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Studies on Antibacterial potential of Dimethyl Cardamonin

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Abstract: **The invitro antibacterial activity evaluation of DMC was done on five different pathogenic bacterial strains (Pseudomonas aeruginosa, Bacillus subtilis, S.aureus, Staphylococcus aureus, E-coli.) using agar disc diffusion technique. The maximum activity was recorded from 100µl of DMC against Pseudomonas aeruginosa and minimum activity was observed in E-coli from 25µl of DMC. The observed antibacterial activities of the compounds could justify the traditional use of the plant for the treatment of different bacterial infections. Thus, further test is recommended on large number of bacterial strains to decide the potentials of the compounds as candidates in development of antibacterial drugs.**

Keywords: **DMC, Pseudomonas aeruginosa, Bacillus subtilis, S.aureus, Staphylococcus aureus, E-coli.**

1. INTRODUCTION

In the last years, scientists have focused on increasing human infections caused by pathogen bacteria and fungi. Microorganisms have unfavorable effects on the quality and safety of life. Synthetic chemicals are widely used against these microorganisms; unfortunately, they develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics [1]. In addition, these antibiotics sometimes cause allergic reaction and immunity suppression. Currently, people heal a lot and although in many cases they are turning to synthetic drugs, but a vast majority is turning to natural products. Therefore the use of essential oils is less damaging to the human health [2] because they are generally few toxic and they do not have side effects.

On the other hand, the food industry at present is facing a tremendous pressure from consumers for using chemical preservatives to prevent the growth of food borne and spoiling microbes. To reduce or eliminate chemically synthesized additives from foods is a current demand worldwide. A new approach to prevent the proliferation of microorganisms is the use of essential oils as preservatives. Essential oils of plants are of growing interest both in the industry and scientific research because of their antibacterial and antifungal properties and make them useful in many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [3].

Staphylococcus aureus are Gram-positive, non-spore forming, facultative anaerobic bacteria that are able to invade via the broken skin or mucous membrane. *S. aureus* causes skin lesions such as boils, furuncules and more serious infections such as pneumonia, phlebitis, meningitis and urinary tract infections. *Pseudomonas aeruginosa*, *S.aureus*, *Bacillus subtilis*, and *E-coli* are the opportunistic pathogens which are involved in the outbreak of various communicable disease and nosocomial infections such as burns, wound infection and urinary tract infection etc., Even though pharmacological industries have produced a number of new antibiotics, in the last three decades, resistant to these drugs by micro organisms. To control the use of antibiotics, we develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs either natural or synthetic. The ultimate goal is to offer appropriate and efficient anti microbial drugs to the patient [4].

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To our knowledge, this is the first detailed study about the compound that shows these anti bacterial properties.

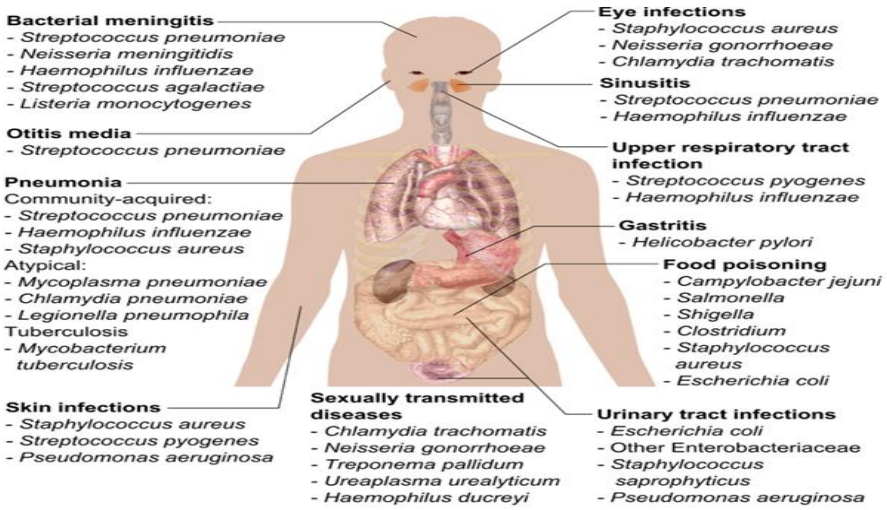

Fig 1: Overview of bacterial infections

Dimethyl cardamonin (DMC- 2,4 Dihydroxy 6 Methoxy 3,5 Dimethyl Chalcone)

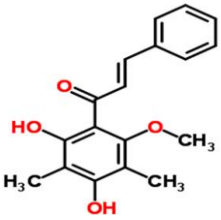

Fig 2: Dimethyl Chalcone

Molecular formula : C_{18} H_{18} O_4

Average Mass : 298.3310

Monoisotopic Mass : 298.121

A member of the class of chalcones that is *trans*-chalcone substituted by hydroxy groups at positions 2' and 4', a methoxy group at position 6' and methyl groups at positions 3' and 5'. Chalcones are precursor compounds for flavonoids biosynthesis in plants, and they can also be synthesized in laboratory. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents having improved potency and lesser toxicity and good pharmacological actions. Chalcones became an object of continued interest in both academia and industry. Nowadays, several chalcones are used for treatment of viral disorders, cardiovascular diseases, parasitic infections, pain, gastritis and stomach cancer, as well as like food additives and cosmetic formulation ingredient [5].

