The combination C/A was superior in terms of antimicrobial susceptibility when compared with other combination groups.

It has successfully improved the antimicrobial susceptibility against bacterial isolates. Overall, it is concluded that the acetonic extract of C/A was the best antibacterial agent candidate to treat acne vulgaris diseases. Further trials might confirm its best possible doses for a prescription to the dermatologists, physicians and, clinicians for the benefits of patients in the field.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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