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Effect of Plant Extract *Eugenia caryophyllus, Cinnamon zeylanicum* on *Antibiotic* resistant from *Staphylococcus aureus*

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Abstract: The aim of this study was determination antimicrobial activity of ethanol extract and Watery of *Eugenia caryophyllus*, *Cinnamon* zeylanicum effect resistant genes in *Staphylococcus aureus* from Tomato in Green house, market in Sulaymani city-Iraq. The isolates were identified through cultural, morphological and biochemical examination, in addition to API Staph system. Susceptibility test to ten antimicrobials were performed for all isolates. The isolates were grouped to 7 antibiogram; The *S. aureus* isolates were 100% resist to Amp, Cef, Ax, Gm, Tet and Tri. Some isolated were resistant to at least one more antibiotics. The Sub Minimum Inhibition Concentration (SMIC) of ethanol extract and watery for *Eugenia caryophyllus* determined (2000 µg/mL), S MIC for *Cinnamon* (2500 µg/mL). Treating *S. aureus* S1, S3 by both medicinal plant, the results showed that Clove extract, appeared high inhibitory effect on the growth of' '*Staphylococcus aureus*, this inhibition converted some resistance genes in S1, S2 to sensitive.

Keywords: Antimicrobial, *cinnamon* ethanol extract, *Eugenia caryophyllus*, *Staphylococcus aureus*, watery extract

INTRODUCTION

Staphylococcus aureus is a type of bacteria commonly found on the skin and hair as well as in the noses and throats of people and animals. These bacteria are present in up to 25% of healthy people and are even more common among those with skin, eye, nose, or throat infections.

Staphylococcus aureus can cause food poisoning when a food handler contaminates food and then the food is not properly refrigerated. Other sources of food contamination include the equipment and surfaces on which food is prepared. These bacteria multiply quickly at room temperature to produce a toxin that causes illness (Life Science Journal, 2009). *Staphylococcus* is killed by cooking and pasteurization. Plants are able to produce different compounds that used to protect themselves against different types of pathogens (Cowan, 1999). Interest in medicinal plants has revived as a consequence of current problems associated with the use of antibiotics (Emori and Gaynes, 1993).

There is a continuing search for new antimicrobials from other sources including plant extracts, these plants then emerged as compounds with potentially significant theatric application against human pathogen (Kathe *et al*., 2003). Most of the investigations show that medical plants used for treating many diseases that are caused by many pathogen, due to its chemical components (Hamilton-Miller *et al*., 1995) in this study. Out of 100 specimens, 30 staph aureus isolates were obtained from different source, of Tomato the effect of ethanol, watery extract of Clove (Eugenia caryophyllus) and Cinnamon examined against species of pathogenic bacteria ('Staphylococcus aureus).

Using the Sub Minimum Inhibitory Concentration (SMIC): The results showed that Clove extract appeared high inhibitory effect genes resistant in S1, S3 which were cleared in (T2, T4).

More ever table (T2, T5) shows that the effect of plant extract ethanol and watery of *Cinnamon* determined to converted some resistant genes to sensitive in (S1, S3).

There is a continuing search for new antimicrobials from other sources including plant extracts, these plants then emerged as compounds with potentially significant theatric application against human pathogen (Kathe *et al*., 2003). Most of the investigations show that medical plants used for treating many diseases that are caused by many pathogen, due to its chemical components (Hamilton-Miller, 1995; Toda *et al*., 1989). On the other hand, in laboratory tests, the chemical eugenic, has been found to be a weak tumor promoter, making clove one of many healing herbs with both pro and anti-cancer effects (Vainker, 2003).

Cloves kill intestinal parasites and exhibit broad anti-microbial properties against fungi and bacteria. Thus, they support their traditional use as a treatment for diarrhea, intestinal worms and other digestive ailments (IIcim and Digrak, 1998).

The anti-microbial effect of clove is attributed to eugenic a phenol compound which is the major active

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constituent of its essential oil (Ali *et al*., 2005). And Cinnamon affect to inhibition bacterial infection the Cinnamon is reputed as a cure for colds. It has also been used to treat diarrhoea and other problems of the diseases. Cinnamon is reputed as a cure for colds.

Cinnamon has been reported to inhibit the growth of several antibiotic resistant strains of bacteria and antibacterial activity of commercial and will cinnamon species has been seen. Cinnamon in concentration as low as 0.02% inhibited mold growth (Ranjan *et al*., 2012).

