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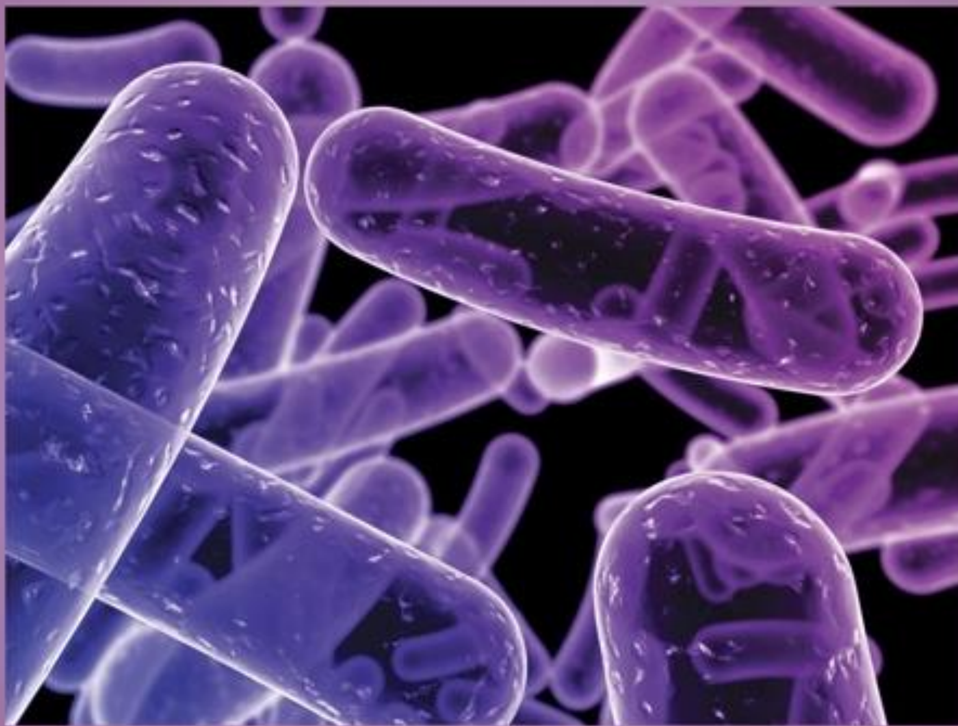

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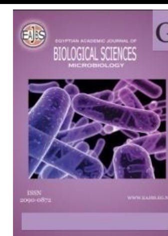

Antibacterial, Antivirulence Activities and Phytochemical Screening of Some Wild&Wasts Plants in Egypt

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ABSTRACT

Natural products have beneficial biological activities and they also do numerous functions as drugs against cancer as well as viral and microbial infections. As a part of a program oriented towards the discovery of bioactive natural products, two wild plants (*Saueda aegyptica*, *chenopdium murale*) and 2 waste plant products (apple, banana peel) were evaluated for their antibacterial activities against *Staphylococcus. aureus, Escherichia. coli* and *Salmonella entrica* using well agar diffusion method. Phytochemical screening tests used to detect the active compounds in them. Then make serial dilution for 2 extracts (banana and Chenopodium) that showed high activity to detect MIC (Minimum Inhibitory Concentration) for *E. coli* of (1.562 mg /ml) in banana and (3.124 mg/ml) in Chenopodium, for *Staph. aureus* (6.25 mg/ml) in banana and (12.50mg/ml) in Chenopodium but MIC test was not enough, antioxidant activity test, biofilm formation and reduction, antihemoytic activity and Transmisiom Electron Microscope (TEM) tests used to confirm the presence of highly active compounds in banana and Chenopodium methanolic extracts.

INTRODUCTION

Bacteria major human illnesses, particularly in Africa and the Middle East areas Resistance and multi-resistant strains of bacteria continue to emerge, requiring the constant search and development of new treatments. The discovery of novel antimicrobial chemicals from diverse sources, such as bacteria, animals and plants, has been pursued extensively. Folk medicine is one of these useful sources of information and healing. New useful compounds might be discovered by conducting systematic screening of them (Tomoko *et al.,*2002). Some 77% of the active ingredients in plants were found after the ethnomedical applications of the plants began to be studied, according to one estimate (Cordell *et al.,*2000). There are an estimated 250,000 to 500,000 plant species on Earth, but only a tiny fraction (1 to 10 percent) of these are consumed as food by humans and other animal species, leaving a vast potential for the creation of medicinal plant products (Heinrich&Gibbons *et al.,*2001). Antimicrobials derived from plants, as opposed to manufactured medications, offer fewer side effects and a greater therapeutic potential for treating a wide range of infectious disorders (Iwu *et al.,*1999).

