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Chemical and biological analysis of essential oils and pectins of banana, cantaloupe peels, guava pulp and formulation of banana pectin gel

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Abstract
The peels of both *Musa paradisicae* var. s*apientum* (banana), *Cucumis melo* L. (Cantaloupe) and pulp of *Psidium guajava* L. (guava) were studied for their essential oils, pectins, antimicrobial and anticancer activities. All tested samples exerted marked activities against Gram +ve, Gram -ve bacteria, fungi, dermatophytes, prostate (PC-3), breast (MCF-7) and colon (HCT-116) carcinoma cell lines. Banana essential oil showed the best activity against all tested micro-organisms, breast carcinoma cell line whereas guava pulp oil exerted the best activity against prostate and colon carcinoma cell line. Banana pectin gels were prepared using Carbopol 934, hydroxyl ethyl cellulose (HEC) and sodium carboxy methyl cellulose (Na CMC) as gelling agents. They were evaluated for their physical properties, anti-microbial and wound healing activities. F6 formulation containing 0.5% carbopol 934 was selected, it has been significantly shown to enhance wound healing compared to panthenol. The stimulation of healing may be due to the immune-modulatory activity of pectin.

Keywords: Banana, cantaloupe, guava, antimicrobial, anticancer gel formulation

1. Introduction
Musaceae, banana family, consists of Zingiberales having spirally arranged leaves, separate male and female flowers and pulpy fruits comprising 6 genera with 45 species (Kumar 2002) [28] while Cucurbitaceae, cucumber family or vine crop family, contains herbaceous annuals or perennials with storage roots and mostly moist vines, rarely grow as trees shrubs or bushes (Kumar 2002) [28] comprising 100 genera and 850 species (Deyo and Malley, 2008) [12] and Myrtaceae, cucumber family, consists of moderate-sized, small trees or shrubs comprising 80 genera and 3000 species (Kumar 2002) [28]. *Musa paradisicae* var. s*apientum* (Banana), *Cucumis melo* L. (Cantaloupe) and *Psidium guajava* L. (guava) are food crops cultivated in tropical and subtropical regions (Kumar 2002) [28]. They are rich in biologically active phytoconstituents like essential oils, sterols/triterpenes, carotenoids, pectins and flavonoids (Abou-ziad 1998; Mittal *et al*., 2010) [1, 30]. Several parts of the plants as leaves, fruits, peels have been investigated for their essential oil components, as limonene, citral, 1,8-cineole were identified in *Cucumis melo* fruit while 6-nonenyl acetate, cinnamyl acetate, nonenol in its peel (Howat and Senter, 1987; Beaulieu and Grimm, 2001; Nattaporn and Pranee, 2011) [21, 6, 32]. Limonene, octanol, *α*-copaene, *α*-humulene, *β*-bisabolene for example have been identified in *Psidium guajava* fruit (Jordan *et al*., 2003; Soares *et al*., 2007) [25, 37], caryophyllene, selinene, viridiflorol, limonene and 1, 8-cineole in its leaves (Karawya 1999; Soliman *et al.*, 2016) [26, 38]. Several biological activities have been previously reported on different parts of the plants as antioxidant activity of *M. paradisicae, C. melo* peels, *P. guajava* fruit and its pulp (Escrig *et al*., 2001; Someya *et al*., 2002; Hassimetto *et al*., 2009; Ismail *et al*., 2010; Calderon *et al*., 2011; Agarwal *et al*., 2012) [16, 39, 19, 23, 11, 4], prostate anticancer and antimicrobial activities of *M. paradisicae* peels and essential oils of *P. guajava* leaves, respectively (Karawya 1999; Akamine *et al*., 2009) [26, 3]. The biological importance and the few phytochemical studies reported on these species growing in Egypt, encouraged the authors to undertake this study.

2. Materials and Methods
2.1. Plant materials
Samples of ripe fruits of banana (*Musa paradisiaca* L. var. *sapientum* Kuntze), cantaloupe (*Cucumis melo* L.) and guava (*Psidium guajava* L.) were collected from trees in gardens, Horticultural Research Station, El-Qanater, Qalyoubia, Egypt during November/December 2012/2013 for banana and guava and March/April 2014/2015 for cantaloupe. The identity of

the fruits was confirmed by Dr. Abdel Halim Abdel Mogali, Agricultural Museum, Giza, Egypt. The peels of banana and cantaloupe and pulp of guava were then air-dried in the shade, reduced to powder and kept in tightly-closed containers. Voucher specimen (Reg. no. 55a, b, c, respectively) were kept in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

2.2 Experimental animals
Adult male rats, of 300 g body weight were obtained from the animal house of National Organization for Drug Control and Research Institute Giza, Egypt. The adopted protocol and implemented experiments were approved by the local Animal Ethics Committee of faculty of Pharmacy, Helwan University, Egypt, and were carried out in accordance to the international Guide for the Care and Use of Laboratory Animals.

