THE ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF LOCAL WHEAT BRAN EXTRACT AGAINST PATHOGENIC BACTERIA AND YEAST

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ABSTRACT

The current study was aimed to investigate the antimicrobial and antibiofilm efficiency of local wheat bran active metabolites over locally isolated pathogens including *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. Local wheat Ibaa99 bran extract consists of phenolic compounds, inulin and phytic acid. The antibacterial activity was examined by well diffusion assay and the minimum inhibitory concentration method by the concentration 1mg/ml for Wheat Bran Extract. On the other hand, antibiofilm dispersal consideration have been completed by Microtiter plate method. The results revealed that Ibaa99 wheat bran extract has antimicrobial activity in average diameter zone, highest inhibition observed over *C. albicans* isolate with 60 ±0.1 mm. Moreover, the minimum inhibitory concentrations of wheat bran extract were 1 mg/ml against both *E. coli* and *S. aureus* isolates. The outcomes of biofilms suppression show high reduction in biofilms, the highest dispersal recorded for *Candida albicans* with OD (0.07). In conclusion, the extract of local wheat bran is a has antimicrobial effect and biofilm suppressor and represent a promising cheap by- product as alternative medication that can be used to treat many gastrointestinal pathogens.

Keywords: Ibaa99, WBE, antibacterial, antifungal, biofilm.

فعالية مستخلص نخالة الحنطة المضادة للمايكروبات والخمائروتشكيل غشائها الحيوي

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المستخلص

هدفت الدراسة الحالية الى التحقق من فعالية مستخلص نخالة حنطة محلية المضادة للمايكروبات و المضادة لتشكيل الغشاء الحيوي لعدد من مايكروبات الجهاز الهضمي المعزولة محليا والتي تتضمن بكتريا الايشيريشيا القولونية السالبة لصبغة غرام و المكورات العنقودية الذهبية الموجبة لصبغة غرام بالاضافة الى خمائر المبيضة البيضاء. درس المضاد البكتيري للمستخلص النباتي بطريقتي اِخْتِبار اِنتِشار الحفر وطريقة التركيز المثبط الأدنى و كمضاد للغشاء الحيوي بطريقة صفيحة المعايرة الدقيقة, و أظهرت النتائج ان مستخلص نخالة الحنطة اباء 99 مكون من المركبات الفينولية, الانيولين وحامض الفايتك و له فعالية مضادة للبكتريا والفطريات بالطريقتين باستخدام تركيز 1 ملغم /مل للمستخلص النباتي ، وكان اعلى معدل قطر تثبيط ضد عزلة خميرة المُبيَضّة البيضاء مع قطر تثبيط يبلغ 60 ملم. كما بينت نتائج فعالية المستخلص النباتي فعالية ضد ذات المايكروبات بطريقة التركيز المثبط الادنى , حيث بلغ التيركيز المثبط للمستخلص النباتي ضد عزلات بكتريا الايشيريشيا القولونية وعزلات المكورات العنقودية الذهبية ايضا 1 ملغم/مل. وسجلت فعالية تشتيت عالية المركبات الفعالة لنخالة الحنطة ضد لغشاء الحيوي حيث دون اعلى تشتيت بنسبة امتصاصية بلغت (0.07) لخمائرالمبيضة البيضاء. بالامكان الاستنتاج اهمية للمستخلص النباتي كمصدر من منتج ثانوي متوفر ورخيص كمضاد مايكروبي ومضاد للاغشية الحيوية.

الكلمات المفتاحية :اباء99 ، مستخلص نخالة الحنطة، المضاد البكتيري، المضاد الفطري، الغشاء الحيوي.