Plants that contain a broad range of secondary metabolites, such as tannins, terpenoids, alkaloids, and polyphenols, are typically thought to be better in their antibacterial properties (Cowan *et al.,*1999).

There is a strong correlation between the variety and amount of natural product ingredients and their biological activity. Therefore, determining the chemicals that may be responsible for any biological activity simultaneously would speed up the selection process for the plants in question. Dental and gum infections may be treated using the stems and leaves of this plant from the Chenopodiaceae familySuaeda aegyptiaca. This plant can also be used as a snuff to treat dizziness, migraines, and nausea as well as to relax the nervous system and improve impaired eyesight (Ghazanfar *et al.,*1994). Chenopodium murale belongs to the Chenopodiaceae family, which has a broad range of uses in folk medicine, including as an anthelmintic and diaphoretic, as an emmenagogue and as an abortifacient, as well as for the treatment of asthma, catastrophia, and migraines (Watt and Breyer *et al.,*1962) (Vasishita *et al.,*1989). It was found that the Musaceae family, which includes the banana (Musa paradisiaca), was associated with numerous medical applications for the banana peel. Inflammatory and pruritic symptoms are alleviated, as are bacterial infections. Insect bites are said to be alleviated by this product. There are several health benefits of eating apples, which are found in both the fruit and the peel of the fruit (Boyer *et al.,*2004) (Wolfe *et al.,*2003) These plants will be tested for their antibacterial and antiviral properties against bacterial strains in this study, which is the goal of this research. drug production.

MATERIALS AND METHODS

Collection of Plant Samples:

Peels of fruits the golden delicious apple (Malus domestica) and the banana (Musa paradisiaca). from the local market, samples of plants *Suaeda. aegyptiaca(S.aegyptiaca) , Chenopodium. murale(Ch.murale)* from Egypt's western desert of El-Fayoum and Egypt's desert of the 10th of Ramadan were also obtained. These samples included In Benha University, at the Department of Plant and Microbiology at the Plant and Flora Laboratory, all of these plant samples were recognised.

Running tap water was used to wash the aerial sections of *S.aegyptiaca*, *Ch.murale*, and the peels of banana and apple. Electric grinders were used to grind the samples into fine powder after they had been air-dried for seven days at room temperature of 25°C ± bn gn 2. They were stored at a temperature of 4°c in plastic bags until they were needed (Harbone *et al.,* 1975).

Crude Extracts Are Made by Preparing:

According to the everyday practices of traditional healers, these customary preparations were made. There were three different solvents (water, acetone, and methanol) used to macerate 50 g of powdered plants in 250 ml of solvents for 24 hours each. Filtration of the mixture was carried out as a last resort. Filtered using Whitman No. 1 paper. Using a rotating evaporator, extracts were concentrated under vacuum at a low temperature of 40°C (ROTAVAPOR, R11, BUCHI). It was tagged and stored in a refrigerant at 4°C until use.

Screening for Phytochemicals at This Stage:

A phytochemical study of the plant extracts was carried out using methanol, aqueous, and acetone extracts. Qualitative approaches such as those developed by (Trease and Evans *et al.,*1989) , (Sofowora *et al.,*1982) , (Harborne *et al.,*1973) were employed to study phytochemicals.

Microorganisms for Examination:

ASU's Cairo MIRCEN provided the Staphylococcus aureus (ATCC 6538), and Salmonella enterica (ATCC 25566), and Escherichia coli (ATCC 10536), utilised in the study for testing.

Antimicrobial Activity Test: The agar plate diffusion test was used to assess the extracts' quantitative antibacterial activity (Perez *et al.,*1990).

Overnight incubation of each bacterial strain in Mueller Hinton broth resulted in viable inoculum. Bacterial cultures were carried out on standard Mueller-Hinton agar. To test the extract, 0.1 mL of the diluted inoculum (10^{ml} CFU/mL) of the tested organism was placed on agar plates and then

dissolved in sterile DMSO at a concentration of 100 mg/mL In the agar medium, six 6-mm-diameter wells were punched and filled with plant extract. For 24 hours, the plates were incubated at 37 degrees Celsius. The positive control in this experiment was DMSO. Inhibition zones smaller than 7 mm in diameter were not evaluated since the well was only 6 mm in diameter. Determination of Minimum Inhibitory Concentration (MIC) was used for all tests.

