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Effect of Eugenol Derived Chemical Compound on Pathogenic Bacterial Isolates

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Abstract: ***This research aimed to determine the antimicrobial effect of new synthesized chemical derivative of plant extracted and purifiedeugenol, Different pathogenic bacterial samples were isolated from 50 specimens (blood, stool, pharynx swab) of both sexes from age 12-60 year. Four bacterial species were isolated and identified: Staphylococcus aureus (methicillin resistant), Streptococcous pyogenes, Salmonella typhi , Shigella spp. The purified eugenol was chemically derived producing new synthesized derivative: derived eugenol compound 2 DEC.2. (methoxy -4- nitro phenol citrate) Anti-microbial effect of new purified eugenol derivative against the isolated bacterial species was accomplished with different concentrations 10%, 5%, 1%, 0.5%, 0.01%. Staphylococcus aureus was the most sensitive bacteriato attack by DEC.2. and The interaction between DEC.2. and 10% concentration was significant and showed the highest inhibitions DEC.2. (39mm). Immunological test resulted in IgA and IgA was nearly reaching normal value in group treated with DEC2 and IgM showed normal value in mice treated with DEC.2. at 10%.***

Keywords: eugenol pathogenic bacteria immunological test

1. Introduction

Expansion of natural drugs has been primed for thousands of years. Kind of plant phenol eugenol was a very extensive biological action. Eugenol derivatives form readily retrieved natural products, in particular eugenol which may containsulphur-containing phytochemicals, formed by relieving the oxygen in the isocyanate group with a sulfur [1, 2] countless herbs and spices have been standard for their preservative or therapeutic property for millennia fixed oils present in plant matter have been qualified as most important source of antimicrobial activity illustrate against bacteria and fungi [3]. Plants have been used for medicinal purposes for as long as history has been recorded. Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the amalgamation of useful drugs. There are between 35, 000 and 70, 000 plant species that have been used for medicinal purposes in the world [4].

2. Materials and Methods

Bacterial media
Blood agar, MacConkey agar, S.S.agar, Desoxycholate agar, Xylose-lysine-desoxycholate agar, Hektoen enteric agar, Bismuth sulfite agar all media were ready made and it was organized according to its specific weight per liter, then media were dissolved in distilled water then sterilized in autoclave. Each medium was prepared according to the prescribed dry weight dissolved in 1 liter of distilled water then the dissolved media placed on hot plate to exclude the precipitate followed by autoclaving process [6]

Two types of plants were used clove *Eugenia caryophyllatum* and *Cinnamon sp.*, both plants were identified in the national Iraqis herbarium, the used plant part in this study were bark of *cinnamon* and bud of clove. The oils extracted from the *Eugenia and cinnamon* were prepared by using the Soxhlet apparatus.

Eugenol isolationand purification from fixed oil
20 ml of clove fixed oil were treated in the following events clove oil was purified using citric acid. A 150 ml of clove oil and NaOH were added under specified ratios of variables. The saponification answer occurred for 22 minutes, and then follows by decantation for 8 hours, then neutralized with hydrochloric acid and was reacted for 5 minutes. This progression was continued by decantation for 15 minutes. The eugenol product was purified by using distillation for 1.5 hours. Result of the reaction treated with dichloromethane (250ml) and kept for 24 hours. Then the liquid extract was filtered and evaporated to afford light brown oil10 ml of clove oil was added to 75 ml aqueous 1 N potassium hydroxide solution was added and shaken thoroughly. Then it was heated on a water bath with occasional shaking for 10 minutes. The flask was refrigerated the contents of the flask were poured into a separatory funnel and the non-phenolic portion was separated. The aqueous layer was filtered through a filter paper and the filtered solution was acidified with dilute hydro-chloric. The extract layer was dried with anhydrous sodium sulphate and evaporated under reduced pressure to get of eugenol as a pale yellow liquid eugenol [5]

Derivation of DEC.2. (Methoxyg-4- nitro phenol citrate)
Mixture of 26.6 g (0.13 mol) of eugenoule and 39.6 g, the filtrate was washed successively with 5% HCI, 5% $NaHCO_3$, solution, and water. The solution was dehydrated over anhydrous MgSO4. The residue obtained after removal of ether was distilled under reduced pressure [7].