2.3 Instruments
Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector with a direct capillary interface and fused silica capillary column HP-5MS (30 m X 0.32 mm X 0.25 µm film thickness) (Agricultural Pesticide Lab, Giza, Egypt), was used for GLC analysis of essential oils, HPLC chromatograph (Win Chrome ver. 1.3 equipped with column KROMACIL C_{18}) (Regional Center for Mycology and Biotechnology, Al-Azhar university, Cairo, Egypt), was used for sugars analysis of pectins, Clavenger apparatus for hydro-distillation of essential oils and Refractometer for identification of refractive indices of essential oils (National Organization for Drug Control and Research, Giza, Egypt). ELISA reader (SunRise, TECAN, Inc, USA) was used for determination of number of viable cells in the cytotoxic activity.

2.4 Chemicals
Authentic sugars: glucose, galactose, rhamnose, xylose and galactouronic acid used in HPLC analysis of pectins were obtained from Regional Center for Mycology and Biotechnology, Al-Azhar university, Cairo, Egypt. All other chemicals, solvents and reagents used in chromatography were of analytical grade.

2.5 Micro-organisms
Staphylococcus aureus (RCMB000105) and *Bacillus subtilis* (RCMB010069); Gram +ve bacteria, *Escherichia coli* (RCMB010054) and *Pseudomonas aeruginosa* (RCMB010048); Gram -ve bacteria, *Aspergillus fumigatus* (RCMB02569), *Candida albicans* (RCMB05038) and *Geotricum candidum* (RCMB05098); fungi, *Trichophyton mentagrophytes* (RCMB0927); as dermatophyte were obtained from Regional Center for Mycology and Biotechnology (RCMB), Cairo, Egypt.

2.6 Cell lines for study of cytotoxicity
Human breast carcinoma cells (MCF-7), Prostate carcinoma cells (PC-3) and Colon carcinoma cells (HCT-116) were obtained from the American Type Culture Collection (ATCC), Rockville, MD. The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamicin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and subcultured two to three times a week**.**

2.7 Investigation of essential oils
The ripe fresh peels of both banana and cantaloupe and the ripe fresh pulp of guava were subjected to hydrodistillation for 3 hours, using a Clavenger-type apparatus. The oils were then collected, dried over anhydrous sodium sulphate followed by GLC/MS analysis (Egyptian Pharmacopeia 1984) [14] (Table 1). Identification of the oils constituents was achieved by Wiley and Nist mass spectral data in addition to the published data in Adams, 2009 [2]. The estimation of each peak was done using a computing integrator adopting the internal normalization procedure.

The collective percentages of different classes of volatile components in essential oils were calculated (Table 2) and the reported biological activities of major volatile compounds identified in banana, cantaloupe and guava essential oils were summarized in table 3.

Table 1: GLC/MS analysis of essential oils of banana, cantaloupe peels and guava pulp

