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AL– Badri & AL-Janabi

المجلة العراقية لبحوث السوق وحماية المستهلك

TESTING THE INHIBITORY EFFECT OF TRICIN AGAINST SOME FOODBORNE BACTERIA AND ESTIMATE ITS PHENOL COEFFICIENT

Samah R. H. AL – Badri [*1], ***NIDHAL M.S. AL-Janabi***[2]
[1] Ministry of Agriculture, Department of plant Production, Republic Iraq , Baghdad , Iraq. samah_r2016@yahoo.com
[2]Professor, PhD. Department of Food Science, Agricultural Engineering Sciences College, Baghdad University, Baghdad, Iraq. Nidhal.m@coagri.uobaghdad.edu.lq

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ABSTRACT

The inhibitory effect of the hot aqueous extract of commercial jasmine rice bran (HAE) and the purified tricin compound on the growth of some food-borne pathogenic bacteria (*Escherichia coli, E. coli* O157:H7, *Salmonella typhimurium, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella* spp, *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis*) and compared with phenol coefficient using several dilutions of water: purified tricin (70:1, 90:1, 1:100, 1:120 and 1:150). and water: purified tricin (70:1, 90:1 and 1:100),at the same concentration, sterilized with microbial filters .Tricin purified showed higher efficiency then the aqueous extract HAE against growth of gram-positive and negative bacteria. The highest significant inhibition activity of purified Tricin compound was at P≤0.05 effect against *B. subtilis* , *B. cereus* and *S. aureus* with inhibitory diameter of 29, 27.5 and 27.5 mm respectively. White the aqueous extract HAE had loupe effectiveness than it against the same bacteria with a diameter of inhibition7,8.5 and 10.5 mm respectively After incubation at 37°C for 24 h, the last reading was the highest inhibition activity of the extract HAE. As for the results of the susceptibility of tricin as an antiseptic towards Gram-negative bacteria *S. typhi* and Gram-positive *S. aureus* compared to phenol using several dilutions of each, high dilutions had a clear inhibition in the growth of *S. aureus* and *S. typhi* bacterial isolates, especially when compared with phenol when The higher dilution (1:100) showed bacterial growth, while we did not find growth for these isolates at the same dilution coefficient of tricin for periods (5, 10, and 15) min After incubation at 37°C for 48 h. *S. typhi* was less sensitive than *S. aureus* towards phenol and tricin.

Key words: Antimicrobial activity, Phenol, Rice bran, Tricin.

اختبار التأثير التثبيطي لمركب Tricin ضد بعض البكتريا التي تنتقل عن طريق الاغذية وتقدير المعامل الفينولي له

سماح راشد حمادي البدري [1]، ***نضال محمد صالح الجنابي*** [2]
[1] قسم الانتاج النباتي، وزارة الزراعة ، بغداد، العراق samah_r2016@yahoo.com
[2] الاستاذ الدكتور، قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق nidhal.m@coagri.uobaghdad.edu.iq

الخلاصة

تم مقارنة التأثير التثبيطي للمستخلص المائي لنخالة الرز الياسمين التجاري (HAE) و مركب Tricin المنقى منها تجاه نمو بعض البكتريا الناقلة للأمراض عن طريق الاغذية (*Escherichia coli* و *Escherichia coli* O157:H7 و *Salmonella typhimurium* و *Salmonella typhi* و *Psedomonas aeruginosa* و *Klebsiella pneumonia* و *Shigella spp* و *Staphylococcus aureus* و *Bacillus cereus* و *Bacillus subtilis*) ومقارنة بالفينول باستخدام عدة تخافيف لكل من الماء : مركب Tricin المنقى (70: 1، 90: 1، 1:100،

[*] **The research is extracted from the doctoral thesis of the first researcher.**