A broth microdilution technique, authorised by the Clinical and Laboratory Standards Institute (NCCL/CLSI, 2007), was used to determine the (MICs). Using sterile distilled water, the concentration of the test material was lowered to 100 (mg/ml). In order to produce the microtitre plates for Staph. aureus and *E. coli,* (100µL) of Mueller Hinton Broth (MHB) was added, followed by (100µL) of Banana and Chenopodium plant extracts. Serial dilutions (from 100 to 0.097mg/ml) were used to obtain the extracts for testing. Boiling and distilled water was only found in the control wells). Each plate was placed in an incubator at 37°C for 24 hours. The MIC was determined to be the lowest concentration of the plant extract tested that inhibited bacterial growth.

Antioxidant Activity Is Tested in This Experiment:

The DPPH for free radical scavenging test was used to measure the antioxidant activity of Banana and Chenopodium plant extracts in triplicate, and the average results were used.

Activities of Radical Scavenging by the DPPH:

Methanol solution of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical freshly synthesised (0.004 percent w/v) was made and kept at 10 °C in the dark for storage. To 3 (ml) of DPPH solution, we added a 40-ml aliquot of each of the plant extract solutions from Banana and Chenopodium. For the duration of the experiment at (515 nm), data was collected every minute until the absorbance level stabilised (16 min). The DPPH radical without an antioxidant (the control) and the reference chemical ascorbic acid were also tested for their absorbance. The DPPH radical's percentage inhibition (PI) was determined using the following formula:

$$PI = [\{(AC-AT) /AC\}x\ 100]$$

Where, AC= Absorbance of control at t=0 min and AT = absorbance of the sample +DPPH at t = 16 min (Yen and Duh *et al.,*1994).

(Antivirulence activity) Protection Against Infection:

Tissue culture plates are used to conduct biofilm formation assays on bacterial isolates.

present a quantitative test using the TCP approach (Christensen *et al.,*1985). Essentially, bacteria obtained from new agar plates were injected into 10 (ml) of trypticase soy broth with 2% glucose. Incubation at 37oC for 24 hours was performed on the inoculated broths. They were diluted at 1:100 with a new medium and resuspended. (200µL) of the diluted cultures with 0.5 McFarland standard were placed in individual wells on 96-well flat-bottom polystyrene tissue culture treatment plates (Sigma-Aldrich, Costar, USA). A sterile broth medium was used to inoculate the negative control wells. It took 24 hours to incubate the plates at 37°C. After incubation, the free-floating bacteria were gently tapped out of each well. It was done four times, using 0.2 ml of pH7.2 phosphate buffer saline. Crystal violet was used to stain the biofilm generated by bacteria adhering to the wells (0.1 percent). Deionized water was used to remove any remaining discoloration, and the plates were then left to dry. Micro ELISA microtiter-plate reader (Sun Rise–TECAN, Inc. ®, USA) at wavelength was used to measure optical density (O.D.) of stained adherent biofilm (570nm). The experiment was done three times in a row in triplicate. Biofilm formation was interpreted in accordance with the parameters of the study (Stepanovic *et al.,*2007).

Assay for the Suppression of Biofilms:

Utilising The Tissue Culture Plate (TCP) Technique: At doses below the MIC, the anti-biofilm properties of extracts from the Banana and Chenopodium plants were investigated.

To acquire the preceding concentrations in (100µL), an aliquot of two-fold serial dilutions was made on a 96-well microtiter plate with trypticase soy broth with 2% glucose TSBGlc. The bacterial suspensions were then injected onto the plate at a final concentration of 50 µL; 5 105 cfu/ml. Distilled water was used in the formulation of TSBGlc (negative control). Plant extract-free TSBGlc was utilised as the inoculant in this experiment (positive control). After 24 hours of incubation at 37°C, the microplate reader was used to analyse the influence of the extracts on bacterial growth (570nm). Plant extracts were then evaluated and compared to a positive control for the production of bacterial biofilms (Lin *et al.,*2011).

Immunoassay for Hemolysis:

A new technique of hemolysis analysis was devised (Larzabal *et al.,* 2010). Sub-MIC quantities of banana and Chenopodium extracts were used to lyse human red blood cells cultured in *Staph. aureus* cultures and the optical density of the cells were compared to positive control.