2. MATERIALS AND METHODS

DMC was purchased from supplied by Sigma Aldrich Chemicals Co. Ltd. All the other chemicals used were of analytical grade and purchased from commercial sources.

Collection of bacteria:

The 24 hrs grown bacteria *(Pseudomonas aeruginosa*, *Bacillus subtilis*, *S.aureus*, *Staphylococcus aureus* ,*E-coli*) were collected from Microbiology Laboratory, Doctors Diagnostic Centre, Tiruchirappalli, Tamilnadu, India.

Preparation of nutrient broth:

To the 2 liter conical flask, suspend 8 g of the nutrient broth medium in one liter of distilled water. Heated to boiling to dissolve medium completely. Dispense into appropriate containers and sterilize in autoclave at 121 °C (15 Ibs pressure) for 15 mins. The prepared medium should be stored at 2-8 °C. The color is amber, slightly opalescent [6].

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Standardization of the test organisms:

A loop full of test organism was inoculated on nutrient broth and incubated for 24 hrs. Exactly 0.2 mL of 24 hrs grown culture of the organisms was dispensed into 20 mL sterile nutrient broth and incubated for 3-5 hrs to standardize the culture to a concentration of $1X10^6$ colony forming units per mL (CFU/mL) before use of culture [7].

Preparation of Muller Hinton Agar medium:

To the 2 liter conical flask, suspend 38 gm of the muller hinton agar medium in one liter of distilled water. Heated to boiling to dissolve medium completely. Autoclave at 121 °C for 15 mins. Cooled to room temperature. Poured cooled muller hinton agar into sterile petri dishes on a level and the horizontal surface to give uniform depth. Allowed to cool to room temperature. Stored the plates at 2-8°C [8].

Antibacterial activity:

The antibacterial screening of DMC was carried out by disc diffusion method [4]. 10% w/v test solution of DMC were prepared by dissolving 500 mg of each extract separately in 5mL of sterile 10% Dimethyl Sulphoxide (DMSO). From this 25, 50 and 100 µL extracts contains 25, 50 and 100 mg, respectively and fresh latex were taken for the analysis of antibacterial activity. The DMC were loaded at different concentrations (25, 50 and 100 mg) in the well on preinoculated Mueller Hinton Agar (MHA) plates with respective bacterial cultures and incubated at 37 °C for 24 hrs. Streptomycin (10 µg) was used as a positive control and the solvent 10% DMSO was used as negative control for this study. After incubation the diameter of zone of inhibition (mm) around the well was measured using zone reader.

Statistical analysis:

The results of the present study were subjected to statistical analysis and the results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

Table 1: Antibacterial activity of DMC against Gram – positive and Gram – negative bacteria

Bacteria	DMC in three different concentration Zone of inhibition(mm in Diameter)				
Concentration	**Control**	**Positive Control (Streptomycin 10µg)**	**25(µl)**	**50(µl)**	**100(µl)**
Pseudomonas aeruginosa	**NIL**	**18.5±0.68**	**22.3±1.0**	**24±0.6**	**26.3±0.68**
Bacillus subtilis	**NIL**	**12.5±0.67**	**19.75±1.0**	**21.4±0.13**	**23.3±0.68**
S.aureus	**NIL**	**14.75±0.48**	**19.66±0.51**	**20.1±0.13**	**22±0.01**
Staphylococcus aureus	**NIL**	**14.3±0.65**	**18.45±0.70**	**18.14±0.20**	**19.0±0.13**
E-coli	**NIL**	**14.4±0.62**	**17.0±0.13**	**18.4±0.65**	**18.24±0.33**