1:120 و 1:150)، والماء: مركب الفينول (70: 1، 90: 1 و 1:100) وبنفس التركيز، معقمة بالمرشحات الميكروبية، اظهر Tricin المنقى كفاءة اعلى من المستخلص المائي HAE تجاه نمو البكتريا السالبة والموجبة لصبغة غرام، اذ كانت اعلى فعالية تثبيط معنوية لمركب Tricin المنقى عند مستوى$P\leq0.05$ تجاه بكتريا *B.subtilis* و *B. cereus* و *S. aureus* بمعدل قطر تثبيط 29 و 27.5 و27.5 ملم على التوالي، في حين بلغت فعالية للمستخلص المائي HAE ضد هذه العزلات بمعدل قطر التثبيط 7،8.5 و 10.5 ملم على التوالي بعد الحضن على درجة حرارة 37 م^0 لمدة 24 ساعة، وكانت القراءة الأخيرة هي أعلى نشاط تثبيط للمستخلص HAE .اما بالنسبة لنتائج قابلية المركب كمادة مطهرة تجاه بكتريا السالبة لصبغة غرام *Salmonella typhi* والبكتريا الموجبة لصبغة غرام *Staphylococcus aureus* مقارنةً بالفينول باستخدام عدة تخافيف لكل منهما، التخافيف العالية (الاقل تركيزاً بالمركب تريسين) كان لها تثبيطاً واضحاً في نمو العزلات البكتيرية *S. aureus* و *S. typhi* اي لم يشاهد حدوث اي نمو لهذه العزلات، خاصة لو قورنت مع الفينول عند التخفيف الاعلى (100:1) الذي ظهر نمو بكتيري في حين لم نجد نمو لهذه العزلات عند نفس معامل التخفيف لمركب Tricin المنقى للفترات الزمنية الثلاث المدروسة (5 و 10 و15) دقيقة التوالي بعد الحضن على درجة حرارة 37 م^0 لمدة 48 ساعة. كما لوحظ بان بكتريا *S. typhi* اظهرت تحسساً اقل من بكتريا *S. aureus* تجاه الفينول ومركب Tricin المنقى.

الكلمات المفتاحية : فعالية المضاد للأحياء المجهرية، فينول، نخالة الرز، تريسين.

INTRODUCTION

Rice bran is a by-product of the rice manufacturing process (the process of whitening and removing the husk). It constitutes (10-12)% of the weight of the grain and includes the casings of the grain, the cap, the aleurone layer, as well as the embryo **(Musa & Farouk, 2012).** Several studies indicated the importance of the nutritional value of rice bran, as it contains It contains protein, ash, vitamins, minerals and biologically active substances. It is a good source of dietary fiber, as it contains approximately 21-27% and 1.9% soluble dietary fiber **(Lilitchan *et al*., 2008)** Plants globally can be good sources of antimicrobial compounds because they contain a group a wide range of structurally complex compounds **(Salih *et al*.,2015 ; Al- wendawi *et al*., 2012).** Research indicated that rice bran extracts have a role in the treatment of diarrhea caused by microorganisms, including *Vibrio cholera, Salmonella spp, Shigella spp, E. coli*, and *S. aureus*, by preventing the growth of these organisms, which cause abnormal symptoms **(Kondo *et al*., 2011). Ghoneum & Agrawal (2011)** found that the lowest inhibitory concentration of rice bran extract against *V. cholera* was 0.976 mg/ml.

Tricin a flavone 4, 5,7-trihydroxy-3, 5′-dimethoxyflavone (is a flavone, found in edible plants such as rice, oats, barley, and wheat **(Wang *et al*., 1998)**. It has many biological activities including antioxidant **(Renuka & Arumughan, 2007)**, and anti-inflammatory, antiviral **(Lazeeza, 2021; Sakai *et al*.,2008)**, and antihistamic **(Kuwabara *et al*., 2003)**. **Mi *et al*.,(2016)** proved that tricin has it should mentioned the kinds bacteria, fungicidal, and insecticidal activity. In addition to other functions such as its effectiveness as an anti-influenza in vivo **(Mi,*et al*., 2016)**, and the virus is a cytomegalic anti- human cytomegalovirus (HCMV) **(Yazawa *et al*., 2018)**, reduces intestinal adenocarcinomas **(Murayama *et al*., 2012)**, and is a tyrosinase inhibitor so its potential pharmaceutical applications can be expanded **(Mu *et al*., 2013).** The study aimed to measure the inhibitory effect of the aqueous extract of commercial jasmine rice bran HAE and the purified tricin compound by estimating the phenolic parameter of tricin.

MATERIALS AND METHODS

Preparation of rice bran samples

Rice bran samples of the commercial jasmine variety were collected from Husking sites in Al-Najaf Governorate for the year (2021).

Extraction

Maceration Method Aqueous Extract (AE)

The water extraction of rice bran of the commercial jasmine variety was carried out according to the method described by **Al-Alani *et al*., (2007)**, as 2 g of rice bran was extracted with 100 mL of distilled water at a boiling point at 70 ^{o}C, and left for 3 h on a magnetic stirrer, then it was filtered through filter paper (Whatman No.1) and concentrated by using rotary evaporator at 60°C. The concentrated extract was Poured into a Petri dish and place in an electric oven at a temperature of 40°C for 24 h to dry. The dried powder was scraped off and collected in dry bottles and kept in the refrigerator until use.