The main goal of this study was determination the antimicrobial activity of ethanol and Watery extract of *Eugenia caryophyllus* and Cinnamon effects on resistant genes in bacteria *S. aureus* that isolated from Tomato in Green houses in Sulaymani city/Iraq market.

MATERIALS AND METHODS

Local bacterial isolates: Thirty isolates of staph aureus were isolated from green house in Sulaymani City, Iraq during June, 2012 to May depending Culture, Morphological and biochemical analysis according to (Andrews and Hammack, 2000).

Principally by applying coagulate, catalase and production of golden pigment on milk agar, in addition to Mannitol salt agar, Gram stain for each isolate moreover API Staph system performed.

Antibiotics used: The susceptibility of the thirty *S. aureus* isolates agents ten antimicrobial agents (Amikacin AK, Ampicillin Amp, Cephalosporin Cef, Ciprofloxacin Cip, Amoxicillin Ax, Gentamycin Gm, Rifampicin Rif, Nitrofrodantin Nit, Tetracycline Te, Trimethoprim, Tri was determined by means of disk diffusion method (Atlas *et al*., 1995).

Alcohol extract of *Eugenia caryophyllus* (Clove), Cinnamon Ethanol extract, Watery extract were prepared according to McClure (1975), 50 g of dried powder using soxhelt extraction techniques. The hot extract was concentrated to dryness at 50°C in the vacuum oven. One gram portion of the sample was reconstituted in 100 mL of sterile distilled water and sterilized by membrane filtration.

Determination of MIC: The antimicrobial activity of ethanol extracts of Eugenia caryophyllus (Clove), *Cinnamon was* tested using serial dilution. *S. aureus* (S1, S3) isolate inoculums were standardized using standard curve prepared (Cruickshank *et al*., 1975). Suspensions were further diluted to obtain the 1×10^5 CUF inoculums. Using spectrophotometer and by account of viable cells on nutrient agar (Atlas *et al*., 1995).

RESULTS

Thirty isolates of *Staph aureus* were obtained from different sample from Tomato in Green houses, market in Sulaymani city, Iraq. These isolates were identified culturally, morphologically and biochemically. The API Staph system was performed to support the identification process. These isolates were screened for ten antibiotics and according to the type Table 1 antibiogram of *Staphylococcus aureus* isolated from Tomato in Green houses, Market in Sulaymani city-Iraq and the number of antimicrobial resistance in *S. aureus* isolates were grouped to 6 antibiogram Table 1. The *S. aureus* isolates were 90% resist to Amp, Cef, Rif. 100% to Te Tri, Ax and Nit antibiotics, Differed in resistance to Ak, Cip. And Gm All isolates are multi resistant (resist to more than two antibiotics) that result was cleared in (T1) The results showed that in Table 2 alcoholic extract of *Eugenia caryophyllus* antimicrobial activity, effect on antibiotic resistant in S1, (*Staph aureus* Ak Gm, Te Rf, Ax, converted from resistant to sensitive and above antibiotic were effected by 2000 µg\mL SMIC alcoholic extracted of *Eugenia-caryophyllus* on (S3) the result showed in Table 2. The resistant genes were affected inAk, Amp, Cip, Te, Cef and Ax in (T3) by 2500 µg\mL SMIC ethanol extracts of *Cinnamon* Determined effect on resistant genes in (S1) In (T3) show also the effect On genes in (S3) Am, Cip Te, Rf, Ax and Nit converted genes from resistant to sensitive.

So that the result of watery extract of *Eugenia caryophyllus* were shown in (T4) for (S1, S3) the

Table 1: Antibiogram

Group of Saureus (T1) antibiogram	Isolated number	Ak	Amp	Gm	Tri	Cip	Te	Rf	Cef	Ax	Nit
1	1, 3	R	R	R	R	R	R	R	R	R	R
2	2, 5, 6, 8, 10, 13	S	R	S	R	S	R	S	R	R	R
3	16, 20, 26, 27, 29, 30	S	R	R	R	S	R	R	R	R	R
4	4, 7, 9, 11, 14, 18, 19, 21, 22	R	R	R	R	R	R	R	S	R	R
5	15, 17, 23, 25, 28	S	S	S	R	R	R	R	R	R	R
6	12, 24	R	R	R	R	S	R	R	R	R	R

Table 2: Effect of SMIC alcoholic extract of *Eugenia caryophyllus* (Clove)

T2 alcoholic extract of (Clove) SMIC 2000 µg/mL	Ak	Amp	Gm	Tri	Cip	Te	Rf	Cef	Ax	Nit
(S1) SMIC 2000 µg/mL	S	R	S	R	R	S	S	R	S	R
(S3) SMIC 2000 µg/mL	S	R	S	R	R	S	S	R	S	R

Effect Obtained in genes resistant in (Amp, Cip, Rf, Cef were converted to sensitive but did not clearly affect on, Ak, Gm Tir, Te, Ax and Nit.