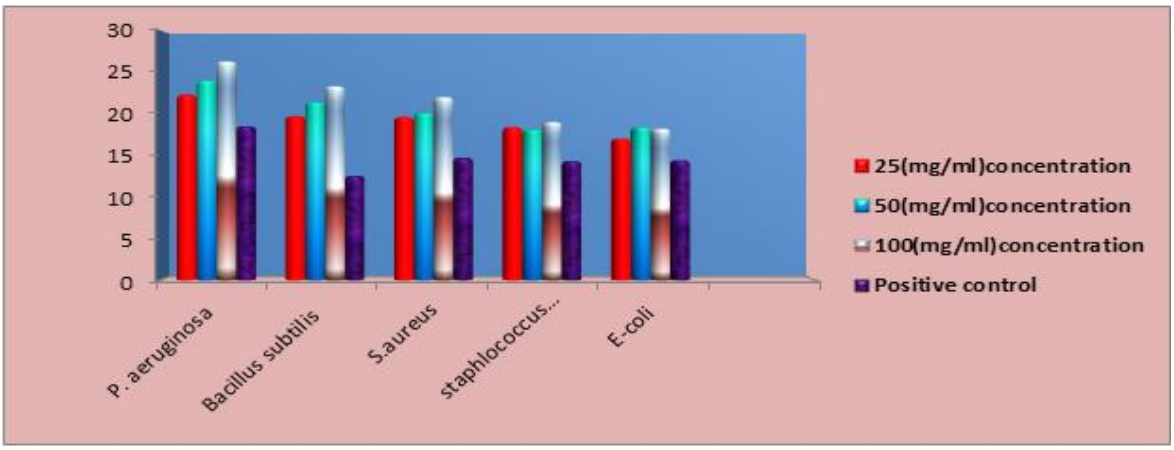

Fig 3: Graphical representation of antibacterial activity of DMC against Gram – positive and Gram –negative bacteria

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invitro tests were carried out to evaluate antibacterial activities of the compound (DMC) using disc diffusion methods against five bacterial species such as *Pseudomonas aeruginosa*, *S.aureus*, *Bacillus subtilis*, *Staphylococcus aureus* and *E-coli.* The activities of the compounds were expressed in terms of growth inhibition zones (given in mm). From the Table 1 and figure 3 it was concluded that the antibacterial activities of the compound (DMC) was higher than that of the reference drug (streptomycin) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* species used in the experiment and their growth inhibition values were also comparable to each other. The highest concentration of DMC showed largest zone of inhibition against the *Pseudomonas aeruginosa* (26.3±0.68) and *Bacillus subtilis* (23.3±0.68) respectively.