Peak No.	RT	Compound	Mass spectral data			Kovate index	%area		
			M^-	Base peak	Major peaks*		Banana peel	Cantaloupe peel	Guava pulp
1	4.55	Isopentyl acetate	130	70	70,55,61,87	876	3.84	-	-
2	6.22	Butyl butanoate	144	71	71,56,89	994	0.80	-	-
3	10.23	n-Octanol	130	41	56,69,84,98	1068	-	-	0.59
4	10.81	Ethyl benzoate	150	105	105,77,122,150,51	1173	-	-	0.48
5	11.10	Hexyl butanoate	172	71	71,89,56,129	1196	3.31	-	-
6	11.50	Ethyl octanoate	172	88	88,57,101,70,127	1197	-	-	0.35
7	11.95	Hydrocinnamyl alcohol	136	117	117,91,77,65,136	1227	-	-	3.11
8	12.02	Hexyl isovalerate	186	85	85,103,57,69	1244	5.43	-	-
9	12.24	Isoamyl hexanoate	186	70	70,55,99,117,143	1249	9.74	-	-
10	12.66	2-Phenyl ethyl acetate	164	104	104,91,65,78,51	1254	-	-	0.11
11	12.84	2-Undecanone	170	58	58,71,85,170	1294	0.59	-	-
12	13.32	*p*-Vinyl guaiacol	150	135	135,77,107,150,51	1309	3.72	-	-
13	14.18	Eugenol	164	164	164,149,131,103,77	1359	27.60	-	-
14	16.74	Hydrocinnamyl acetate	178	117	117,91,77,65,179	1368	-	-	43.87
15	16.76	*α*-Ylangene	204	105	105, 119, 161, 93	1375	-	0.23	-
16	16.80	*β*-t-Damascenone	190	69	69, 121, 105, 91, 190	1384	-	1.05	-
17	16.95	Ethyl decanoate	200	88	88,101,55,73,157	1395	-	-	0.80
18	17.18	Methyl eugenol	178	178	178,163,147,107,91	1403	2.36	-	0.44
19	17.50	trans-Caryophyllene	204	93	93,133,79,69,105	1417	-	0.50	2.87
20	17.83	trans-Cinnamyl acetate	176	115	115,134,105,176,92	1446	-	-	4.06
21	18.03	*α*-Humulene	204	93	93,80,121,147,107	1454	-	-	0.48
22	18.22	trans-Ethyl cinnamate	176	131	131,103,176,77,147	1467	-	-	1.48

23	18.44	*α*-Curcumene	202	119	119, 132, 105, 202, 145	1480	-	0.42	-
24	18.70	*α*-Muurolene	204	105	105, 161, 204, 119, 91	1500	-	0.36	-
25	18.91	Butylated hydroxy toluene	220	205	205,220,57,145,86	1515	10.63	26.14	18.51
26	19.04	*δ*-Cadinene	204	161	161, 204, 119, 105, 134	1523	-	0.19	-
27	19.09	cis-Calamenene	202	159	159,202,128,144,115	1529	-	-	1.26
28	19.36	*E*-Nerolidol	222	69	69,93,107,81,161	1563	-	-	3.37
29	19.69	trans-Isoelemicin	208	208	208,193,177,165,69	1570	8.06	-	-
30	20.07	Globulol	222	109	109,69,161,81,189	1590	-	-	7.00
31	20.14	Hexadecane	226	57	57, 71, 85, 99, 226	1600	-	1.45	-
32	20.33	Ledol	222	122	122,69,109,81,161	1602	-	-	1.41
33	20.43	Tetradecanal	212	57	57,82,69,96,110	1612	0.65	-	-
34	20.80	*α*-Cadinol	222	95	95,121,204,161,105	1624	-	12.50	0.57
35	20.82	*α*-Muurolol	222	161	161, 43, 119, 204, 105	1646	-	4.95	-
36	20.89	Heptadecane	240	57	57, 71, 85, 99, 240	1700	-	1.12	-
37	20.93	*E*-Isoamyl cinnamate	218	131	131,103,77,147,70	1741	0.35	-	-
38	21.04	2*E*,6*E*-Farnesol	222	69	69,81,93,107,136	1743	-	-	0.61
39	21.86	Ethyl myristate	256	88	88,101,55,157,213	1795	-	-	0.39
40	22.52	n-Octadecane	254	57	57, 71, 85, 99, 254	1800	-	0.46	-
41	22.76	Isoamyl dodecanoate	270	70	70,55,183,201	1845	1.18	-	-
42	22.77	n-Nonadecane	268	57	57, 71, 85, 99, 268	1900	-	2.41	-
43	22.78	Methyl palmitate	270	74	74,87,143,227,270	1921	0.32	-	0.30
44	23.99	Methyl palmitoleate	268	55	55, 69, 83, 96, 236	1934	-	1.79	-
45	24.15	Ethyl palmitate	284	88	88,101,73,157,284	1993	0.68	-	0.76
46	24.44	Methyl linoleate	294	67	67,81,95,109,294	2085	-	4.73	0.24
47	24.48	Ethyl oleate	310	88	88, 101, 55, 70, 157	2196	-	7.36	-
48	25.12	n-Heneicosane	296	57	57,71,85,99,296	2100	0.30	10.96	-
49	26.48	n-Docosane	310	57	57,71,85,99,310	2200	0.38	3.99	-
50	26.58	Tricosane	324	57	57, 71, 85, 99, 324	2300	-	0.48	-
51	27.23	Tetracosane	338	57	57,71,85,99,338	2400	0.89	3.01	0.19
52	28.23	Pentacosane	352	57	57,71,85,99,352	2500	0.94	5.65	0.26
53	29.30	Hexacosane	366	57	57,71,85,99,366	2600	1.33	-	0.28
54	29.42	Heptacosane	380	57	57, 71, 85, 99, 380	2700	-	3.46	-
55	31.03	Octacosane	394	57	57,71,85,99,394	2800	3.01	-	-
56	31.90	Nonacosane	408	57	57,71,85,99,408	2900	3.34	-	-
57	32.71	Triacontane	422	57	57,71,85,99,422	3000	2.51	-	-
58	34.43	Dotriacontane	450	57	57,71,85,97,355	3200	0.74	-	-