The result of watery extract of *Cinnamon* is shown (T5) the genes effected in S1were Ak, Amp, Cip, Te, Cef and Ax, but the antibiotic converted in S3were observed Amp, Cip, Te. Rf. Ax and Nit.

DISCUSSION

Thirty *Staph aureus* isolated from green house in Sulaymani city. The Isolated bacteria were screened for ten antibiotics and the results papered that most isolates were resistant to most antibiotics (T1) the isolates considered multi resistant bacteria.

High level of microbial contamination Bacterial isolates may include Staphylococcus aureus, fungal isolates consist of Penicillum spp., Aspergillus, Escherichia coli containing dirty soil organic material. The organic material into the tomato fruits, storage is also affected. Focus should be drawn towards ensuring Good Agricultural Practices (GAP) with regards to water quality during the food chain especially in areas where the cold-chain is inefficient or absent Microorganism that are capable of causing human disease may be found on raw produce. These according to Hernandez-Brenes (2002a), may form a part of the fruit or vegetable micro-flora as incidental contaminants from the soil, dust and surroundings; or may be introduced through poor production and Handling practices such as the application of untreated manure, the use of irrigation water practices. These isolates were screened for ten antibiotics and according to the type and number of antimicrobial resistance *S. aureus* isolates were grouped to 6 antibiogram table (T1) The S. *aureus* isolates were 90% resist to Amp, Cef, Rif., 100%, to Te Tri, Ax and. Nit antibiotics, differed in resistance to Ak, Cip. All isolates are multi resistant (resist to more than two antibiotics) that result was cleared in (T1).

The SMIC2500 µg/mL ethanol extracts of *Cinnamon* show in Table 3 effect on resistant genes in (S1) Ak, Amp. Cip, Te, Cef and Ax also the Result is shown in S3 the genes responsible for ethanolic extract on Amp, Cip, Te, Rf, Ax and Nif in Table 4. It is cleared that watery extract affected antibiotic resistance genes the genes effected in S1were Ak Amp, Cip, Te Cef and Ax but the antibiotic converted were in S3) observed, Amp, Cip, Te, Rf, Ax and Nit. And there are no growth observed when SMIC of plant extract is applied to agar plates containing, above antibiotic, while the bacteria were resistant before treating with such extract and the resistance were reduced.

Table 2 showed the SMIC of *E. caryophyllatathe* the SMIC of alcoholic extracts with S1 for *E. caryophyllatathe* 2000 µg/mL Effect on Ak, Gm, Te, Rf and Ax SMIC of alcoholic extracts with S3 for *E. caryophyllatathe* 2000 µg/m the result genes determined also in (S3) converted genes responsable from resistant to sensitive Ak, Gm, Te, Rf and Ax the result.

Table 5 is show the effect of SMIC2000 µg/mL Watary extract of *Eugenia- caryophyllatathe* for ((S1, S3) is cleared the activity on Amp, Cip, Rf and Cef. In this study determined the effect of E. caryophyllatate Alcoholic extract which is the major active (Adel and Sabiha, 2010) on genes resistant in *Staph aureus* (Nascimento *et al*., 2000), mentioned that the extracts of clove inhibited 64.2% of the tested microorganisms, with higher activity against antibiotic resistant bacteria which was about 83% and it is worth mentioning that the results were similar to our results of curing test ore than Ethanol extracts of *Cinnamon* and the difference Between Clove and *Cinnamon* the material compound as curing agent, maybe is due to the absence of phenols.

The anti-microbial effect of clove is attributed to eugenic a phenol compound which is the major active constituent of its essential oil (Ali *et al*., 2005), in this study obtain that the alcoholic extract of clove more effect (Adel and Sabiha, 2010) than *Cinnamon*, the watery extract in cinnamon more effect than watery extract of clove.

