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EVALUATION OF ANTIBACTERIAL ACTIVITY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF WATERMELON WHITE RIND EXTRACTS

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ABSTRACT

Watermelon white rind is thrown to the environment as agro waste producing ecological and environmental problems. The present study is an attempt to evaluate the medicinal properties of the rind to generate valuable health-promoting products. Aqueous and ethanolic extracts were prepared and screened for antibacterial activity against three pathogenic bacterial strains: two Gram-negative bacteria (*E. coli and Salmonella sp.*) and one Gram-positive bacteria (*Staphylococcus aureus*). Both ethanolic and aqueous extracts of watermelon white rind have antagonistic effect against *E. coli* and *Salmonella sp.,* While *Staphylococcus aureus* was resistant to both extracts. The diameters of inhibition zone of ethanolic and aqueous extracts against *E. coli* were 5.75±0.96 mm and 3.00±0.82 mm, respectively. Whereas, the diameters were 8.50±0.58 mm and 4.25±0.96 mm against *Salmonella sp.*, respectively. GC-MS analysis revealed the existence of methionine, L-aspartic acid, glycyl-D-asparagine, 9-cis-retinoic acid, stearic acid allyl ester and ascorbic acid permethyl. These active compounds contributed definitely to the antibacterial activity of ethanolic and aqueous extracts against the used pathogenic bacteria. As a result, the white rind could be properly handled and utilized to maximize the safe waste management.
Keywords: Watermelon white rind, GC-MS technique, antibacterial activity.

INTRODUCTION

Watermelon (*Citrullus lanatus* var. *lanatus*, family Cucurbitaceae) is a flowering plant originally from southern Africa. Egypt is one of the top five watermelon producers with total amount of 1,874,710 tones yearly (FAO, 2012). Watermelon pulp serves as a good source of phytochemical and lycopene acting as antioxidant during normal metabolism and protects against cancer (Perkins-Veazie et al., 2006). The rind is thrown as agro waste producing ecological and environmental problems. We intended in the present study to evaluate the antibacterial activity of ethanolic and aqueous watermelon white rind extracts against some Gram-positive and Gram-negative pathogenic bacteria. The present study pointed to the bioactive compounds of the rind that can be utilized to afford new antibacterial agents from natural sources and consequently helping in waste management and reduction of pollution.

MATERIALS AND METHODS

Pathogenic bacterial strains

The bacterial strains of *Escherichia coli, Salmonella sp.* and *Staphylococcus aureus* were provided from Microbiology Department, Faculty of Agriculture, Ain Shams University. The tested bacteria were grown in buffered peptone water (pH 7.2) and incubated for 24 h at 37°C to achieve viable cell count of 10^8 cfu/ml.

Watermelon white rind

The watermelon white rind was collected from restaurants in Cairo and Giza governorates. It is cut into small pieces, dried at 40˚C and pulverized into fine powder.

Preparation of aqueous extract

Aqueous extraction was carried out by decoction process as described by Johnson et al. (2011). Twenty five gram of the dried powder was mixed with 125 ml of hot distilled water in a flask and boiled for 15 min. The extract was filtered through whatman filter paper, kept in a clean sterilized container and stored at 4˚C till further investigation.

Preparation of the ethanolic extract

Soxhelet extraction of the rind was performed using 25 g of the dried powder mixed with 125 ml ethanol in a ratio of 1:5 (w/v) as described by Johnson et al. (2011).

Antibacterial assay

The antibacterial activity of the ethanolic and aqueous extracts was carried out by well diffusion method (William, 1989). Mueller-Hinton agar was the selected media for preparing the test plates. One hundred micro liter (100µl) of the microbial suspension was taken and spread onto Mueller-Hinton agar. Five hundred micro liter (500 µl) of both extracts are placed in holes in the agar layer seeded with the above mentioned bacteria. Negative control received 500 µl of ethanol 70% (Liviu et al., 2010).The inoculated plates were incubated at 37°C for 48 h. Estimation of the antibacterial potencies of the extracts was accomplished by measuring the zone of inhibition diameters in millimeters against the tested organisms. All tests were performed in triplicate.