RT: Retention time, M^-: Molecular ion peak

Table 2: Collective percentages of different classes of volatile components in essential oils of banana peel, cantaloupe peel and guava pulp

Constituents	Banana peel	Cantaloupe peel	Guava pulp
Hydrocarbons	13.44%	34.71%	5.34%
- Aliphatic	13.44%	33%	0.73%
- Terpenoidal	--	1.71%	4.61%
Oxygenated compounds	79.26%	58.53%	88.45%
- Ethers	8.06%	--	--
- Esters	25.65%	13.89%	52.84%
- Ketones	0.59%	1.05%	--
- Aldehydes	0.65%	--	--
- Alcohols			
• Aliphatic	--	--	3.70%
• Sesquiterpene	--	17.45%	12.96%
- Other oxygenated compounds	44.31%	26.14%	18.95%
Total	92.70%	93.24%	93.79%

Table 3: Reported biological activity of major volatile compounds identified in banana, cantaloupe and guava essential oils

Serial No	Compound	%Area	Biological activity	Reference
1	Eugenol	27.6 (banana)	Antiseptic and anti-inflammatory allowing its use by dentists, antibacterial, antifungal	Bennis *et al.*, 2004 [7]
			protects against cardiovascular diseases by inhibiting the aggregation of platelets	Broadhurst and Duke, 1997 [10]
2	Hydrocinnamyl acetate	43.87 (guava)	Antibacterial, Antifungal	Aziz *et al.*, 2013 [5]
3	Butylated hydroxyl toluene	10.63 (banana) 26.14 (cantaloupe) 18.51 (guava)	Antioxidant	Yehye *et al.*, 2015 [41]
			Antiviral, antimutagenic and anticarcinogenic	Ohno *et al.*, 1984 [33]
4	*α*-Cadinol	12.5 (cantaloupe)	Antifungal	Ho *et al.*, 2011[20]
5	Heneicosane	10.96 (cantaloupe)	Antimicrobial, antitumor	Hsouna and Trigui, 2011[22]

2.8 Investigation of pectins

Powdered peels of both banana and cantaloupe and guava pulp, were separately extracted with hot water (70º-90ºC) for 30 min at a sample-to-water ratio of 1: 20 by magnetic stirring. At the end of extraction period, extract water temperature had reached ambient. The extract was filtered through cotton piece and the filtrate was reduced in volume by rotary evaporation. Pectin was precipitated from the extract by ethanol addition in a ratio of 1: 4 (v/v) (extract : ethanol), allowing to stand for 24 h at 5ºC, the precipitate was removed by centrifugation (10,000 RPM for 20 min), dried in a desiccator over anhydrous calcium chloride then turned to powder (Pazur 1986) [34] and assayed for their degree of esterification, percentage of galacturonic acid and methoxy groups (Bhatty 1993) [8] (Table 4).

2.9 Hydrolysis of pectins

The dried pectins (20 mg) were separately dissolved in 10 ml of 50% ethanol, 10 ml of 2N hydrochloric acid and refluxed on a water bath for 30 min. The aqueous acidic solution was neutralized by sodium carbonate solution then evaporated to dryness by rotary evaporator and the residue was dissolved in 50% ethanol/water and reserved for examination for sugars composition by HPLC (United States Pharmacopeia 2011) [40].