Immunologicaltest:
The examined protein diffusing in agarose gel contain IgG, IgM, IgA, immuno-complex well form, perceptible as ring around the well. Its diameter proportional directly to concentration of analyzed protein corresponding to diffusion time, at the end of 72 h. the square diameter will be linear proportional to concentration of samples. Tests were accomplished with LTA labs technologies. Italy.

3. Results and Discussions

Yield of plant extracted fixed oils:

Yields of fixed oils extracted from *Eugenia caryophyllatum* (clove), *Cinnamon* spp. by soxhlet apparatus, shown in table 1. Highest yield of fixed oils from clove was76% (v/w) and the lowest yield from Cinnamon which was 24% (v/w).

Table 1: Yield of fixed oils extracted from each plant used in this study

Plant part	Plant extracted part dry weight		Oil yield in v\w
Eugenia caryophyllata	250gm	19 ml	76%
Cinnamon sp.	250gm	6ml	24%

Inhibitions of derived eugenol compound on bacteria

The interaction between DEC.2. and 10% concentration was significant and showed the highest inhibitions DEC. 2 *Staphylococcus aureus (*39mm).Diameter of inhibitions showed the highest value for *Shigella* (30mm) at 10%.Diameter of inhibitions showed the highest value when *Salmonella typhi* was treated DEC.2. (27mm) at 10%

Diameter of inhibitions showed the highest value when *Streptococcus Pyogenes* was treated with, followed by DEC.2. (30mm), at 10%

Major significance and interactions shown between the concentrations of DEC.2. at p≤ 0.05.

According to the statistical analysis which shown in and many significant differences appeared between the different derivatives and other differences appeared among the conc. used against *Staphylococcus aureus* at p≤ 0.05.

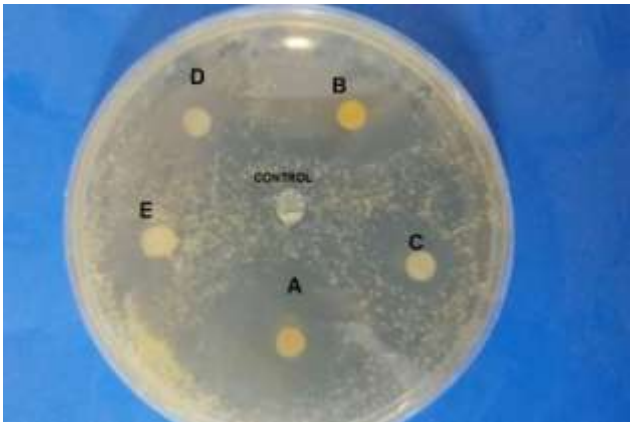

Figure 1: Inhibitions zone diameter in mm as an effect of different concentration of most effective compound DEC.2. on *staphylococcus aureus* (A-10%, B- 5%, C- 1%, D- 0.5%, E- 0.01%).

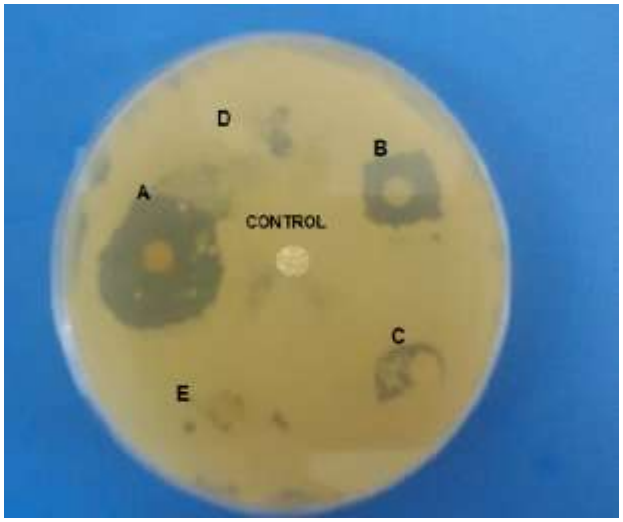

Figure 2: Inhibitions diameter in mm as an effect of different concentration of DEC.2. on *Shigella* spp. (A-10%, B- 5%, C- 1%, D- 0.5%, E- 0.01%).