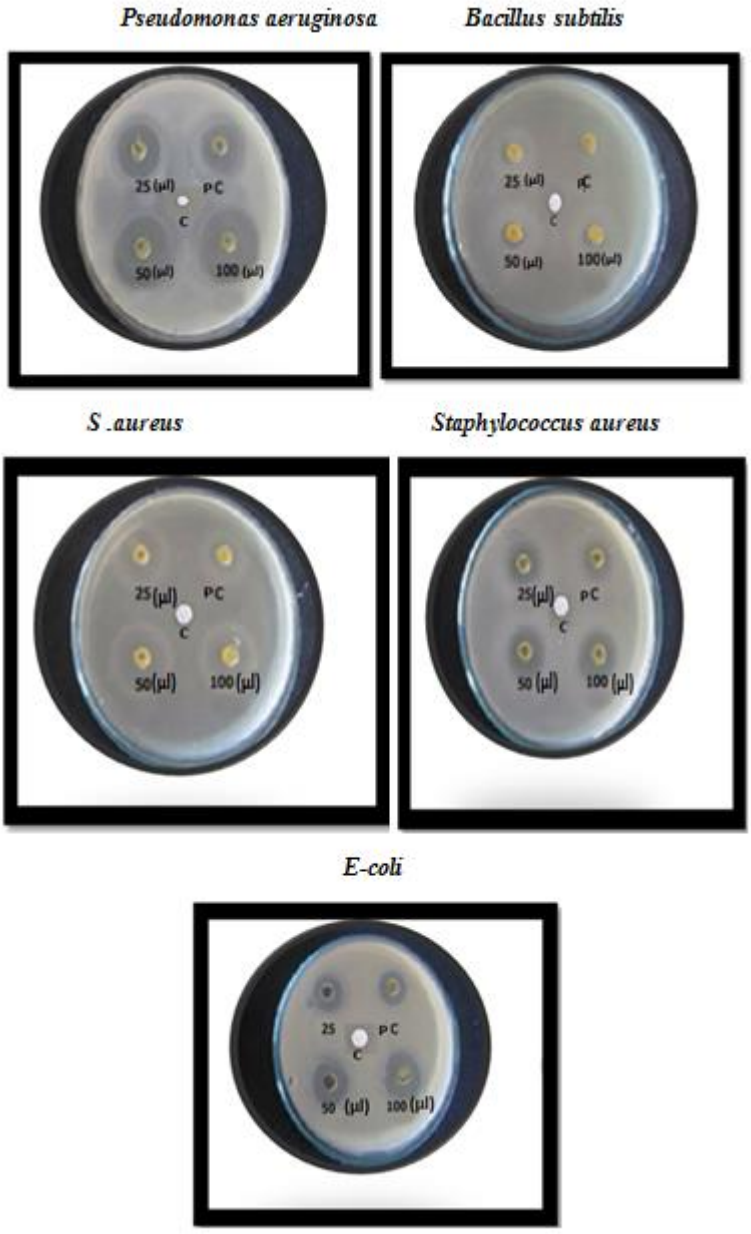

Fig 4: Anti bacterial activity on various concentration of DMC

E. coli are common member of the normal flora of large intestine. It is predominant facultative organism in the gastrointestinal tract and colonizes the tract within hours or few days. It is responsible for causing diarrhea which is characterized by rapid onset of watery non bloody fluid. *Pseudomonas sp*. is the epitome of an opportunistic pathogen to human. It causes urinary tract infection, respiratory system infection, dermatitis soft tissue infection, gastrointestinal infection and a variety of systemic infection. *S. aureus* is a facultative anaerobe that grows by aerobic respiration or by

fermentation which yields lactic acid. These are pathogenic to human beings. They cause a wild range of superlative infection as well as food poisoning and toxic shock syndrome. *Salmonella sp*. includes a large number of pathogens of human beings as well as mammals. These are pathogenic when acquired by oral route. Broadly they may cause enteric fever, septicemia and enteritis. The enteric fever and septicemia are caused by thousand of *Salmonella* [9, 10, 11].

According to Shaik Khadar Yazdan *et al.*, [12] isolated or synthesized chalcones that possess antimicrobial activity by the presence of a reactive α, β-unsaturated keto function in chalcones was found to undergo conjugate addition with a nucleophilic group like a thiol group in an essential protein, thus partly contributing for their antimicrobial activity, which may be altered depending on the type and position of the substituents on the aromatic rings.

Chalcones, extensively existed in edible plants, are usually regarded as the open-chain flavonoids, in which the two aromatic rings are linked by a three-carbon α, β-unsaturated carbonyl system [13]. They have exhibited various biological activities and been demonstrated to be the compounds responsible for the antibacterial effects [14, 15, 16].

Nowakowska [17] has summed the anti-infective activity of synthetic and naturally occurring chalcones and discussed the structure requirements of effective antibacterial chalcones. Hydroxyl groups especially the one at 4' position are likely to induce and enhance the activity, while the methoxyl group tends to reduce or eliminate the activity and that the lipophilicity of ring A substituents plays important role in modulating the activity. In the more recent review by Sahu [18] the necessary of hydroxyl group at 4 positions and the importance of lipophilic substituents such as prenyl and hexyl groups in the molecular structure were inferred and the presentation of electron-attracting groups was also regarded as the positive factor for antibacterial activity [19].

Husain *et al*., [20] has emphasized the bischalcone derivatives synthesized and investigated on their activity against *E. coli, P. aeruginosa,* and *C. albicans*. The structure-activity relationship revealed that the compounds having free hydroxyls were more active than those of the compounds having methoxy groups and the presence of electron withdrawing groups like chloro increased the activity.

The recent studies have showed that both the C-benzylated dihydrochalcone and the dihydrochalcone dimer were found to be inactive for antibacterial activity against *M. tuberculosis* [21]. Based on the above reviews, the newest literatures were discussed here to make an update. At first, it has been demonstrated that DMC showed antibacterial activity against *Pseudomonas aeruginosa, S.aureus, Bacillus subtilis, Staphylococcus aureus* and *E-coli.* The results indicated that the DMC was found to be the remarkable antibacterial activity in this study according to their inhibition action against all tested pathogenic bacteria. It is hoped that this study would lead to the establishment of some new and more potent antibacterial drugs from natural origin and native plants. However, there are no reports in literature to compare and contrast our result.

Sohly *et al.*, [22] isolated prenylated chalcones from the leaves of *Malclura tinctoria* possessing antifungal activity. Stevaz *et al.*, [23] isolated a 2',4'-dihydroxy-3' -methoxychalcone from the methanolic extract of *Zuccagnia punctata* which exhibited antifungal activity. Tsukiyama *et al.*, [24] isolated a retrochalcone, licochalcone-C from *Glycyrrhiza infanta* which showed potent antibacterial activity.

4. CONCLUSION

Based on the result of the above study on the DMC we conclude that the compound shows superior antibacterial activity against the following microorganisms such as *Pseudomonas aeruginosa, Bacillus subtilis, S.aures, Staphylococcus* and *E-coli*. The maximum activity was recorded from 100µl of DMC against *Pseudomonas aeruginosa* and minimum activity was observed in *E-coli* from 25µl of DMC. These antibacterial properties can be used for the therapy of infectious diseases caused by pathogenic microbes. These results suggest that the chalcone derivatives have excellent scope for further development as commercial antibacterial agents.

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