Isolation of tricin crystals

It was isolated according to the method described by **Takeru *et al*., (2009)**, 100 mL of distilled water was added to the rice bran of the commercial jasmine variety (2 g) at boiling point at 70 ^{o}C and left for 3 h on a magnetic stirrer, then filtered through filter paper (Whatman No.1). An extract was fractionated using a separating funnel by taking 35 mL of the previously prepared aqueous extract, with 35 mL of ethyl acetate solvent, then 2 ml of the ethyl acetate fraction was

Passed through silica gel column 60 with dimensions (inner diameter 3 X 56 cm, 400 g), with a degassing process, and washing the column with solvents n-hexane, ethyl acetate and methanol, respectively, at a flow rate of (2 mL/ min). This process yielded nine fractions (A-I) of ethyl acetate solvent. The nine parts were collected and concentrated by rotary evaporator to a quarter of their original size at a temperature of 40 °C and left in the refrigerator for 48 h for the purpose of obtaining crystals of tricin compound that precipitated in a Petri dish after adding chloroform to it.

Antimicrobial efficacy

Study of the inhibitory activity of commercial jasmine rice bran extract HAE and Tricin purified from it against some pathogenic bacteria: The study of inhibitory effectiveness included two phases:

The first stage: the test isolates were activated in Nutrient Broth (NB) medium at 37 °C for 18 h by using ten types of bacteria, including seven Gram-negative bacteria (*E. coli, E. coli* O157:H7, *S. typhimurium, S. typhi, P. aeruginosa, and K. pneumoniae* and *S. spp*) and three Gram-positive bacteria (*S. aureus, B. cereus and B. subtilis*) Obtained from the Department of Microbiology / College of Science - University of Baghdad **(Al-Jumaily, 2022; Al-Hamdani, 2022).**

The second stage: the method of filter paper discs diffusion mentioned by **Saleh *et al*.,(2023)** was adopted, by spreading 0.1 mL of activated bacteria on medium Nutrient Agar (NA) in a sterile glass diffuser, each disc containing 10 µL of aqueous extract of rice bran HAE and tricin purifier sterilized with (0.45µm) Millipore filters. The dishes were incubated at delete 37 °C for 24 h, after which the diameter of the clear zone was measured.

Testing the efficacy of purified Tricin against *S. aureus* and *S. typhi* in comparison with phenol

The method described by **Shital & Sneha, (2020)** delete the full stop was used to determine the phenol coefficient through:

A series of dilutions were prepared of water: purified tricin (70:1, 90:1, 1:100, 1:120 and 1:150), and water: phenolic compound (70:1, 90:1 and 1:100), and sterilized by Millipore (0.45µm).From each dilution 4.5 mL was transferred in to a tube and 0.2 mL of test bacteria was inoculated separately, the tubes were inoculated at 37 °C for periods of (5, 10 and 15) min. About (0.1) mL of test tubes incubated at different times are transferred to glass dishes and sterile NA medium cooled to 45 °C is poured onto them, and incubated at 37 °C for 48 h. The phenol coefficient is calculated using the following equation:

$$\text{phenol coefficient} = \frac{\text{The dilution factor of the compound that shows inhibition}}{\text{Dilution coefficient of phenol under the same conditions}}$$

The inhibitory activity against bacteria is calculated according to the following equation:

$$\text{inhibitory efficacy} = \frac{\text{number of live cells}}{\text{Compound concentration x exposure time}}$$

Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

RESULTS AND DISCUSSION

Effect of hot aqueous extract of commercial jasmine bran and tricin purified from it on the growth of bacteria:

The results showed (Table 1) that the inhibitory effect of the aqueous extract of commercial jasmine rice bran HAE and tricin against some food spoilage microorganisms and foodborne pathogens.