Received:13 /8/2023, Accepted:2/12/2023, Published:December 2025

INTRODUCTION

Wheat (*Triticum aestivum* L.) is part of the most substantial cereal crops and covers vast area of crops on Earth. wheat culture is essential and represent the first strategic crop for the cultivated fields and agricultural production in Iraq (7). The bran, as well recognized at the same time as grain coat, representing the outer layers of the kernel, the seed coat is composed of many layers that together provides the protection to the central fragment of the grain, the main bioactive compounds of wheat bran (WB) are the phenolic acids and flavonoids groups (5). According to Alara *et al.* (2), phenolic compounds which exist in WB are classified into several classes are toxic to microorganisms due to the attendance of the hydroxyl groups on the phenol components. Furthermore, a promising polyphenols compounds among Ferulic acid, Gallic acid, *p*-coumaric all together with Rutin has antibiofilm activity as well as antimicrobial properties (10). Gastrointestinal infections (GIs), mostly obvious as clinical syndromes, involving enteric fever, acute vomiting, and diarrhea, induced by the digestion of pathogens as well as the deactivation of normal microbiota (4), Diarrhea caused by enteropathogenic microbes recorded an essential global health burden especially in developing nations and a major cause for children mortality and morbidity (21). Microbial biofilms are considerably convoluted microbial ecosystems depend on microorganisms boned to a surface and established in an organic polymer matrix of microbial source (23). The aim of the current work is to extract bioactive compounds from the bran as a by- product of the milling process of local wheat varieties and investigate the antibacterial, antifungal and antibiofilm effects as well.

MATERIALS AND METHODS

Preparation of wheat bran extract : Samples of local cultivars wheat grains were collected from AL Dourah Silo, Grain Board of Iraq (GBI) belong to the harvest season 2021/2022, The samples were identified for specific variants type as subjected to the scientific laboratory identification according to the variant's characteristics through the physical properties analysis including kernel dimensions (grain width and length), shape, color, sphericity, thousand kernel weight (TKW) and bulk density (17). The sample of wheat grains was subjected for milling process according to Al-Kharkhi and Mousa (3). Afterward, Wheat Bran active compounds were Extracted with aqueous ethanol solvent ratio (ethanol: water, 80:20, v: v) with percentage (wheat bran: solvent, 1:10) then shacked for 6hrs by a shaker under room temperature, then it purified by Buchner filtered through a No. 2 Whatman filter paper on a and evaporated under rotary vacuum. Furthermore, the extract was collected and subjected for second shake process for only one hour under 70ºC, the resulted extraction gathered in steel tray and kept under laboratory temperature and environment for one week until it completely dried following Tian *et al.* (22). Finally, wheat bran extract was Identified by High Performance Liquid Chromatography (HPLC) depending on the standards available for wheat bran bioactive compounds following Radovanović *et al*. (21).

Collection and isolation of pathogenic samples: Exactly 203 clinical specimens, 75 sample for *Escherichia coli,* 71 samples for *Staphylococcus aureus* in addition to 58 samples for *Candida albicans* were gathered from children's patients of both sex (males and females) aged between 2 to 10 years old suffering from gastrointestinal infections and diarrhea who settled in Al-Yarmouk Teaching Hospital, Baghdad, Iraq. Moreover, each pathogenic microbe was grown on its selective medium and then incubated under each required condition, *E. coli* specimen was incubated aerobically at 37°C for 24hours on MacConkey agar, *S. aureus* on Mannitol salt agar at 37°C for 24hours and *C. albicans* was grown on Sabouraud dextrose agar (SDA) at 37°C for 24hours. Furthermore, after incubation all isolates were submitted for primary identification. Pathogenic isolates were subjected for microscopic, biochemical (oxidase and catalase) tests, also the isolates were subjected for VITEK 2 compact system for confirmation the diagnosis (21).