2.10 Antimicrobial study

Antimicrobial study was carried out using the agar disc diffusion method (Scott 1989) [36]. Samples were individually tested against a panel of Gram-positive and Gram-negative bacterial pathogens, yeast and fungi. Pathological tested bacteria, yeast (1 x 106 CFU/ml) and fungi (1 x 104 spore/ml) were spread on nutrient agar (NA), Sab. dextrose agar (SDA) and malt extract agar (MA), respectively. After the media had cooled and solidified, wells (6 mm in diameter) were made in the solidified agar and loaded with 100 µl of samples. The inoculated plates were then incubated for 24 h at 37ºC for bacteria and yeast, 48 h at 28ºC for fungi. Ampicillin, gentamycin and amphotericin B were used as standards for Gram-positive bacteria, Gram-negative bacteria and fungi, respectively, as positive controls and DMSO without the extracts was used as a negative control. After incubation, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm). The experiment were performed in triplicate and the data was expressed as mean ± SD. Minimum inhibitory concentrations (MIC) was determined using the broth micro-dilution method using 96-well micro-plates (Saini *et al.*, 2005; Bhuiyan *et al.*, 2011) [35, 9] (Table 5).

2.11 Cytotoxic study

Potential cytotoxicity was evaluated using viability assay (Mosmann 1983; Gangadevi and Muthumary, 2007) [31, 17]. The cytotoxic activity was evaluated on PC-3 (prostate carcinoma cell line), MCF-7 (breast carcinoma cell line) and HCT-116 (colon carcinoma cell line). Tumor cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamicin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well micro titer plate incubated for 24 h at 37ºC in a humidified incubator with 5% CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100µl from different dilutions of tested sample in fresh maintenance medium and incubated at 37ºC. A control of untreated cells was made in the absence of tested sample. A positive control containing Doxorubicin drug was also tested as reference drug for comparison. Six wells were used for each concentration of the test sample. Every 24 h the observation was made under the inverted microscope. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and the absorbance at 590 nm was read using ELISA reader after well mixing. The absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using ELISA reader as previously mentioned and the percentage of viability was calculated. The 50% inhibitory concentration (IC_{50}) (the concentration required to cause toxic effects in 50% of intact cells) was estimated from graphic plots (Table 6).

Table 4: Analysis of pectins content in banana, cantaloupe and guava

Parameter	% Pectin	DE	% GA	% MG
Banana peel	3.5	95.2	81.52	12.40
Cantaloupe peel	5.75	92.3	88.32	13.00
Guava pulp	4.6	87.5	77.64	10.85

DE: Degree of esterification, GA: galacturonic Acid, MG: Methoxy groups

Table 5: MIC values (µg/ml) for oils and pectins of banana, cantaloupe and guava

Tested microorganism	Banana peel		Cantaloupe peel		Guava pulp		Standard
	Oil	Pectin	Oil	Pectin	Oil	Pectin	
G+ve bacteria							Ampicillin
S. aureus	15.63	15.63	31.25	31.25	31.25	250	3.9
B. subtilis	3.9	15.63	3.9	15.63	7.81	125	1.95
G-ve bacteria							Gentamicin
E. coli	3.9	7.81	15.36	7.81	31.25	125	31.25
P. aeruginosa	3.9	15.63	62.5	31.25	125	250	125
Fungi							Amp.B
A. fumigatus	15.63	31.25	31.25	62.5	15.63	500	3.9
C. albicans	15.63	125	62.5	250	125	1000	3.9
G. candidum	3.9	125	7.81	125	15.63	500	1.95
Dermatophyte							Amp.B
T. mentagrophytes	500	500	1000	500	1000	NA	31.25

G: Gram reaction, *S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, A. fumigatus: Aspergillus fumigatus, C. albicans: Candida albicans, G. candidum: Geotricum candidum, T. mentagrophytes: Trichophyton mentagrophytes,* Amp. B: Amphotericin B, NA: no activity.

Table 6: Potential cytotoxicity expressed as IC_{50} for oils and pectins of banana, cantaloupe and guava

Cell Line	Plant	IC_{50}		
		Essential oil	Pectin	Standard (Doxorubicin)
Prostate tumor cell line (PC-3)	Banana	9.49	44.3	0.71
	Cantaloupe	21.5	36.4	
	Guava	5.1	43.2	
Breast tumor cell line (MCF-7)	Banana	5.49	>50	0.44
	Cantaloupe	11.6	44.1	
	Guava	5.89	>50	
Colon tumor cell line (HCT-116)	Banana	11.7	>50	0.46
	Cantaloupe	35.6	48.1	
	Guava	8.67	>50	