From the results obtained in this study, the following can be concluded:

Table 3: T3 of SMIC alcoholic extract of *Cinnamon zeylancum* T

T3 SMIC 2500 µg/mL alcoholic extract of *Cinnamon zeylancum* T	Ak	Amp	Gm	Tri	Cip	Te	Rf	Cef	Ax	Nit
(S1) SMIC 2500 µg/m	S	S	R	R	S	S	R	S	S	R
(S3) SMIC 2500 µg/m	R	S	R	R	S	S	S	R	S	S

Table 4: Effect of SMIC watery extract of *Eugenia caryophyllus* (Clove)

T4 SMIC Watary extract of (Clove)	Ak	Amp	Gm	Tri	Cip	Te	Rf	Cef	Ax	F
(S1) SMIC 2000 µg/mL	R	S	R	R	S	R	S	S	R	R
(S3) SMIC 2000 µg/mL	R	S	R	R	S	R	S	S	R	R

Table 5: Effect of SMIC watary extract of *Cinnamon zeylancum* T

Watery extract of *Cinnamon zeylancum* T T5	Ak	Amp	Gm	Tri	Cip	Te	Rf	Cef	Ax	Nit
(S1) SMIC 2500 µg/mL	S	S	R	R	S	S	R	S	S	R
(S3) SMIC 2500 µg/mL	R	S	R	R	S	S	S	R	S	S

R = Resistant; S = Sensitive

- ***Staphylococcus aureus* causes human infection food poisoning:** It can be isolated from vegetable and contamination food
- *Staphylococcus aureus* appear resistance to most widely used antibiotics and some isolates to all tested antibiotic
- The SMIC of ethanol and watery plant extract of clove and Cinnamon have affected as curing agents on resistant genes and reduced their resistance
- Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases which caused by resistant microbes.

REFERENCES

Adel, K.K. and S.S. Sabiha, 2010. Genetic site determination of antibiotic resistance genes in *Pseudomonas aeruginosa* by genetic transformation. Brit. J. Pharmacol. Toxicol., 1(2): 85-89.

Ali, S.M., A.A. Khan, I. Ahmed, M. Musaddiq, K.S. Ahmed, H. Polasa *et al*., 2005. Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. Ann. Clin. Microbiol. Antimicrob., 4: 20.

Andrews W.H. and T. Hammack, 2000. Bacteriological Analytical Manual. US Food and Drug Administration, FDA.

Atlas, R.M., A.E. Brown and L.C. Parks, 1995. Laboratory Manual Experimental Microbiology. Mosby-year Book, Inc., St. Louis.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Cruickshank, R., J.D. Duguid, B.P. Marmion and R.H.A. Swain, 1975. Medical Microbiology the Practice of Medical Microbiology. 12th Edn., Vol. 1, Churchill Livingstone, London.

Emori, T.G. and R.P. Gaynes, 1993. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin. Microbiol. Rev., 6: 428-442.

Hamilton-Miller, J.M.T., 1995. Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrob. Agents Ch., 39(11): 2375-2377.

Hernandez-Brenes, C., 2002a. The importance of training for improving the safety and quality of fresh fruits and vegetables. In: Improving the safety and quality of fresh fruits and vegetables: A training manual for trainers. University of Maryland.

IIcim, A. and M. Digrak, 1998. The investigation of antimicrobial effect of some plant extract. J. Biol., 22: 1.

Kathe, W., S. Honnef and A. Heym, 2003. Medical and Aromatic Plant in Albania. Bosnia-Herzegovnia, Bulgaria, Croatia and Romania: A study of the collection of and trade in Medicinal and Aromatic Plants (MAPs), relevant legislation and the potential of MAP use for financing nature conservation and protected areas. Federal Agency for Nature Conservation, Bonn. BfN-Skripten no. 91.

Life Science Journal, 2009. 6(3): 80- 82, ISSN: 1097-8135.

McClure, J.W., 1975. Physiology and Function of Flavonoids. In: Harborne, J.B., T.J. Mabry and H. Mabry (Eds.), the Flavonoids. Academic Press, New York, pp: 970-1055.

Nascimento, G.G.F., J. Lacatelli, P.C. Freitas and G.L. Silva, 2000. Antibacteria activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol., 31(4): 886-891.

Ranjan, S., N. Dasgupta, P. Saha, M. Rakshit and C. Ramalingam, 2012. Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish. Adv. Appl. Sci. Res., 3(1): 495-501.

Toda, M., S. Okubo, R. Hiyoshi and T. Shimamura, 1989. The bacterial activity of rea and coffee. Lett. Appl. Microbiol., 8: 123-125.

Vainker, S., 2003. Southeast Asian exports since the 14th century: Cloves, pepper, coffee. J. Early Modern History, 6: 296-307.