Determination of Tests for Time-Kill:

Tested extracts of Banana and Chenopodium Methanolic extract at MBC concentrations (6.2 and 1.562 mg/ml) against Staph. Aureus and E. coli were diluted in sterile distilled water and dialyzed. 25 milliliters were utilised in total, including 12 milliliters of tryptic soy broth (TSB), 10 milliliters of dialyzed filtered extract, and 2.5 milliliters of inoculum. And it was incubated at a constant temperature of 35-37°C. (0, 3, 6, 12 and 24h) All experiments were replicated and the average results were reported. A bacterial suspension was collected and the influence of the tested extract on the bacterial growth was examined using the spectrophotometric assay at optical density (620nm) (OD620nm) (Souza *et al.,*2006).

Electronic Microscope Transmitting Electrons (TEM):

Banana was used to study the effects of treatment and non-treatment on bacterial cultures. At (MICs) concentrations of metabolic extract. Each bacterial type's non-treated cultures were included as a control, and then all of them were examined by TEM as a whole.

Sample Preparation for TEM Analysis:

An automated tissue processor dried the samples by dilution using repeated dilutions of ethanol (Leica EM TP).

Afterward, the CO2 critical point drier was used to dry the samples (Tousimis Audosamdri-815). The gold sputter-coated samples (SPI-Module). the exanimated samples were obtained by scanning electron microscopy at the Regional Center for Mycology and Biotechnology (JEOL-JEM-1010) in high vaccum mode, Cairo, Egypt.

RESULTS

Phytochemical Screening Results:

Results of phytochemical screening of water, methanol and acetone plant extracts revealed the presence of alkaloids, flavonoids, saponins, tannin and Phenol compounds in different concentrations as in (Table 1).

The concentrations of the various classes of Secondary metabolite vary among the extracts evaluated. The concentrations of the constituents are In order of methanol > aceton > water.

Table (1) screening of phytochemical compounds in plant extracts.

Plant extracts		Phytochemical screening test s								
Apple		Terpenoids	Flavonoids	Phenolic compounds	Tannins	Alkaloids Mayer's	Wagner's	Protein	Sterols	Saponins
	Aqu.	+	+	-	-	-	-	+	+	-
	Acet.	+	+	-	-	-	+	++	++	-
	Meth.	+	++	-	-	++	++	+++	+++	+
Banana	Aqu.	-	++	++	+	-	+	++	+	+
	Acet.	++	+++	+++	-	-	+	+++	++	-
	Meth.	++++	++++	++++	+++	+++	-	++++	++++	+++
Suaeda	Aqu.	-	+	+	+	-	-	-	+	-
	Acet.	-	-	+	+	-	+	-	-	-
	Meth.	++	++	++	++	-	++	-	++	++
Chenopodium	Aqu.	+	+	+	+	+	-	-	-	+
	Acet	+	++	++	++	+	-	-	-	++
	Meth	++	+++	+++	+++	++	-	-	-	+++

As (-) not detected ND, (+) low concentration, (++) moderate concentration, (+++) high concentration and (++++) very high concentration. Meth=methanol Acet=aceton aqu =Aqueous

Antibacterial Activity:

The antibacterial screening of plant extracts showed the activity against the bacteria.Aqueous extract of apple has no activity, in banana and Suaeda and Chenopodium found in *St.aureus* only. While in acetone extract it showed inhibition zones range (from 9mm to 20 mm) for *E. coli* and (from 7.5mm to 18mm) for *St.aureu*s (Table 2) and it showed inhibition zones in salmonella in Chenopodium and apple extracts.

The methanolic extracts showed inhibition zones ranges (from 11 mm to 18mm) for *St. aureus* and (from 11 mm to 20 mm) for *E. coli* and no inhibition zone detection for Salmonella Species.

The result indicates the effectivity of methanol>aceton >aqueous extracts in order. The inhibition zones were calculated at 3 replicates.

Table 2: Showed the (inhibition zones in mm) for the plant extracts.

Plant extracts \ Bacterial strain		Inhibition zones in (mm)		
		E. coli	St. aureus	S. enterica
Apple	Aqu.	-	-	-
	Acet.	13	11	10
	Meth.	14	11	-
Banana	Aqu.	-	7.5	-
	Acet.	16	11	-
	Meth.	20	18	-
Suaeda	Aqu.	-	10	-
	Acet.	16	-	-
	Meth.	11	11	-
Chenopodium	Aqu.	-	12	-
	Acet.	9	7.5	8
	Meth.	18	15	-

Meth=methanol Acet=aceton aqu =Aqueous

MIC:

Minimum inhibitory concentrations (MICs) of Banana and Chenopodium methanolic extracts.

The results presented in Table (3) and figure (1) showed the (MICs) values of Banana and Chenopodium methanolic extracts against the growth of *Staph. aureus* and *E. coli* isolates. It was found that The Banan extract had the highest activity against *E. coli* and *Staph.* aureus at lower activity mg/mL were required for growth inhibition when compared with Chenopodium methanolic extract.