2.12 Preparation of banana pectin gels

Different gels were prepared using Carbopol 934 (0.25 and 0.5%), HEC (3 and 5%) and Na CMC (3 and 5%) as gelling agents (Table 7). The polymer was dispersed into a beaker containing the calculated amount of purified water and stirred using a magnetic stirrer until no lumps were observed. Stirring speed was reduced to break the foam and propylene glycol (10%) was added followed by pectin. For gels containing Na CMC, the polymer was dispersed in the purified water and kept in a refrigerator overnight. In formulae containing carbopol 934 adequate amount of triethanolamine was added to neutralize the free carboxylic acid groups of carbopol 934 to pH 6.5 - 7 ± 0.2 (Lucero *et al.,* 1994) [29].

2.13 Evaluation of the prepared gels

The prepared formulations were examined for their physical characteristics namely; color, clarity, homogeneity and phase separation, spreadability, rheological properties, anti-microbial and wound healing activities (Tables 8, 9, 10).

Test for spreadability

Spreadability was determined using an adapted in-house apparatus (Jain *et al.,* 2007) [24]. It consists of a wooden block provided by a pulley at one end. The spreadability was measured on the basis of “slip” and “drag” characteristics of the gels. A ground glass slide was fixed on this block, excess of gel (about 2 g) under study was placed on it. The gel was sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with a hook. A weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to a pull of 20 g weight with the help of a string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. Spreadability (S) was calculated from equation $S = M.L/t$ where M is the weight (g) tied to the upper glass slide, L is the length (cm) moved on the glass slide, and t is time (sec) (Table 8).

Assessment of rheological properties

The rheological properties were evaluated using a rotational Brookfield viscometer of cone and plate structure (Ekong *et al.,* 2001) [15]. About 0.5 g of the tested formula was applied to the plate and left until the temperature of the cone reached (25ºC±1ºC). Measures were taken over a large range of shearing rates (from 0.75 to 1875 sec $^{-1}$) corresponding to 0.1 to 250 rpm. The viscosities and degree of pseudoplasticity (Farrow's constant) were determined. To study the flow behavior of different gel bases, apply Farrow's equation (Dolz *et al.,* 1988) [13] Log G = N Lof F – Log ɳ where G is shear rate (sec-1), F: is shear stress (dyne/cm2), ɳ is viscosity (c.p.), N: is Farrow's constant.

Evaluation of wound healing process

This activity was evaluated using excision wound method (Khan *et al.,* 2014) [27]. Rats were anaesthetized with (300 mg/ kg body weight) of chloral hydrate via intraperitoneal injection. The dorsal surface of rat was shaved, cleaned with 70% ethanol. Excision wounds were made by cutting out a predetermined dorsal area (approximately 22 mm diameter) of skin from the shaved area using toothed forceps and pointed scissors. The entire wound was left open. Adult male rats were randomly allocated into 3 groups. Each group consisted of 6 rats. The tested formula 6 that showed the best antimicrobial activity (Table 9) was topically applied after excision of the skin and continued daily for successive 21 days. One ml of formulated gel was applied topically to each animal once a day. All groups of animals were treated in the similar manner. The animals were treated according to the following scheme:

Group1: Rats having wounds were kept untreated, served as control group.

Group 2: Rats having wounds treated topically with gel formula 6 of banana pectin (1 mg/ml) applied after excision of the skin.

Group 3: Rats having wounds treated topically with panthenol 5% emulgel applied after excision of the skin, served as reference.

The rate of wound contraction was measured as percentage reduction of wound size at every other day from each rat wound until wound closure. Progressive decrease in the wound size was monitored periodically using transparency paper and a marker. The wound area was assessed graphically to monitor the percentage of wound closure which indicates the formation of new epithelial tissue to cover the wound. Wound contraction was expressed as reduction in percentage of the original wound size (Table 10).