Gas chromatography analysis (GC/MS)

Analysis of the ethanolic extract of watermelon white rind was carried out using GC (Agilant Tecnologies 7890A) interfaced with an apolar Agilent HP-5ms (5% phenyl methyl ploy siloxane) capillary column (30 m x 0.25 mm i.d. and 0.25 µm film thickness). The flow of carrier gas (helium) was maintained 1 ml/min during the run. The components were verified by matching their mass spectra and retention time with the database of National Institute of Standard and Technology (NIST) library. The name, molecular weight and structure of the components of the test materials were ascertained.

Statistical analysis

The mean values and standard deviation were calculated from the data obtained in triplicate using Minitab 14 program. Also, results were analyzed using Two-sample T-Test and One-sample T-Test. The results were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Antagonism

The antibacterial activity of ethanolic and aqueous watermelon white rind extracts are shown in Figures (1-4) and Table (1).

Antibacterial activities were found to have range of 5.75±0.96 mm and 3.00±0.82 mm against *E. coli* in case of ethanolic and aqueous extracts of watermelon white rind, respectively. While, inhibition diameters against *Salmonella sp.* valued 8.50±0.58 mm and 4.25±0.96 mm , respectively. Whereas *Staphylococcus aureus* was resistant to both extracts. Control (ethanol 70%) had no inhibitory effect on the studied bacterial strains. The antibacterial screening data revealed that the rind had good bacterial inhibition against *Salmonella sp. and Escherichia coli*. The fact that *E. coli* is already known to be multi-resistant to drugs, give a good impression about the effectiveness of the rind extracts to inhibit this type of Gram- negative bacteria. Surveillance data showed that resistance in *E. coli* is consistently the highest for antimicrobial agents in the domain of human and veterinary medicine (FDA, 2010).

Fig. 1. Inhibition zone of watermelon rind ethanolic extract against *Escherichia coli*

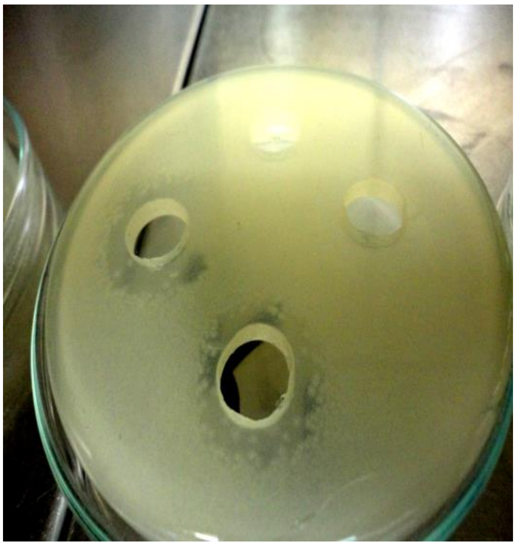

Fig. 2. Inhibition zones of watermelon rind aqueous extract against *Escherichia coli*

Fig. 3. Inhibition zones for watermelon rind ethanolic extract against *Salmonella sp.*

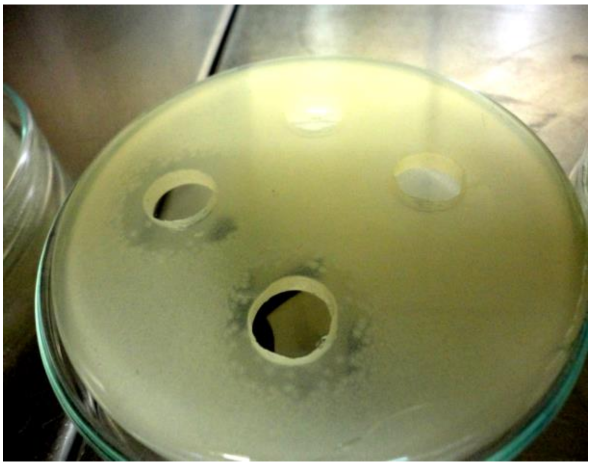

Fig. 4. Inhibition zones of watermelon rind aqueous extract against *Salmonella sp.*