Table 3: MICs (mg/ml) of Banana and Chenopodium methanolic extracts against bacterial isolates**.**

Bacterial isolates	MIC	
	Chenopdium extract Conc (mg/ml)	Banana extract Conc.(mg/ml)
E.Coli	3.124	1.562
Staph. aureus	12.50	6.25

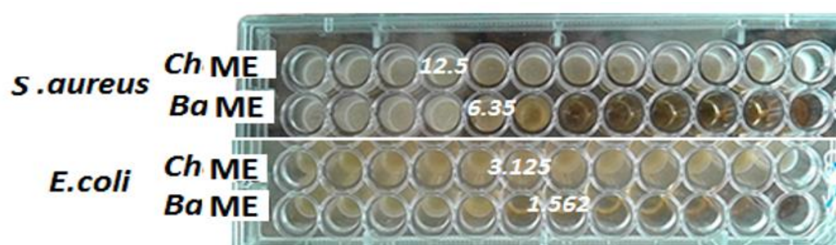

Fig. (1) Microtiter ELISA plate showing MIC of Banana and Chenopodium methanolic extracts against the growth of tested Staph. Aureus and E. Coli bacteria by microdilution method.

Antioxidant activity

Antioxidant Activity of Banana and Chenopodium Extract Using DPPH Free Radical-Scavenging Activity:

The total antioxidant activity of the banana extract was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical oxidation inhibition system as a natural antioxidant agent. Furthermore, the banana extract showed in vitro antioxidant high activity while Chenopodium extract showed antioxidant low activity in vitro by neutralizing the DPPH radical (Table, 4).

Table 4: Antioxidant activity of Banana and Chenopodium methanolic extracts total extract using DPPH scavenging**.**

Extracts (ug)	DPPH scavenging %	
	Banana Extract	Chenopodium Extract
3200	92.25	54.25
1600	81.16	41.16
800	72.92	32.92
400	61.62	22. 35
200	50.45	14.45
100	38.74	8.64
50	23.11	0
25	15.50	0
0	0	0
IC50	150 ug	2400 ug

The absorbance values were converted to scavenging effects (%) and data plotted as the means of triplicates scavenging effect (%) values.

The Antivirulence:

The activity of Banana and Chenopodium Methanolic Extracts Biofilm Formation of Tested Bacterial Strains:

At 570nm, biofilm positive phenotype was characterised as an OD 0.17 (O.D.570). Strong biofilm formation was defined as O.D.570 0.7–1.0; moderate biofilm formation was defined as O.D.570 0.3–0.4, and weak biofilm formation was defined as O.D.570 0.3. Table (5) and Fig. (2) exhibit the findings: Staph. Aureus, which had an optical density of 1.10 O.D., was shown to be the strongest biofilm-producing strain. While E. coli, 0.45 O.D. was a moderate biofilm producer.

Table 5: Biofilm formation of bacterial isolates using (Tissue culture plate method by ELISA reader).

Antivirulence activity Bacteria isolates	Growth (O.D.620nm)	Biofilm (O.D.570 nm)	Biofilm production
Negative control (O.D.620nm)	0.09	-	-
E.coli	1.2	0.45	Moderate
Staph.aureus	1.4	1.10	Strong

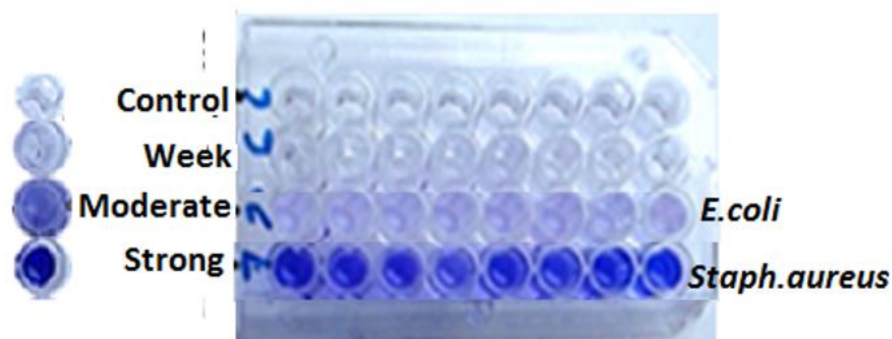

Fig. (2) Microtiter ELISA plate showing biofilm formation of Staph. Aureus and, E. Coli were grown overnight in polystyrol microtiter wells in TSB supplemented with 2% glucose. The cells that adhered to the plate after washing were then visualized by staining with crystal violet.