Antibacterial assay of wheat bran extract: Well diffusion method: The agar well diffusion method was applied to screen the

antimicrobial activity of the crude wheat bran extract, the plant material was dissolved in 2% of Dimethyl sulfoxide (DMSO), then 1mg/ml of the plant extract was applied against three pathogenic microbes (*E. coli, S. aureus* and *C. albicans*). Only 100µL of the newly activated microbial isolates were prepared at turbidity equivalent to 0.5 McFarland and spread with a sterile cotton swab over petri dishes containing sterile Muller Hinton agar. Moreover, 5.0 mm wells were made using a sterile cork borer into the agar plates followed by filling the wells with wheat bran extract. The plates were placed in a cold area for 15-30 min to allow extract distribution throughout the agar medium, followed by incubation at 37°C for 24hours. Later, the plates were examined and measured for zones of inhibition appearance (15). Measurements were calculated in millimeter (mm) as follow: (Inhibition zone (mm)= Diameter of growth inhibited zone-Diameter of the well) according to Pan *et al.* (19).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is the lowest concentration of wheat bran extract (WBE) expressed in mg/L as resazurin solution was prepared by dissolving 337.5 mg of resazurin powder in 50 ml sterile distilled water then the inoculum of pathogenic organisms (*E. coli, S. aureus* and *C. albicans*) suspensions were prepared and adjusted to the McFarland standard required by the test (0.5McF) by using DensiCHEK Plus. Afterward, the minimum inhibitory concentration has been quantified by using microtiter plate method and the twofold dilution method as well, 100ul of Muller Hinton broth dispensed into the wells of a microtiter plate, then 100ul of appropriate 1g/ml of wheat bran extracts, Using the micropipette set at 100ul, mix the product into the wells in first column 1 by sucking up and down for 6 times. 100ul removed from column 1 then added to column number 2. The procedure has been repeated down to column 7. The same set of tips used for the entire dilution series Furthermore, the plates incubated at 37^{o}C, when satisfactory growth is obtained (24 hours), 10 µl resazurin added to each well and incubated for an additional 2 hours at 37^{o}C. Change in color was noticed and inhibitory concentration. have been recorded. MIC has been completed.

Detection of pathogenic microorganisms' biofilm: The Congo red agar assay (CRA) is a qualitative method for detection of biofilm producing microorganisms which depends on colonies color change grown on Congo red agar medium (14). Additionally, the tube method is a qualitative method for additional detection of biofilm producing by yeast has been applied for *C. albicans* biofilm formation. In other hand, Microtiter plate (MtP) method is a quantitative assay used to determine biofilm through the microplate reader and data were calculated according to Kırmusaoglu (14).

Antibiofilm assay : The microtiter plate assay was used to determine the antibiofilm efficiency of wheat bran extract according to (14). After incubation, part of a grown colony of each isolate was suspended in normal saline, then the concentration of all isolate's suspensions was equilibrated with 0.5 McFarland standards. Furthermore, 180 µl of Mueller-Hinton broth enriched with 1% glucose was put in each of the 96 wells, and then, 20 µl of wheat bran extract was added to it. Notably, each well was washed twice with 200 µl of PBS and dried for 30 min at 60°C prior to staining with 150 µl of crystal violet for 15 min. After staining, the plates were again washed twice with PBS. After that, the microplate was kept at room temperature for drying; the dye bound to the cells was re-solubilized by eluting from attached cells with 150 µl of 96% ethanol per well. Thereafter, the microtiter plate was covered with the lid and left still for at least 10 min at room temperature. Stained biofilms were measured at 560 nm using microtiter reader. Each strain was examined for triplicate and mean was taken; data were calculated as ODc= Average OD of negative control + (3 × standard deviation of negative control). OD isolate= Average OD of isolate – ODc. Additionally, for investigation the capability of antibiofilm activity when data resulted if OD ≤ ODc means no biofilm production, ODc< OD ≤ 2 × ODc= Weak biofilm, 2 × ODc< OD ≤ 4 × ODc= Moderate biofilm and if 4 × ODc< OD leads to strong biofilm formation.