Table 1: Antibacterial activity of watermelon white rind extracts against the selected pathogenic bacteria

Bacterial strain / Extracts	Inhibition zone diameter (mm)		
	E. Coli	***Salmonella sp.***	***Staphylococcus aureus***
Ethanolic extract	5.75±0.96	8.50±0.58	0.00
Aqueous extract	3.00±0.82	4.25±0.96	0.00

The inhibition zone diameters of ethanolic and aqueous extracts against *Salmonella sp.* as well as against *E. coli* were statistically significant (p=0.002) and (p=0.007), respectively.

Statically, there was a significant difference ($p<0.05$) and (p=0.003) between ethanolic and aqueous extracts in comparison with the control, respectively, in case of *Salmonella sp.* In case of *E. coli,* there was a significant difference (p=0.001) and (p=0.005) between ethanolic and aqueous extracts in comparison with the control, respectively.

The GC-MS identification of the various compounds present in the rind is illustrated in Fig. (5) and Table (2). GC-MS analysis revealed the existence of methionine (RT: 4.31); L-aspartic acid (RT: 5.03); glycyl-D-asparagine (RT: 21.09); 9-cis-retinoic acid (RT: 22.88); stearic acid, allyl ester (RT: 23.82) and ascorbic acid, permethyl (RT: 24.71).

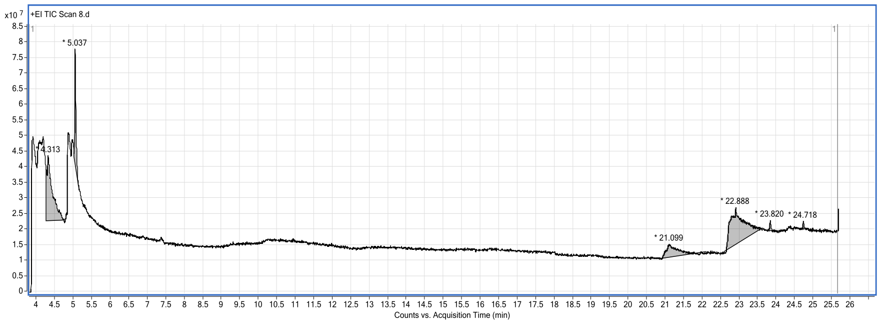

Fig. (5): GC-MS chromatogram of the watermelon white rind extract

Table 2. Phytochemical constituents of watermelon white rind by GC-MS spectra

The identified compounds belong to the categories of amino acids, fatty acids and vitamins which are diverse groups of biologically active chemical constituents present in the rind that contribute to its antimicrobial activities. The obtained data are in agreement with Andreea et al. (2011) who reported the activity of amino acids complexes against bacterial strains and proposed its application as potential antibacterial agents, in the field of disinfection, food packaging and piping of drinking water.

Contrarily to Kabbani *et al.* (2004) who cited that Gram-positive bacteria are known to be more susceptible to amino acids complexes than gram-negative bacteria, our finding demonstrated that ethanolic and aqueous rind extracts showed antimicrobial activity towards Gram-negative bacteria (*E. coli and Salmonella sp.).*