It is clear that the purified tricin was superior significant to the aqueous extract HAE at the level of $P \leq 0.05$ in its effect against all bacterial isolates and the two yeasts tested with growth inhibition diameters, with varying effect, and it is inferred from the results that tricin showed a higher significant at the level of $P \leq 0.05$ effect against Gram-positive bacteria *B. subtilis* with growth inhibition diameters 29 mm, and the highest effect was also against Gram-negative bacteria, *K. pneumoniae*, with an inhibition diameter 28 mm, while the compound showed an inhibitory effect with equal inhibition diameters of 27.5 mm towards the growth of both *S. aureus* and *Bacillus cereus*, Gram-negative bacteria. *S. typhimurium* and *S. typhi* with an average diameter of inhibition of 25 mm, and tricin showed the lowest inhibitory effect on the growth of deleted *E. coli*, which was characterized by its least sensitivity towards the compound among other tested isolates with an average diameter of inhibition of 22 mm. Tricin being a poly phenolic flavonoid compound **(Saleh & Hammadi, 2017).**

While the aqueous extract had the highest effect HAE against *S. aureus* with an inhibition diameter of 10.5 mm, which is less than that achieved by purified tricin in inhibiting its growth, also the highest effect was deleted against Gram-negative bacteria *S.typhimurium* with an inhibition diameter 9 mm, the extract showed the lowest inhibitory effect In the growth

of *S. typhi, K. pneumoniae, S. spp*, and Gram-positive bacteria *B. subtilis*, which were distinguished by their least sensitivity towards the extract among the other tested isolates with an inhibition diameter 7 mm each, the extract had an inhibitory effect with an equal inhibition diameter of 8.5 mm against the growth of both *B. cereus, E. coli* O157:H7, and *P. aeruginosa*. The reason for the decrease in the inhibitory effectiveness of the aqueous extract may be due to its content of components that represent a nitrogen source that helps bacteria in their growth, and this has been proven by **Farhan *et al*., (2020) ; Mahamed ,(2019)** that rice bran is a good source of proteins, minerals, fatty acids, fiber, deleted, and essential amino acids (tryptophan and histidine). Methionine, cysteine, and arginine and micronutrients magnesium, calcium, phosphorus, manganese, B-9 vitamins, folic acid, and vitamin E.

Table (1): Inhibitory effectiveness of extract HAE of rice bran commercial jasmine variety and tricin purified against some bacteria

	Microscopic organism	Average diameter of inhibition zones (mm)	
		Commercial Jasmine Rice Bran Extract HAE	Purified Tricin
1	*E. coli*	8	22
2	*E. coli O157:H7*	8.5	24
3	*S. typhimurium*	9	25
4	*S. typhi*	7	25
5	*P. aeruginosa*	8.5	23
6	*K. pneumoniae*	7	28
7	*S. spp*	7	27
8	*S. aureus*	10.5	27.5
9	*B. cereus*	8.5	27.5
10	*B. subtilis*	7	29
LCD value		3.29*	5.076*
* ($P \leq 0.05$)			

The difference in the susceptibility of bacteria to plant extracts between negative and positive is due to the composition of the cell wall and the organization of the outer membrane of Gram-negative and Gram-positive bacteria, due to the differences in the outer layers of the wall of Gram-positive and Gram-negative bacteria, negative bacteria contain outer membranes, exceptionally there are no In positive bacteria **(Verma *et al*., 2013)**, however, and antibacterial substances easily damage the cell wall and the cytoplasmic membrane of the cells, which leads to the exit of the cytoplasm to the outside of the cells and its coagulation, and as a result cell death **(Nazzaro *et al*., 2013).**

Studying the susceptibility of the tricin purified as antibacterial:

The susceptibility of tricin purified as antibacterial against gram-negative bacteria *S. typhi* and gram-positive bacteria *S. aureus* was studied because they are known pathogens and cause infections in hospitals **(Chukwuebuka *et al*., 2018)**, compared to˝ phenol˝, it was found that the phenolic coefficient of the purified tricin is (1), meaning that the effect of the tricin is similar to the effect of phenol. the value of the phenol coefficient is a means to determine the effectiveness of the disinfectant. Disinfectants that have one or more phenol coefficients have more effectiveness than phenol and vice versa **(Mbajuika *et al*., 2014)**, so purified tricin can be used as a disinfectant, it was effective in killing the pathogenic microorganisms used this study, in addition because it is a non-toxic compound.

(Table, 2) shows the results of the effect of phenol on *S. aureus* and *S. typhi* deleted, where no growth was observed for both deleted within the dilutions (1:70, 1:90, and 100:1) at

deleted periods (10 and 15) min, wile the growth significant at the level of P≤0.05 of *Staphylococcus aureus* and *Salmonella typhi* was observed in Petri dishes at dilutions (1:100), (1:90, and 100:1), respectively, at the period of time (5) min, as show in Figure(1 A) and Figure(3 A).

Table (2): Comparison of tricin with phenol against *S. aureus* and *S. typhi*.