RESULTS AND DISCUSSION

Identification of wheat bran active compounds: Two and halve kilograms of grains sample was collected and identified as Ibaa 99 variety type, 500 gm of wheat bran (20%) was obtained after milling process. Exactly 30 grams (6% of wheat bran amount) of bioactive compounds were obtained after the extraction of active compounds from ibaa99 bran. In advance, the identification of the bioactive metabolites of this variety concluded within (Table.1). Al-Kharkhi and Mousa (3) in other study were separated wheat bran from other parts of wheat kernels by same techniques for their own experiments without mentioning an exact amount.

Table 1. The total content of bioactive metabolites for Ibaa99 Wheat bran

Phenolic Compounds/ ppm (mg/L)		Inulin/ppm (mg/L)	Phytic Acid/ppm (mg/L)
Vanillic Acid	2162		
Ferulic Acid	973		
Rutin	919	598	3.12
P – Coumaric Acid	560		
Apigenin	445		
Total	5059		

Identification of pathogenic microbes

Colonies of *E. coli* isolates appeared as small round donut-shaped and semi-mucoid, with pink color and found to be positive for catalase test and negative for oxidase test, three of *E. coli* isolates were chosen and confirmed by VITIK 2 compact system. Moreover *S. aureus* isolates were appeared to be large, round and yellow colonies. Microscopic observation confirmed that they were Gram- negative, the isolates were found to be positive for catalase test and negative for oxidase test examination of the suspected *S. aureus* revealed that they were Gram- positive cocci, 3 isolates were confirmed by VITIK 2 compact system. *C. albicans* appeared to be large creamy, smooth, and convex colonies. Microscopic examination of the suspected *C. albicans* revealed that they were Gram- positive and confirmed by VITIK 2 compact system. Haji *et al.* (8) used exact method for further diagnosis of pathogenic isolates gathered from Iraqi patients.

Antimicrobial effect of wheat bran extract: The inhibitory effect of wheat bran extract (WBE) against three *E. coli* isolates depending on the well diffusion method illustrated that all isolates were affected by WBE based on (Fig. 1), the highest inhibition zone recorded was (38 mm) in dimeter and much close diameter was (32.5 mm), while the lowest inhibition zone diameter was (29.5 mm). Moreover, the inhibitory effect of WBE towards the three *S. aureus* isolates explicated that that all isolates were affected by WBE as shown there in (Fig. 2), the highest inhibition area was recorded (60 mm) in diameter average, while the lowest inhibition zone diameter was (34.5 mm). The inhibitory impact of WBE over *C. albicans* isolate depending on the well diffusion method leaded to inhibition circle measured exactly (60 mm) diameter as Showed by (Fig. 3). May be no previous experiments handled any extraction of raw wheat bran active compounds mainly phenolic compounds and its effect towards conclusively *E. coli* like the current study did, the closet study has been done by Mohammed *et al.* (15), when used phenolic compounds from worm wood against *Escherichia coli* bacteria.

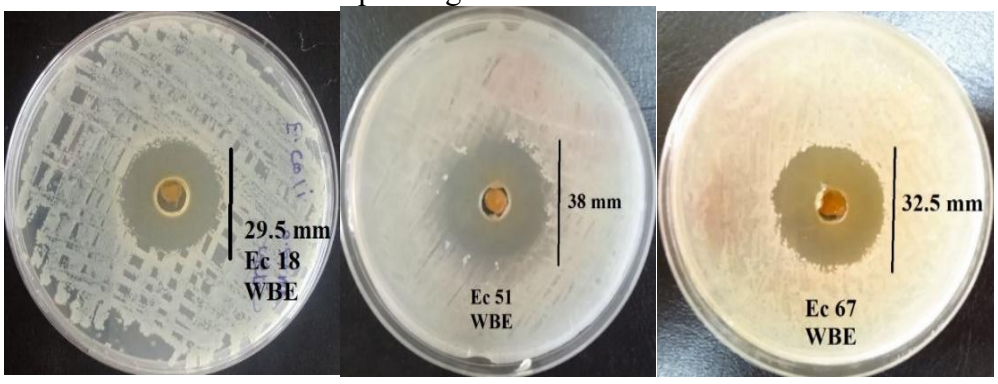

Figure 1. The antibacterial activity of Ibaa99 wheat bran extract (WBE) by well diffusion technique against *E. coli* isolates symbolled (Ec 28, Ec 51 and Ec 67 at 37ºC for 24hrs