Ta Effect of DEC.2. on bacteria

Treatment concentration	Effect of DEC.2. on Salmonella typhi	Effect of DEC.2. on Streptococcus pyogenes
Con. 10 %	27	30
	±	±
	2.598	3.073
Con. 5 %	15	28
	±	±
	1.443	2.868
Con. 1 %	15	15
	±	±
	1.443	1.536
Con. 0.5 %	2	2
	±	±
	0.192	0.205
Con. 0.01 %	0	0
	±	±
	0	0
LSD P ≤ 0.05 of concentration	4.472	5.638

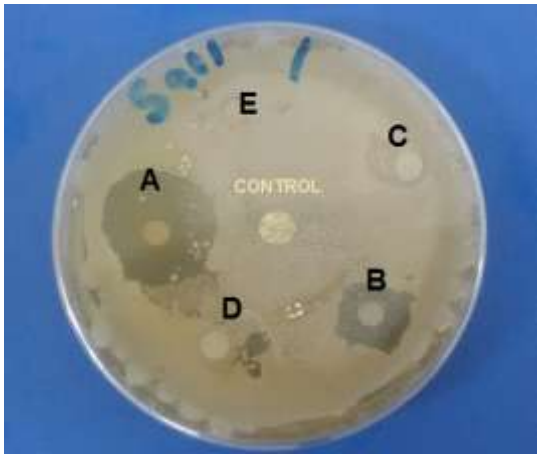

Figure 3: Inhibitions zone diameter as an effect of different concentration of DEC.2. on *Salmonella typhi* (A-10%, B- 5%, C- 1%, D- 0.5%, E- 0.01%).

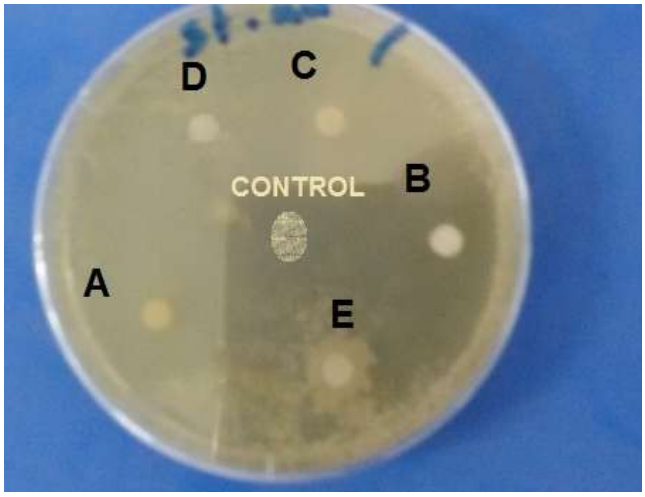

Figure 4: Inhibitions zone diameter in mm as an effect of different concentration of DEC.2. on *Streptoococcus pyogenes* (A-10%, B- 5%, C- 1%, D- 0.5%, E- 0.01%).

Table 3: Effect of derived eugenol compound DEC.2. on bacteria

Treatment concentration	Effect of DEC.2. on Shigella spp.	Effect of DEC.2. on Staphylococcus aureus
Con. 10 %	30	39
	±	3.555
	3.352	30
Con. 5 %	18	±
	±	2.735
	2.011	22
Con. 1 %	20	±
	±	2.006
	2.235	15
Con. 0.5 %	19	±
	±	1.367
	2.123	5
Con. 0.01 %	1	±
	±	0.069
	0.069	6.548
LSD P ≤ 0.05 of concentration	5.966	22.2

Antibodies concentration according to IFCC

According to the used procedure which was used the group 1 treated with DEC.2., group 2 treated twice with DEC.2. twice at concentration 10% and group 3 were with 5 mice that is control not with *E-coli* infected not treated.

