J.Agric.Chem.and Biotechn., Mansoura Univ Vol. 6 (6): 167 - 175, 2015

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ETHANOLIC BANANA PEEL AND LEMON SEED EXTRACTS

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ABSTRACT

In the present work, the antibacterial activity of banana peel and lemon seed ethanolic extracts had been evaluated against two gram negative bacteria *Escherichia coli and Salmonella sp.* and one gram positive bacteria *Staphylococcus aureus*. Results showed that banana peel and lemon seed extracts had an inhibitory effect against the used Gram -positive and Gram -negative bacterial strains and that the activity against the bacterial strains was associated with increasing the amount of the prepared extracts. The results of GC-MS analysis ensured the presence of various bioactive compounds. Banana peel extract contained hydroquinone, n-hexadecanoic acid, α-bisabolol, trans-2,4-dimethoxycinnamic, sinapyl alcohol, Cis-13-eicosenoic acid and Phytol. Lemon seed extract contained n-hexadecanoic acid, α-bisabolol, hydroquinone, (S) – (-) citronellic acid, Butylated hydroxytoluene , Ascorbic acid, premethyl and 2,5- dihydroxybenzoic acid. The total antioxidant activity was evaluated and found to be 292.88±3.80 and 291.86 ±6.76 mg AAE/g for banana peel and lemon seed extracts, respectively. The total phenolic content was 76.75±0.84 and 80.62±0.17mg GAE/g for banana peel and lemon seed extracts, respectively. Results showed that banana peel and lemon seed had good antioxidant and antibacterial potential. This study developed an antibacterial formulation that can be used as natural food preservative and would scope for future utilization of the waste products for therapeutic purpose.

Keywords: Banana peel, lemon seed, total antioxidant activities, total phenolics, antibacterial assay, gas chromatography mass spectrometer analysis

INTRODUCTION

Fruit peels and seeds are thrown into the environment as agro waste. Their utilization in preparing new antibacterial agents will be economic and reduce pollution. The peels of the fruit are very rich in bioactive components, which are considered to have a beneficial effect on health, they contain various antioxidant compounds (Raza, 2014). These peels are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which include antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral activities (Genitha Immanuel, 2014).

Banana (Musa paradaisica) is grown worldwide and consumed as ripe fruit or used for culinary purposes. Peels form about 18-33% of the whole fruit and are not being used for any other purposes. It is thus significant and even essential to find applications for these peels as they can contribute to real environmental problems (Zhang *et al.,* 2005). The manipulation of food processing wastes is now becoming a very serious environmental issue. Potential applications for banana peel depend on its chemical composition.

Banana peel is rich in dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids and potassium (Emaga *et al*., 2007). Banana and tomato peels have been reported to be a good source of carotenoids (Baysal *et al.*, 2000). Reports are also available on medicinal benefits of banana peel extract which include relief from pain, swelling, itching, bruising, wrinkles and sunburn (Edwards, 1999). However, there is limited information about the nutritional composition and antioxidant activities of banana peel (Emaga *et al.,* 2008 and Gonzalez-Montelongo *et al*., 2010).

Banana peels have been shown to be a good suppressor of food-borne pathogens including *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis and Escherichia coli* (Mokbel and Hashinaga, 2005) and could potentially be applied into food systems in the future. Devatkal *et al*. (2014) also added that the preservative capability of banana peel water extract from similar banana variety in reducing lipid oxidation process in raw meat was comparable to synthetic antioxidant such as butylated hydroxy toluene (BHT). Banana peel demonstrated the presence of various phenolic compounds such as gallocatechin and anthocyanins like peonidin and malvidin. Phenolic compounds are secondary metabolites, which have been associated with flavour and colour characteristics of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (Kaur and Kapoor, 2001).

The citrus peels are rich in nutrients and contain many phytochemicals that can be efficiently used as drugs or as food supplements. Since there is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative drug that is regarded as safe.

Properly dealing with discarded products can reduce the potential for environmental pollution. Recent research revealed that fruit peels and seeds (Prasad *et al.,* 2010) may potentially possess antimicrobial property, representing a potential area of future investigation. This study monitored the antibacterial and antioxidant activities of ethanolic extracts of banana peel and lemon seed, as well as identified the chemical constituents of these wastes, allowing them to be used as natural safe preservatives.

MATERIALS AND METHODS

Plant material

Banana peel and lemon seed were collected from juice stores in Cairo and Giza Governorates during the year 2014. Lemon seed was air dried for several days. Banana peel was washed with distilled water, dried in the oven at 40°C. Seed and peel were powdered using a lab grinder and stored at 4°C till use.