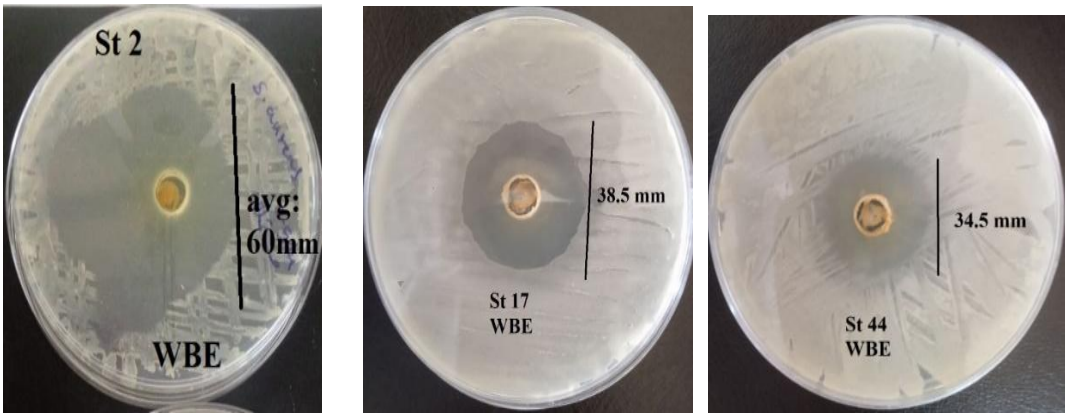

Figure 2. The antibacterial activity of Ibaa99 wheat bran extract (WBE) by well diffusion assay against *S. aureus* isolates (symbolled St 2, St 17 and St 44) at 37ºC for 24hrs

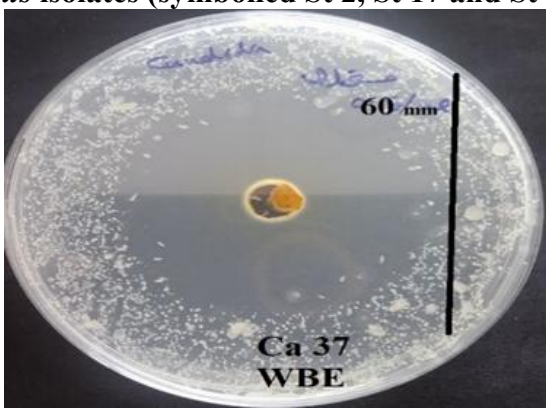

Figure 3. The antifungal activity of Ibaa99 wheat bran extract (WBE) over *Candida albicans* isolate (symbolled Ca 37) by well diffusion method at 37ºC for 24hrs

The Minimum inhibitory concentration

By using microtiter plate and the twofold dilution starting from the concentration 1 g/ ml WBE, the outcomes showed the plant extract had minimum inhibitory concentration for all tested microorganisms, 1 (mg/ml) for both *E. coli* and *C. albicans* while it registered 2 (mg/ml) for *S. aureus* isolates numerated within (Table. 2), color of pigment had transformed from blue to pink or pale pink. By covalent manner, Călinoiu and Vodnar (5) examined the MIC of fresh wheat bran over several bacterial indicators.