	Dilutions	*Staphylococcus aureus*			*Salmonella typhi*		
		Time / min			Time / min		
		5	10	15	5	10	15
1	70:1	-	-	-		-	-
2	90:1	-	-	-	+	-	-
3	100:1	+	-	-	+	-	-
P- value		NS	NS	NS	0.047*	NS	NS
* (P≤0.05)							

(-) no growth of bacteria, (+) growth of bacteria.

(Table, 3) shows the results of the effect of the purified tricin on the tested bacteria, where high dilutions (the lowest concentration of the compound) showed a clear inhibition of the growth at the level of P≤0.05 of *S. aureus* and *S. typhi*, i.e. no growth was seen for the tested isolates, especially when compared with phenol at the higher dilution (1:100) showed bacterial growth, while there were no growth for these isolates at the same dilution factor of the purified tricin for the three studied periods (5, 10, and 15) min ,as in Figure(1 B,2 B,3 and 4). It was also observed through this test that *S. typhi* is less sensitive than *S. aureus* towards phenol and tricin, due to the high degree of complexity in the structure of the cell wall, so Gram-negative bacteria are more resistant to the effects of the disinfectant compared with Gram-positive bacteria **(Mamman *et al*, 2005)**.

Table (3): Effect of tricin against *S. aureus* and *S. typhi* during different time periods.

	Dilutions	*Staphylococcus aureus*			*Salmonella typhi*		
		Time / min			Time / min		
		5	10	15	5	10	15
1	70:1	-	-	-	-	-	-
2	90:1	-	-	-	-	-	-
3	100:1	-	-	-	-	-	-
4	120:1	-	-	-	-	-	-
5	150:1	+	-	-	+	+	-
P- value		NS	NS	NS	NS	NS	NS
NS: Non -Significant							

(-) no growth of bacteria, (+) growth of bacteria.

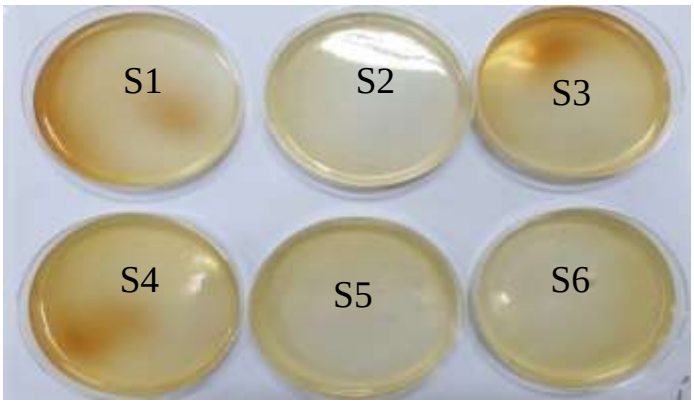
Disinfection is a selective process for eliminating some unwanted organisms and preventing their transmission, reducing bacterial contamination in a contaminated environment and disinfecting the skin or hands **(Mbajuika *et al*., 2014).**Antibacterial are of high quality, low cost, less toxic and have a broad spectrum of antimicrobial activity **(Santajit & Indrawattana, 2016).**

A **B**

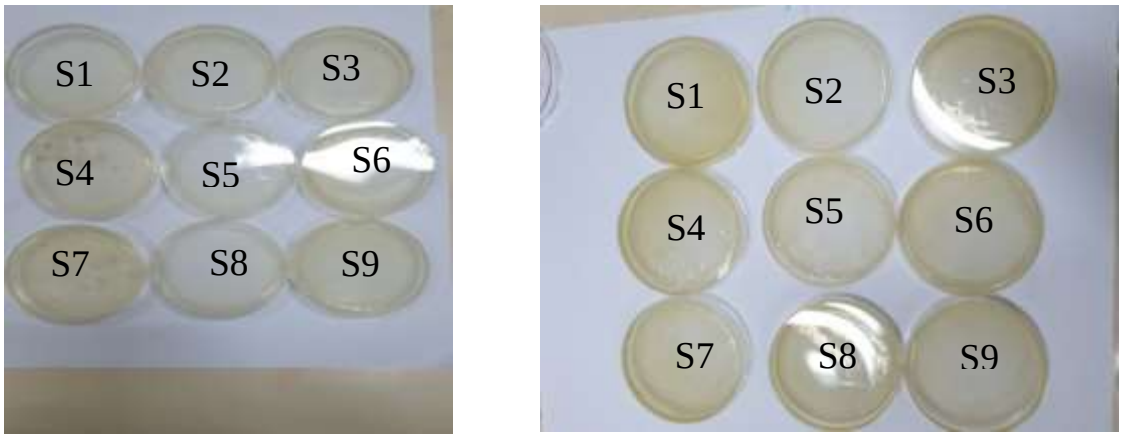
Figure(1): Effect of phenol and purified tricin as antibacterial against *S. aureus*