Biofilm Inhibition Activity:

The Banana and Chenopodium methanolic extracts were investigated for their potential to prevent biofilm formation against strong biofilm-producing isolates.

Staphylococcus Aureus Biofilm:

Staph.aureus is one of the most common biofilm-forming pathogens. Using Banana and Chenopodium methanolic extracts at sub-MIC concentrations, researchers were able to reduce the production of Staph. Aureus biofilms in a dose-dependent manner. Banana and Chenopodium methanolic extracts demonstrated potential action against Staph. aureus biofilm development, as shown in Table (6) and depicted in Figs. (2&3). Results revealed that Banana methanolic extract had the greatest anti-biofilm activity, with 67.27 percent, whereas the biofilm reduction percentages for Chenopodium methanolic extracts were only 28,45 percent. (Fig. 3).

Table 6: Antibiofilm formation effect of banana and Chenopodium methanolic extracts againstStaph.aureus.

Staph. aureus	Sub (MICs) concentrations		
	Control 0.0 mg/ml	Banana 6.125 mg/ml	Chenopodium 12.25 mg/ml
Biofilm formation O.D	1.4	1.10	1.10
Biofilm reduction O.D	-	0.36	0.79
(%)	-	67.27 %	28.45 %

***E. coli* biofilm**

Biofilm Banana and Chenopodium methanolic extracts sub (MICs) concentrations effectively reduced biofilm development in *E. coli.* However, the findings revealed that the anti-biofilm activity of the Banana methanolic extract was higher than that of Chenopodium methanolic extract, with a reduction percentage of biofilm of 40 percent (Table 7).

Table 7: Antibiofilm formation effect of banana and Chenopodium methanolic extracts against *E. coli*

E. coli	Sub (MICs) concentrations		
	Control 0.0 mg/ml	Banana 1.562 mg/m	Chenopodium 3.124 mg/ml
Biofilm formation O.D	1.2	0,45	0,45
Biofilm reduction O.D	-	0.27	0.32
(%)	-	40.0 %	28.88 %

Antihemolysis activity against *Staph. aureus*

The Banana and Chenopodium methanolic extracts were investigated to identify a novel anti-hemolytic agent against *Staph.aureus* at (MICs). The results are given in table (8).

Table 8: Antihemolysis effect of banana and Chenopodium extract against Staph. Aureus

Staph.aureus	Sub (MICs) concentrations		
	Control 0.0mg/ml	Banana 1.562 mg/ml	Chenopodium 3.124 mg/ml
Hemolysis O.D	0.395	0.175	0.325
Hemolysis reduction	0.0	0.062	0.295
%	-	64.57%	9.23%

Time kills of Staph. Aureus and *E. coli* by Banana and Chenopodium methanolic extract:

In a microbiological medium, the bactericidal action of Banana and Chenopodium methanolic extracts was examined at various time intervals to determine the time-kill assay. Using the Minimum Bactericidal Concentration (MBC) for Staph. aureus and *E. coli*, the findings showed that the methanolic extracts of Banana and Chenopodium methanolic extracts were effective at 12.5 mg/ml for Staph. aureus and 1.562 mg/ml for *E. coli.* Table (9) shows the optical density of *E. coli* and staph. Aureus control growth was 0.012 and 0.041O.D., respectively, at zero time. While the optical densities (O.D.) with complete extract were 0.014 for one of the bacteria and 0.0110.43 for the other. However, after 48 hours of incubation, the optical densities for Staph. Aureus controls reached 6.234 and O.D., while those treated with Banana or Chenopodium methanolic extracts reached 0-3h and (0-6 h) in the time-kill assay for Staph. aureus, as well as 0-3 h and 0-12 h in the time-kill assay for *E. coli.* Staph. Aureus's time-kill test was shown to be quicker than *E. coli's. Coli.*

Table 9: Time-kill assay of Staph. Aureus and *E. coli* cultures by Banana and Chenopodium methanolic extracts by optical densities at 37 °C for 48h

Time (hrs)	Bacterial growth (O.D.620 nm)					
	Staph.aureus.			E.coli		
	Control	Banana extract	Chenopodium Extract	Control	Banana Extract	Chenopodium extract
0	0.015	0.014	0.014	0.019	0.015	0.017
3	0.385	0.164	0.175	0.455	0.143	0.181
6	0.675	0.0	0.091	1.030	0.0	0.164
12	1.234	0.0	0.0	2.160	0.0	0.086
24	2.556	0.0	0.0	2.160	0.0	0.0
48	6.234	0.0	0.0	8.723	0.0	0.0