Table 2. The Minimum Inhibitory Concentrations of Ibaa99 wheat bran extract (WBE) over pathogenic microbial indicators

Pathogenic isolates	MIC by WBE (mg/ml)
E. coli: **Ec 28, Ec 51 and Ec 67**	**1**
S. aureus: **St 2, St 17 and St 44**	**2**
C. albicans: Ca 37	**1**

Detection of biofilm formation: After incubation of three *E. coli* isolates, three isolates, *S. aureus* and one isolate of *C. albicans* were found to be capable to form biofilm when black colonies were observed for the biofilm production by Congo red agar (Fig. 4). In addition to the tube assay was applied for confirmed *C. albicans* isolate biofilm formation (Fig. 5). Additionally, the results of Microtiter plate (MtP) method for biofilm production by *E. coli*, *S. aureus* and *C. albicans* are shown within (Tables. 3, 4, 5) illustrated the strong formation of chosen isolated. Based on biofilm formation results, pathogenic indicator isolates were chosen for antimicrobial and antibiofilm investigation. Abdulla and Ismael (1) found by their own study that *C. albicans* isolates are biofilm producer by utilizing Congo red agar.

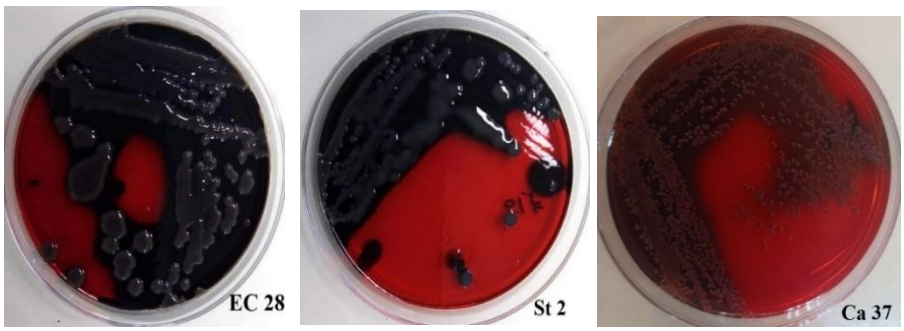

Figure 4. *Escherichia coli* isolate (symbolled Ec 28), *Staphylococcus aureus* isolate (symbolled St 2) and *Candida albicans* isolate (symbolled Ca 37) after incubation at 37°C for 24hrs on Congo red agar, given positive results for biofilm production

Figure 5. *Candida albicans* isolate incubated at 37 °C for 24hrs. given positive result for biofilm production by Tube method

Table 3. The detection of biofilm formation by *E. coli* isolates depending on microtiter plate method

Isolate NO	Isolate OD	Results
Ec 28	0.33	++
Ec 51	0.32	++
Ec 67	0.58	+++
NC	0.05	0

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; NC= Negative control; SD= 0.001

Table 4. The detection of biofilm formation by *Staphylococcus aureus* isolates depending on microtiter plate method

Isolate NO	Isolate OD	Results
St 2	0.58	+++
St 17	0.33	++
St 44	0.15	+
NC	0.05	0

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; NC= Negative control; SD= 0.001

Table 5. The detection of biofilm formation by *Candida albicans* isolate depending on microtiter plate method

Isolate NO	Isolate OD	Results
Ca 37	0.60	+++
NC	0.05	0

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; NC= Negative control; SD= 0.001

Antibiofilm activity of wheat bran extract

Biofilm produced by three *E. coli* isolates has been eradicated by WBE when the recorded averages of biofilm OD values were reduced from averages of each isolate control. However, highest reduction in biofilm was registered OD (0.10) in isolate that already formed strong biofilm (Fig. 6) with OD record (0.49). Moreover, Biofilm produced by *S. aureus* isolates has been eradicated too when the recorded averages of biofilm OD values were reduced from averages of each isolate control. However, highest reduction in biofilm OD (0.11) was registered (Fig. 7) in the isolate already formed strong biofilm with OD record (0.58). Also, Biofilm produced by *C. albicans* isolate has been eradicated in same manner when recorded an average of biofilm OD value (0.07) was reduced from the average of the isolate control (0.58) according to (Fig. 8). Other studies in this field reveals that González-Ortiz *et al.* (7) suppressed biofilm formed by *S. aureus* by Spanish wheat bran extract. In other hand, Torras *et al*. (23) invitro study showed anti-biofilm activity of Wheat extract at a concentration of 0.29 mg/100mL on *S. aureus*, inhibiting its formation, as well as destroying it greatly after 24hours. Phenolic compounds including p-Coumaric and ferulic acids extracted by Kot *et. al.* (13) had suppressor action on *E. coli* biofilm formation.