Desbois and Smith (2010) pointed to the ability of fatty acids (FA) to inhibit the growth of bacteria. The prime target of FA is the cell membrane, where they disrupt the electron transport chain and oxidative phosphorylation. Besides interfering with cellular energy production, FA mode of action may also result from the inhibition of enzyme activity, impairment of nutrient uptake, generation of peroxidation and auto-oxidation degradation products or direct lysis of bacterial cells. Their broad spectrum of activity, non-specific mode of action and safety makes them attractive as antibacterial agents for various applications in medicine, agriculture and food preservation, especially where the usage of conventional antibiotics is undesirable or prohibited. The antimicrobial activity of fatty acids is a function of carbon chain length as well as the presence, number, and orientation of double bonds (Desbois and Smith 2010). Maximum antimicrobial activity of saturated fatty acids is found in fatty acids 12-carbons in length (Brogden et al. 2011). Unsaturated fatty acids are generally more active than saturated fatty acids of the same length (Zheng et al. 2005). Our results revealing that stearic acid exhibited antibacterial activity, is strongly correlated with Laetitia et al. (2014) who reported that fatty acids are able to fight bacteria. Jubie and Dhanabal (2012) demonstrated that long chain fatty acids exhibited considerable antibacterial activity.

Fischer (2013) proved the evidence of cellular membrane disruption, bacterial cell lysis, and flocculation of intracellular contents by fatty acids. The existence of 9-cis-retinoic acid in the rind received attention, as it is present only in traces in plants (IARC, 1999).

Soignet et al. (1998) reported that 9-cis-retinoic acid may affect certain types of cancer including, acute promyelocytic leukaemia , breast carcinoma, colon carcinoma and neuroblastoma.

Fujimura et al. (1998) cited that the mode of action of 9-cis-retinoic acid as anticancer agent (antineoplastic) emerged from its ability to induce apoptosis in a variety of tumour cell lines.

Mehrdad and Salam (2011) suggested that the application of ascorbic acid may have potential as preservative to inhibit the growth of *E. coli* O157:H7 in food.

The study directed to the utilization of watermelon white rind as a new and promising antimicrobial agent derived from plant source and alternatively minimizing environmental pollution.

CONCLUSION

From the foregoing results, the present work proved that ethanolic and aqueous extracts of watermelon white rind possesses antibacterial activity against *E. coli and Salmonella sp*. Hence, the utilization of white rind may be useful in limiting the environmental pollution. Gas chromatography (GC/MS) analysis characterized the bioactive constituents in the white rind that may contribute to its activity. The associated toxicological study and subsequent clinical trials in animal model should be carried out. Further studies are needed to better evaluate the potential effectiveness of the extracts as antibacterial agents.

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تقييم النشاط المضاد للبكتيريا و تحليل كروماتوجرافيا الغاز لمستخلصات القشرة البيضاء للبطيخ

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تعتبر القشرة البيضاء للبطيخ من المخلفات الزراعية التى تسبب العديد من المشاكل البيئية، و تهدف هذه الدراسة الى انتاج مستخلصات مائية و ايثانولية من القشرة البيضاء و اختبارها كمضاد للبكتيريا مما قد يسهم فى تقليل التلوث البيئى. تم اختبار نشاط المستخلصات كمضادات للبكتيريا باستخدام ثلاث سلالات بكتيرية: اثنين سالبة لجرام ايى كولاى و سالمونيلا، وواحدة موجبة لجرام ستافيلوكوكاس اورياس.

أظهرت النتائج نشاط المستخلصات فى تثبيط النمو البكتيرى لسلالتى ايى كولاى و سالمونيلا ، فى حين ان البكتيريا موجبة لجرام ستافيلوكوكاس اورياس كانت مقاومة لكلا المستخلصين.

أكدت النتائج ان المستخلص الايثانولى أكثر تاثيرا على البكتيريا سالبة لجرام من المستخلص المائى.

وجود مركبات فعالة مثل: الميثيونين، حمض الاسبرتك، جلايسيل د. اسباراجين ، GC-M اكد
تحليل S حامض ريتينوليك، حامض الاستياريك و حامض الاسكوربيك و هذة المركبات تحتوى على مجموعة الكربوكسيل (COOH) التى ساهمت فى النشاط المضاد للبكتيريا للمستخلصات المائية والايثانولية.