Effect of Banana methanolic extract on *E. coli* and Staph. Aureus via Transmission Electron Microscope (TEM)

The Staph. Aureus and E. coli were killed by Banana methanolic extract as a bactericidal agent by TEM. Staph. aureus and E. coli were cultured for 24 hours at the proper temperature and conditions with MIC Banana extract. The morphological alterations in cell injury were obtained using TEM. The examined microorganisms suffered substantial damage as a direct result of the banana extract. Smooth, untreated cells (the control) were found to be

undamaged (Figs. 4&5). When bacteria were treated, the Gram-positive and Gram-negative cells shrank and even some were empty, leaving just flaccid remnants. In addition, it looked that the majority of them were melted and bonded together. Giant cells and appendages could be seen on the surface of the treated E. coli bacteria. In *E. coli* (TEM) pictures, pseudomycelium-like structures emerged. Gram-positive and Gram-negative pathogenic bacteria treated with banana methanolic solution suffered physical damage and underwent significant morphological changes, as seen by TEM pictures. extract.

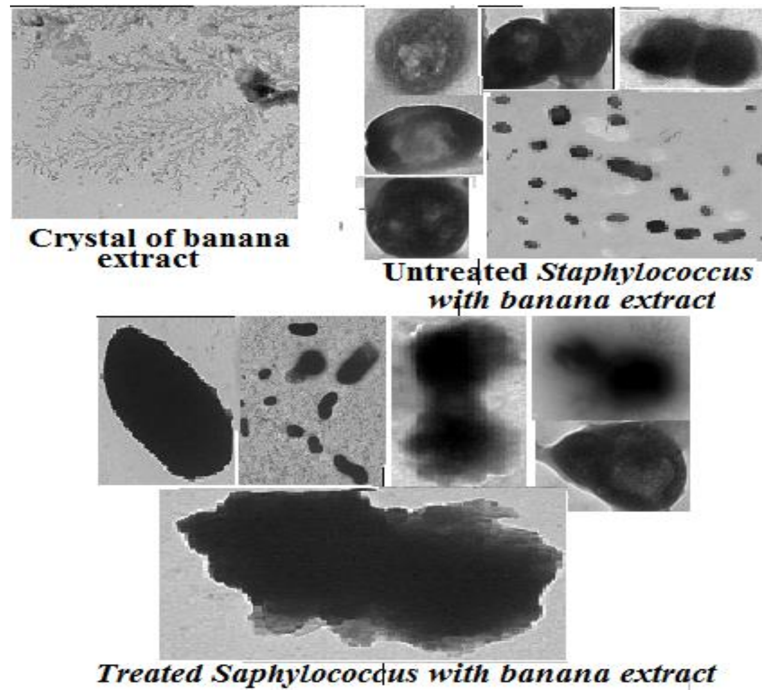

Fig. (4): Photographic Transmission Electron Microscope observed the effect of Banana methanolic extract on Staph. aureus (control: A2, Treated: C1-C3).

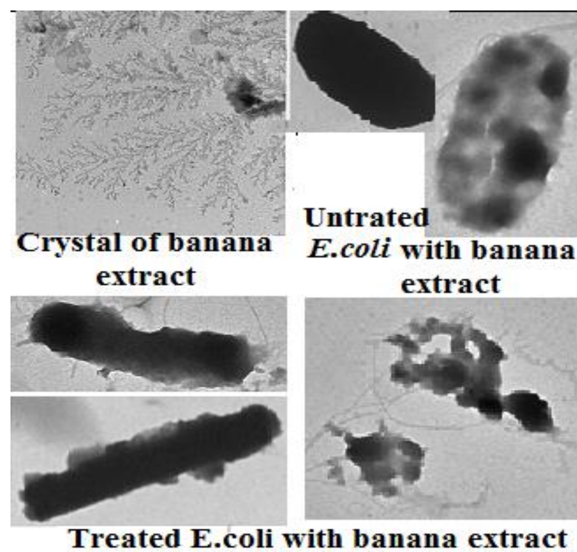

Fig. (5): Photographic Transmission Electron Microscope observed the effect of the banana methanolic extract on *E. coli* (control: A1, Treated: B1-B3).