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.

Preparation of the ethanolic extracts

Ethanolic extracts of banana peel and lemon seed were prepared by soaking the powdered peel and seed in ethanol 90%, then stirring using a magnetic stirrer for 4 hours. The extracts were filtered and the solvent evaporated. The residues were then collected and stored at 4 °C until used.

Antibacterial activity of the ethanolic extracts

The antibacterial activity of the ethanolic extracts was carried out by well diffusion method (William, 1989). Mueller-Hinton agar was the selected media for preparing the test plates. 100 µl of the microbial suspension was taken and spread onto Mueller-Hinton agar. 50-500 µl of the extracts were placed in holes in the agar layer inoculated with the above mentioned bacteria. Negative control (Masih et al., 2012) was included using ethanol 90%. 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.

Determination of total antioxidant activity of the extracts

The total antioxidant capacity of the fractions was determined by phosphomolybdate method according to the procedure described by (Prieto *et al.,* 1999) using ascorbic acid as a standard. The total antioxidant activity was expressed as milligrams of ascorbic acid equivalents per gram of dry weight (mg AAE/g DW).

Determination of the total Phenolic content of the extracts

The total phenolic content in the extracts was determined by spectrometry using ‘‘Folin Ciocalteu’’ reagent assay (Singleton *et al.,* 1965). Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

GC-MS analysis of the extracts

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. 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.

RESULTS AND DISCUSSION

Tables (1) and (2) documented the antibacterial activities of banana peel and lemon seed at different volumes (50-500µl) of the ethanolic extracts against the tested organisms. The extracts of banana peel and lemon seed were found to inhibit the Gram-negative bacteria *E. coli and Salmonella Sp.*

as well as the Gram-positive bacteria *Staphylococcus aureus*. Control (ethanol 90%) had no inhibitory effect on the studied bacterial strains.

Also, it was clear that the activity of the extracts increased by increasing the volume taken and 500 µl gave the highest inhibition zone diameters in both extracts.

500 µl of the ethanolic extract of banana peel produced remarkable clear zone of inhibition valued 20 mm, 20 mm and 15 mm against *E. coli, Staphylococcus aureus* and *Salmonella sp.,* respectively. Lemon seed ethanolic extract produced zone of inhibition of 18 mm, 17 mm and 14 mm against *E. coli, Staphylococcus aureus* and *Salmonella sp.,* respectively.

Table (1) Antibacterial activity of ethanolic banana peel extract against the used bacterial strains

Inhibition zone (mm) / bacterial strains	Banana peel extract				
	50µl	**100µl**	**200µl**	**400µl**	**500µl**
E. coli	12	14	16	20	20
Staphylococcus aureus	11	12	15	15	20
Salmonella sp.	10	12	14	14	15

Table (2) Antibacterial activity of lemon seed ethanolic extract against the used bacterial strains

Inhibition zone (mm) / bacterial strains	Lemon seed extract				
	50µl	**100µl**	**200µl**	**400µl**	**500µl**
E. coli	12	13	15	17	18
Staphylococcus aureus	10	11	12	16	17
Salmonella sp.	6	6	7	8	14

As the plants produce secondary metabolites to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants. The extracts showed a significant antibacterial activity against all the tested organisms, indicating the presence of active phytochemical antioxidant compounds (Mohamed *et al*., 2011).

The total antioxidant activity (TAA) of the extracts is shown in Table (3). The obtained results indicated that the total antioxidant activity valued 292.88±3.80 and 291.86 ±6.76 mg AAE/g for banana peel and lemon seed extracts, respectively. Antioxidants exert their action either by scavenging the reactive oxygen species and free radicals and protecting the antioxidant defense mechanisms. The total phenolic content (TPC) of the extracts is shown in Table (3) and has range of 76.75±0.84 and 80.62±0.17mg GAE/g for banana peel and lemon seed extracts, respectively.

Table (3): Total antioxidant activity (TAA) and total phenolic content (TPC) of the extracts

Ethanolic extract	TAA (mg AAE/g DW)	TPC (mg GAE/g DW)
Banana peel	292.88±3.80	76.75±0.84
Lemon seed	291.86±6.76	80.62±0.17

Phenolic compounds, often present in plant extracts, have antioxidant activity (Emanuel *et al.,* 2011) and play a major role in reducing lipid oxidation and may contribute to antioxidative action. TÜrkoglu *et al.* (2007) suggested that polyphenolic compounds had inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables.