A - Dilutions of phenol coefficient
S1: 70:1 dilution / 5 min
S2: 90:1 dilution / 5 min
S3: 100:1 dilution / 5 min
S4: 70:1 dilution / 10 min
S5: 90:1 dilution / 10 min
S6: 100:1 dilution / 10 min
S7: 70:1 dilution / 15 min
S8: 90:1 dilution / 15 min
S9: 100:1 dilution / 15 min

B - Tricin dilutions
S1: 70:1 dilution / 5 min
S2: 90:1 dilution / 5 min
S3: 100:1 dilution / 5 min
S4: 70:1 dilution / 10 min
S5: 90:1 dilution / 10 min
S6: 100:1 dilution / 10 min
S7: 70:1 dilution / 15 min
S8: 90:1 dilution / 15 min
S9: 100:1 dilution / 15 min



Figure(2): Effect of purified tricin as an antibacterial against *S.aureus*
S1: 120:1 dilution/ 5 min, S2: 1:120 dilution/ 10 min, S3: 1:120 dilution/ 15 min
S4: 1:150 dilution/ 5 min, S5: 150:1 dilution/ 10 min, S6: 150:1 dilution/ 15 min

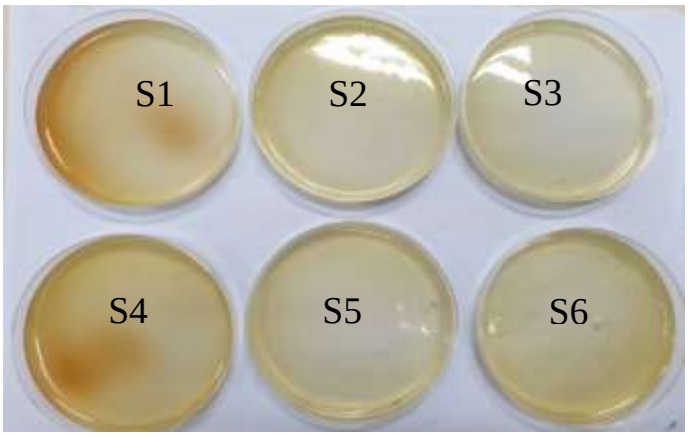


A **B**

Figure (3): Effect of purified tricin as an antibacterial against *S. typhi*

A - Dilutions of phenol coefficient
S1: 70:1 dilution / 5 min
S2: 90:1 dilution / 5 min
S3: 100:1 dilution / 5 min
S4: 70:1 dilution / 10 min
S5: 90:1 dilution / 10 min
S6: 100:1 dilution / 10 min
S7: 70:1 dilution / 15 min
S8: 90:1 dilution / 15 min
S9: 100:1 dilution / 15 min

B - Tricin dilutions
S1: 70:1 dilution / 5 min
S2: 90:1 dilution / 5 min
S3: 100:1 dilution / 5 min
S4: 70:1 dilution / 10 min
S5: 90:1 dilution / 10 min
S6: 100:1 dilution / 10 min
S7: 70:1 dilution / 15 min
S8: 90:1 dilution / 15 min
S9: 100:1 dilution / 15 min



Figure(4): Effect of purified tricin as an antibacterial against . *typhi*
S1: 120:1 dilution/ 5 min, S2: 1:120 dilution/ 10 min, S3: 1:120 dilution/ 15 min
S4: 1:150 dilution/ 5 min, S5: 150:1 dilution/ 10 min, S6: 150:1 dilution/ 15 min

CONCLUSION

1. The filter paper discs diffusion experiment showed that the tricin was distinguished with a higher inhibitory effectiveness than the aqueous extract against gram-positive and negative deleted bacteria.
2. From the results obtained in this study it is inferred that the purified tricin can be used as an alternative to antibiotics in the treatment of pathological conditions caused by some bacteria due to the fact that it has a higher inhibitory effectiveness .
3. It can also be used as a disinfectant, as it was effective in killing microorganisms that cause diseases deleted, because it is a non-toxic compound that can be applied as a preservative in food because it is safe to use.

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