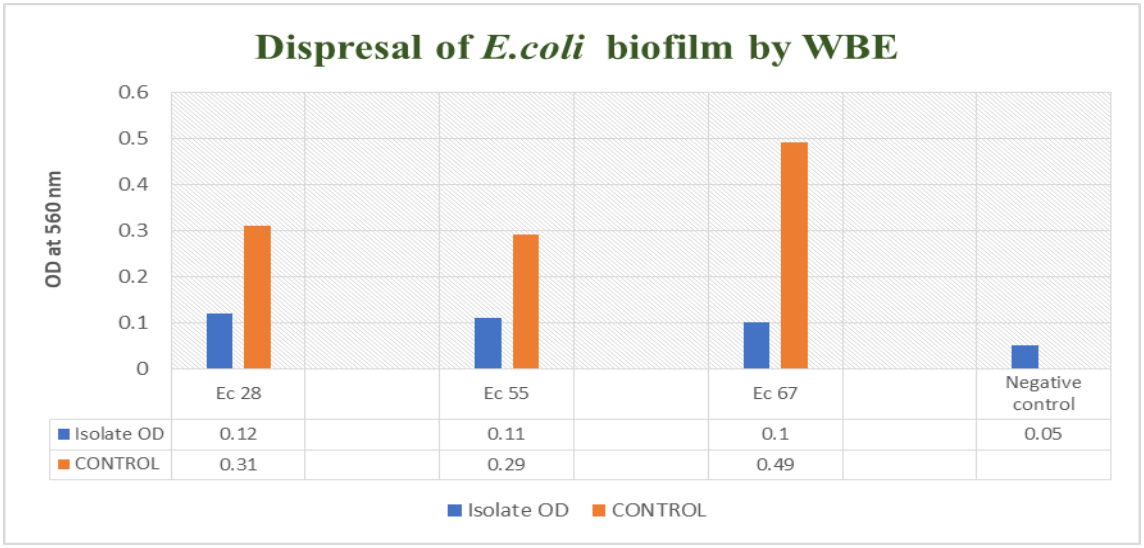

Figure 6. Dispersal of *Escherichia coli* isolates biofilm by Ibaa99 wheat bran extract (WBE) using microtiter plate method