GC-MS analysis of the chemical components of banana peel and lemon seed extracts is shown in Fig. 1-2 and Table (4). The GC-Ms analysis of banana peel and lemon seed extracts showed that the common components in both extracts are Hexadecanoic acid (palmitic acid), α–bisabolol and Hydroquinone. Hexadecanoic acid had known antioxidant and antibacterial properties, cytotoxicity to human leukemic cells, and in vivo antitumor activity in mice (Zhang *et a*l., 2014). α-bisabolol had been reported to have strong bacteriocidal effect, antimicrobial and antiseptic effects (Singh *et al.*, 2011). Hydroquinone is a polyphenol compound exerting antioxidant activity (Uthumporn and Ekkalak, 2015). The common chemical compounds identified in the studied extracts are shown in Fig. 3.

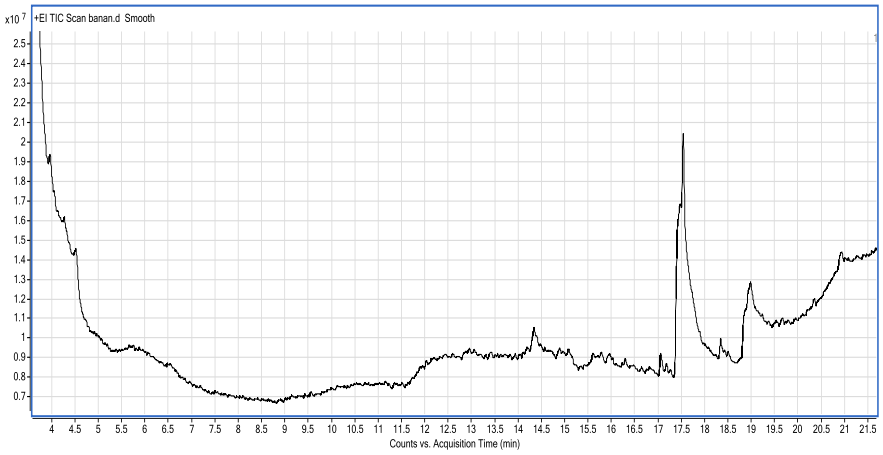

Fig. (1): GC-MS chromatogram of banana peel ethanolic extract

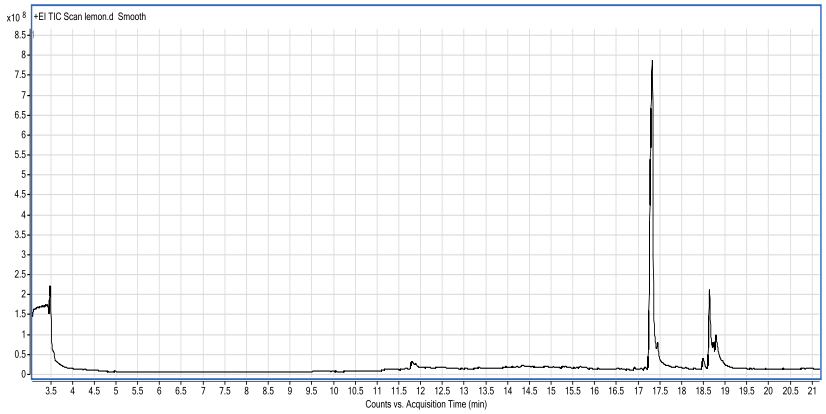

Fig. (2): GC-MS chromatogram of lemon seed ethanolic extract

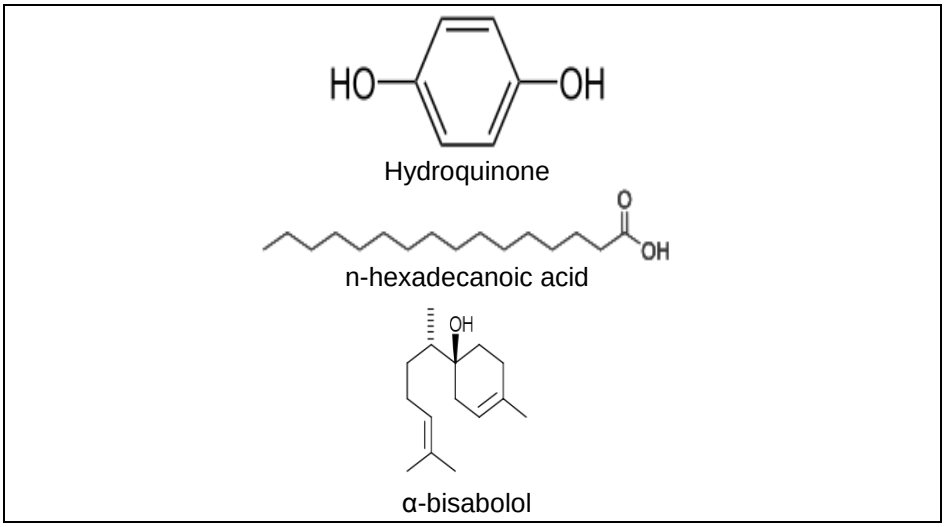

Fig. (3): Common chemical compounds identified in banana peel and lemon seed extracts