DISCUSSION

Plants structures that might lead to novel chemotherapeutic drugs are found in these organisms. Knowing the plant's phytoconstituents and bioactivity is important. Many health disorders and long-term conditions may be treated using certain substances synthesised.Bacterial resistance is more difficult to develop against plant extracts than it is against many routinely used antibiotics, which only target one component of the organism. Smith-Palmer (Smith-plamer *et al.,*2001). Plant extracts are being screened for active chemicals (flavonoids, terpenoids, alkaloids, phenolic compounds, saponins and tannins) that have a significant impact on bacterial strains as the first step in achieving this aim.4 plant extracts were tested for antibacterial activity against S. aureus, *E. coli*, and Salmonella entrica, and inhibition zones were determined to identify which plants may be utilised for future testing and isolation.

In contrast to the other strains, Salmonella entrica was unafraid. MIC In a study by (Cos *et al.,*2006), plant extracts with MIC were found. There is a lot of promise in values below 100µL /mL are very promissing. When tested against *E. coli* and Staph. Aureus, the high MICs of Banana methanolic extracts (1.562 mg/ml = 1562 µL/ ml and 6.25mg/ml= 625 µL/ml, respectively) and Chenopodium methanolic extract (3.124 mg/ml = 3124 µL/ml and 12.50 mg/ml= 1250µL/ml) for E.coli and Staph. aureus, respectively, showed that they were very active. This study's MIC values were insufficient; thus, an antioxidant activity test was performed instead.

Researchers looked into the antioxidant effects of banana crude and methanolic extracts of Chenopodium, and the findings showed that banana extract had higher activity than methanolic extracts of Chenopodium. Different biologically active phytochemicals have been discovered by phytochemical research, which may be linked to the plant's antioxidant power.

In order to produce biofilms, bacteria must transition from planktonic (free-swimming) to surface-bound. In the 1970s, plastic tissue culture plates were used to study biofilm development, and in the 1980s, 96-well tissue culture and Microtiter plates were introduced (Christensen GD, Simpson WA *et al.,*1985) , (Fletcher M , Loeb GI *et al .,*1979) To see whether bacteria can cling to a Microtiter plate's plastic surface at a certain temperature and nutrient level, this test is used. First reported in a scientific journal, the procedure Developed by (Genevaux *et al.,*1996) For St. Aureus at MIC (6.125 mg /ml) in banana (67.27 percent) and Chenopodium at (12.25 mg/ml), methanolic extracts of banana and Chenopodium demonstrated excellent biofilm reduction (28.45 percent). At MIC (1.562 mg /ml) for banana, (40%) and at MIC (3.124mg /ml) for Chenopodium, (28.88%)

Hemolytic Inhibition:

There are a lot of biological and morphological properties in erythrocytes, making them a good candidate for medication delivery since they are the most common human cell type. In particular, erythrocytes are the primary target of PUFA and hemoglobin, which are both redox active oxygen transport molecules and powerful pro-Motors of activated oxygen species. Erythrocyte oxidative mutilation There are multiple causes that may lead to hemolysis, including abnormalities in the membrane lipids and proteins, Hemo-Globinopathies, oxidative medications, excessive transition metals, radiation of different kinds as well as deficits in erythrocytes Coordination of antioxidants (Hamidi M, Tajerzadeh H *et al.,*2013) (Ebrahimzadeh M *et al.,*2009) The goal of this study was to see whether erythrocyte membrane oxidative damage might be prevented by methanolic extracts of banana and Chenopodium. Antihemolytic activity against Staphylococcus aureus was found to be varied in each of them. Banana and Chenopodium methanolic extracts showed effective anti-hemolytic efficacy, according to the results. Despite this, banana (64.57 percent) and Chenopodium (9.23 percent) displayed the greatest suppression of

homolysis at MICs of 1.562 and 3.124 mg/ml, respectively.

Electron Microscope Transmission:

Electron microscopy can be considered as a technique that Allows the attainment of specific and special information about A sample (Marisol Faraldos *et al.,*2002) transmission electron Microscopy or TEM for its acronym in English, is a visual Technique in which an electron beam is focused on a sample to Get an extended version of the image over a fluorescent screen. In this study, TEM technique was applied on banana methanolic extract that showed a high effect on bacterial strains. On *Staph. Aureus*, the cells shrunk in size, damaged and stopped the diffusion and also damaged the cell Wall of *E. Coli.*

Conclusion

The antibacterial study of 4 plants has identified various extracts with activity against several pathogenic bacteria which might explain their ethnomedical uses for the treatment of various infectious diseases. The plants with the greatest potential as targets for bioassay-guided fractionation are peel of banana (*Musa. paradisiaca) and Chenopodium. morale.*

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