IgG: The highest value normally for group 2 showed 1002

IgM: The highest value normally for group 2 showed 109,

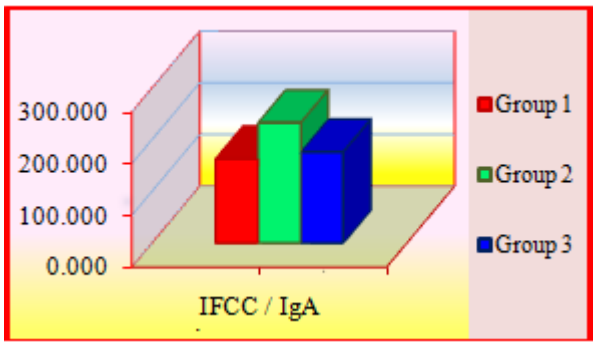

Figure 5: Effect of DEC.2.on IgA

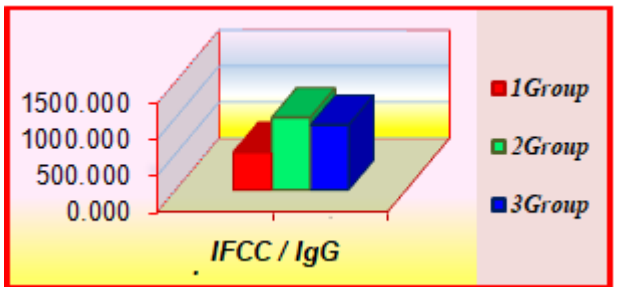

Figure 6: Effect of DEC.2.on IgG

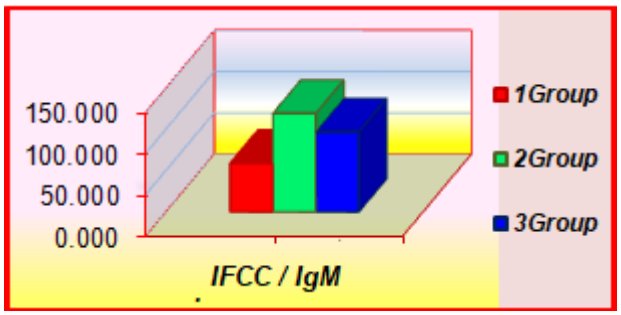

Figure 7: Effect of DEC.2.on IgM

Eugenol derivatives shows highly consequence on bacterial growth that because the presence of active groups in the chemical. That amuse yourself in the inhibitory effect due to the content of the carbonyl group of atoms oxygen that the corporation of the two of electronic that react or link with enzymes of active groups in the body, which cause to the inhibitions of some imperative enzymes and its aromatic ring (Togo, 2002)

Comparable to that of other Gram-positive bacteria, the pathogenicity of *S. aureus* is, to a great level, dependent upon the secretion of numerous extracellular virulence factors. The clinical performance of antibiotics for the treatment of S. *aureus* is conform not only by their particular bactericidal or bacteriostatic activities but also by their effects on the release of virulence factors, many genes encoding virulence factors are coordinately regulated in response to a variety of intracellular and extracellular signals [8]

The most inhibited bacteria was *staphylococcus aureus* when it was treated with the three derivatives this could be discussed as inhibitory concentrations may inter with the translocation of one or more regulatory gene products in *S. aureus*, which in gone affects transcription of exoprotein-encoding genes, [9] Furthermore, sub inhibitory concentrations of Eugenol differentially repress the transcription of S. *aureus* fetoprotein genes and act partially through, it is tempting to speculate that eugenol-induced inhibitions of global regulators. Sub inhibitory of eugenol derivatives differentially stop transcription of exo-protein genes in *staphylococcus aureus* [10]

Most resistant bacteria was *Sallmonella*, according to have been isolated, many have varying metabolic characteristics, rank of virulence, and multi-drug resistance genes that complicate treatment in areas that deffence is prevalent. *S. typhi* has a combination of characteristics that do it an

effective pathogen. This species contains an endotoxin typical of Gram negative organisms, as well as the Vi antigen which is thought to increase virulence. It also produces and excretes a protein known as permit non-phagocytic cells to take up the bacterium, where it is able to live intra-cellular. It is also able to slow down the oxidative burst of leukocytes, making innate immune response ineffective and resistance to antibiotic increased [11]

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