Table (4): GC-Mass analysis of the ethanolic extracts of banana peel and lemon seed

Banana peel		Lemon seed	
Component	**Area sum**	**Component**	**Area sum**
Hydroquinone	46.57	n-hexadecanoic acid	77.89
n-hexadecanoic acid	31.63	α – bisabolol	11.34
α – bisabolol	14.46	Hydroquinone	5.29
Trans-2,4dimethoxycinnamic	3.18	(S) – (-) citronellic acid	2.44
Sinapyl alcohol	2.06	Ascorbic acid, premethyl	1.16
Cis-13-eicosenoic acid	1.44	Butylated hydroxytoluene	0.96
Phytol	0.66	2,5- dihydroxybenzoic acid	0.93

The remaining compounds found in banana peel extract were: Trans-2,4-dimethoxycinnamic, Sinapyl alcohol, Cis-13-eicosenoic acid and Phytol. While, the compounds of lemon seed extract were: citronellic acid, Ascorbic acid, Butylated hydroxytoluene and 2,5- dihydroxybenzoic acid. All compounds had known bioactivity as antioxidant and antibacterial agents.

CONCLUSION

The present work showed that wastes such as banana peel and lemon seeds contain various bioactive chemical compounds. The tested extracts had shown appreciate amounts of total phenols which contribute mainly to their antioxidant activity. Also, the extracts were found to inhibit both Gram positive and Gram negative bacterial strains. Further studies could be made on the use of these extracts in food preservation systems.

REFERENCES

Baysal, T.; Ersus, S. and Starmans, D.A.J. (2000): Super critical CO2 extraction of β- carotene and lycopene from tomato paste waste. Journal of *Agricultural and Food Chemistry*, 48(11), 5507-5511.

Devatkal, S.K.; Kumboj, R. and Paul, D. (2014): Comparative antioxidant effect of BHT and water extracts of banana and sapodilla peels in raw poultry meat. *J Food Sci Technol*., 51(2):387-91.

Edwards, B. (1999): Banana peel extracts composition and method for extraction, *Patent No. WO 99/38479*.

Emaga, T.H.; Andrianaivo, R.H.; Wathelet, B.; Tchango, J.T. and Paquot, M. (2007): Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. *Food Chemistry*, 103, 590-600.

Emaga, T.H.; Robert, C.; Ronkart, S.N.; Wathelet, B. and Paquot, M. (2008): Dietary fibre components and pectin chemical features of peels during ripening in banana and plantain varieties. *Bioresource Technology*, 99, 4346-4354.

Emanuel, V.; Adrian V . and Sultana, N. (2011): "Antioxidant and antimicrobial activities of ethanol extracts of Cynara scolymus (Cynarae folium , Asteraceae Family)." *Tropical Journal of Pharmaceutical Research*, 10(6), 777.

Genitha Immanuel, S.S. (2014): "Extraction of antioxidants from fruit peels and its utilization in paneer." *Journal of Food Processing & Technology* 5(7), 5-9.

Gonzalez-Montelongo, R.; Gloria Lobo, M. and Gonzalez, M. (2010): Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. *Food Chemistry*, 119, 1030-1039.

Kaur , C. and Kapoor, H.C. (2001): Antioxidants in fruits and vegetables – the millennium's health. *International Journal of Food Science & Technology*, 36, 703-725.

Masih, U. ; Shrimali, R. and Naqvi, S.M.A. (2012): Antibacterial Activity of acetone and ethanol extracts of Cinnamon (Cinnamomum zeylanicum) and Ajowan (Trachyspermum ammi) on four Food Spoilage Bacteria. *International Research Journal of Biological Sciences*, Vol. 1(4), 7-11.

Mohamed, M.; Moideen, J. and Kumar, S. (2011): "Antimicrobial activity and phytochemical analysis of citrus fruit peels-utilization of fruit waste." *International Journal of Engineering Science and Technology (IJEST)*, 2(4), 5414–5421.

Mokbel, M.S. and Hashinaga, F. (2005): Antibacterial and antioxidant activities of banana (Musa AAA cv. Cavendish) fruit peels. *Am. J. Biochem. Biotechnol,* 1(3), 125–131.