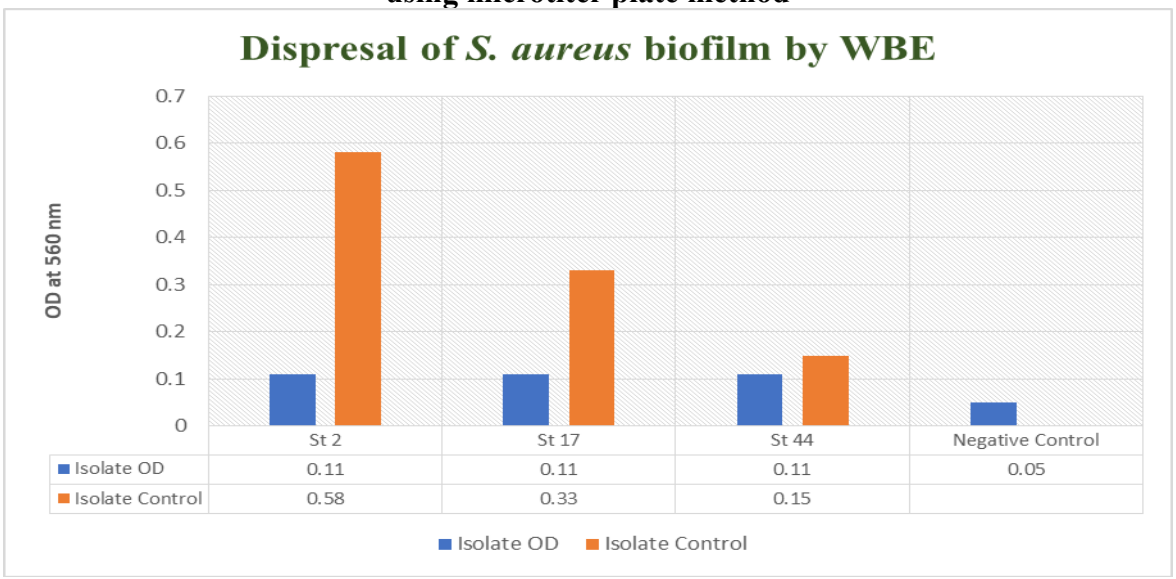

Figure 7. Dispersal of *Staphylococcus aureus* isolates biofilm by Ibaa99 wheat bran extract (WBE) using microtiter plate method

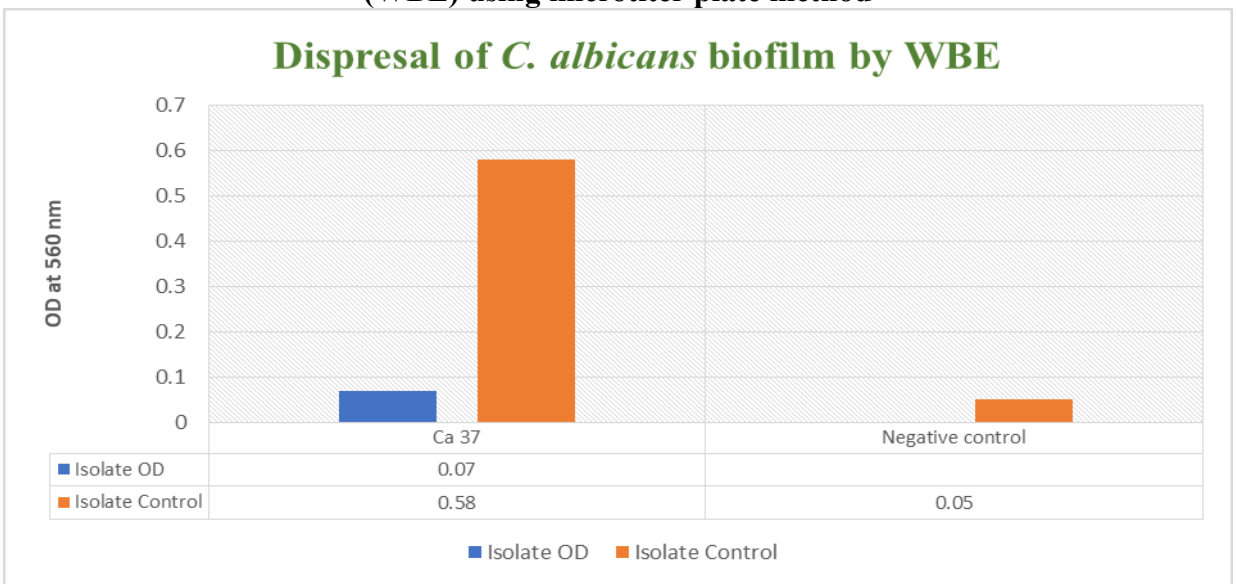

Figure 8. Dispersal of *Candida albicans* biofilm by Ibaa99 wheat bran extract (WBE) using microtiter plate method

CONCLUSIONS

Considering the results of the current work, the outcomes were showed that a significant efficiency of wheat bran extract as antimicrobial and antibiofilm metabolites against the Gram negative, Gram positive and yeast microorganisms as well. The extract of wheat bran active compounds as a cheap source usually consumed and used as a byproduct might considered a promising new aera that could be used within pharmaceutical purposes in instead of common more resisted drugs.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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