Prasad, K.N.; Xie, H.; Hao, J.; Yang, B.; Qiu, S.; Wei, X.; Chen, F. and Jiang, Y. (2010): Antioxidant and anticancer activities of 8 – hydroxypsoralen isolated from wampee [Clausena lansium (Lour.) Skeels] peel. *Food Chemistry*, 118, 62-66.

Prieto, P.; Pineda, M. and Aguilar, M. (1999): Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry,* 269, 337-341.

Raza, M. (2014): "Free radical scavenging activity in methanolic extract of Musa Paradisiaca peel." *International Journal of Research and Engineering,* 2(1),17–19.

Singh, O.; Khanam, Z.; Misra, N. and Srivastava, M.K. (2011): "Chamomile (Matricaria chamomilla L.): An overview." *Pharmacognosy reviews,* 5(9), 82–95.

Singleton, V. L. ; Joseph, A. and Rossi, Jr. (1965): Colorimetry of Total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic,* 16(3), 144-158.

TÜrkoglu, A.; Duru, M.E. and Mercan, N. (2007): Antioxidant and antimicrobial activity of Russula deliaca Fr: *Eurasian Journal of Analytical Chemistry*, 2(1), 54-67.

Uthumporn, K. and Ekkalak, M. (2015): "The Antioxidant Activity from Hydroquinone Derivatives by the Synthesis of Cinnamomium Verum J.Presl Bark's Extracted." *International Journal of Chemical Engineering and Applications,* 6(2), 91–94.

William, M. O'Leary (1989): Practical Handbook of Microbiology, page 634.

Zhang, P.; Whistler, R.L.; BeMiller, J.N. and Hamaker, B.R. (2005): Banana starch: production, physicochemical properties, and digestibility – a review. *Carbohydrate Polymers,* 59 (4), 443-458.

Zhang, Y.; Xiao, Y.; Wang, P. and Liu, Q. (2014): "Compositions and anti-tumor activity of pyropolyporus fomentarius petroleum ether fraction in vitro and in vivo." *PloS one* 9(10):e109599.

التركيب الكيميائى و النشاط المضاد للاكسدة و للبكتيريا للمستخلصات الايثانولية لقشرة الموز و بذور الليمون

نعمات ابراهيم بسيونى
المركز الاقليمى للاغذية و الاعلاف - مركز البحوث الزراعية - جيزة – مصر

تم تقييم المستخلصات الايثانولية لقشرة الموز و بذور الليمون كمضادات للبكتيريا باستخدام ثلاث سلالات بكتيرية: اثنين السالبة لجرام ايى كولاى و سالمونيلا، وواحدة الموجبة لجرام ستافيلوكوكاس اورياس. اظهرت النتائج نشاط المستخلصات فى تثبيط النمو البكتيرى لايى كولاى ، سالمونيلا و ستافيلوكوكاس اورياس. كانت المستخلصات فعالة ضد البكتيريا السالبة لجرام و الموجبة لجرام و ان هذا النشاط مرتبط بزيادة الكمية الماخوذة من المستخلصات التى تم تحضيرها.

اكد تحليل GC- MS لمستخلص قشرة الموز وجود مركبات فعالة مثل: هيدروكينون، حمض هيكساديكانويك ، الفا-بيسابولول ، ٢,٤-داى ميسوكسسينامیك و الكحول سينابايل. احتوى مستخلص بذور الليمون على هيدروكينون، حمض هيكساديكانويك ، الفا-بيسابولول ، حمض السيترونيليك ،بيوتابلاتد هيدروكسى تولوين ، حامض الاسكوربيك و حمض داى هيدروكسى بينزويك.

كما تم تقدير النشاط الكلى المضاد للاكسدة لكل من مستخلص قشرة الموز و بذور الليمون، وكانت القيم بما يكافئ ٣,٨±٢٩٢,٨٨ و٦,٧٦ ± ٢٩١,٨٦ مجم حمض اسكوربيك لكل جرام وزن جاف على التوالى. كان المحتوى الكلى للفينولات بما يكافئ ٠,٨٤±٧٦,٧٥ و ٠,١٧±٨٠,٦٢ مجم حمض الجاليك لكل جرام وزن جاف على التوالى. اظهرت النتائج ان المستخلصلين لهما نشاط قوى كمضادات للاكسدة.

ساهمت هذه الدراسة فى تقديم تحضيرات مضادة للبكتيريا مستمدة من بعض القشور و البذور مما قد يساعد فى استخدامها كمصدر طبيعى لحفظ الاغذية و تقليل التلوث البيئى الناتج من هذة المخلفات.