Table 7: Composition of pectin gels formulated with different polymers

Formula No.	C934 (%)	PG (%)	Pectin (%)	HEC (%)	Na CMC (%)	TEA
1	-	10	0.1	3	-	-
2	-	10	0.1	5	-	-
3	-	10	0.1	-	3	-
4	-	10	0.1	-	5	-
5	0.25	10	0.1	-	-	q.s
6	0.5	10	0.1	-	-	q.s

N.B: Water was added to make 100 g of each gel formula.
HEC: hydroxyl ethyl cellulose, Na CMC: sodium carboxy methyl cellulose,
PG: propylene glycol, TEA: Triethanolamine

Table 8: Physical evaluation of the prepared gels

Formula code	Appearance	pH ± SD*	Spreadability (Mean ± SD, n=3)	η at Minimum Shear Rate (cp)	η at Maximum Shear Rate (cp)	Farrow's Constant
F1	Pale brown clear homogenous gel	5.31 ± 0.012	13.6 ±0.5	1743	636	1.75
F2	Pale brown clear homogenous gel	5.03 ± 0.014	11.4 ±0.4	2975	835	2.15
F3	Pale brown clear homogenous gel	6.78 ± 0.026	16 ±0.35	1967	738	1.52
F4	Pale brown clear homogenous gel	6.75 ± 0.019	14.1 ±0.52	2712	1025	1.8
F5	Pale brown clear homogenous gel	5.70 ± 0.031	14.6±0.42	11145	196.4	2.52
F6	Pale brown clear homogenous gel	6.00 ± 0.028	12.4±0.39	20844	263	2.91

Table 9: Antimicrobial activity of the prepared formulations

Formula	Tested microorganism	
	Staphylococcus aureus Zone of inhibition (mm) (mean ± SD, n = 3)	*Trichophyton mentagrophytes* Zone of inhibition (mm) (mean ± SD, n = 3)
F1	15.2 ± 0.63	13.3 ± 1.2
F2	16.8 ± 0.58	15.3 ± 0.58
F3	15.2 ± 0.72	14.3 ± 1.5
F4	16.4 ± 1.5	15.2 ± 0.63
F5	16.2 ± 0.58	14.2 ± 0.72
F6	17.2 ± 0.72	16.1 ± 1.2
Ampicillin	26.2 ± 1.5	-
Amphotericin B	-	20.3 ± 1.2

3. Results and Discussion

Regarding to essential oils, the yield was 0.106%, 0.025% and 0.017% with refractive indices 1.401, 1.523 and 1.477 for banana peel, cantaloupe peel and guava pulp, respectively. The hydrocarbon fractions constituted 13.44%, 37.71% and 5.34% and the oxygenated fractions constituted 79.26%, 58.53% and 88.45% of banana peel, cantaloupe peel and guava pulp, respectively with eugenol (27.6%), butylated hydroxy toluene (26.14%) and hydrocinnamyl acetate (43.87%) as the most abundant constituents of banana, cantaloupe and guava, respectively.

Pectins yield were 3.5%, 5.75% and 4.6% for banana peel, cantaloupe peel and guava pulp, respectively. Cantaloupe peel had the highest percentage of galacturonic acid (88.32%). Besides galacturonic acid, both banana peel and guava pulp contained L-rhamnose, glucose and galactose in their pectin composition while the pectin of cantaloupe peel consisted of xylose and glucose.

Our data revealed that essential oils and pectins of all studied parts exerted marked effects against Gram +ve, Gram -ve bacteria, fungi and dermatophytes. Only guava pulp had no antidermatophyte activity. Banana oil showed the best activity against all tested microorganisms. Banana oil and pectin showed the same MIC against *Staphylococcus aureus* (15.63 µg/ml) and *T. mentagrophytes* (500 µg/ml). Both oils of banana (3.9 µg/ml) and cantaloupe (15.36, 62.5 µg/ml) and both pectins of banana (7.81, 15.63 µg/ml) and cantaloupe (7.81, 31.25 µg/ml) showed anti-Gram -ve bacterial activity better than standard gentamicin (31.25, 125 µg/ml). Pectins of both banana and cantaloupe showed the same MIC against *E. coli* (7.81 µg/ml) followed by cantaloupe oil (15.36 µg/ml).

All tested samples showed an activity against prostate (PC-3), breast (MCF-7) and colon (HCT-116) carcinoma cell lines. Essential oil of guava pulp exerted the best activity against prostate carcinoma cell line PC-3 (IC_{50}= 5.1µg/ml) and colon carcinoma cell line HCT-116 (IC_{50}= 8.67µg/ml) although essential oil of banana oil exerted the best activity against breast carcinoma cell line MCF-7 (IC_{50}= 5.49 µg/ml).

The study of essential oils, pectins composition, antimicrobial and cytotoxic activities are reported for the first time for the investigated waste parts of these three species, however in previous reports other parts of the plants have been studied. *P. guajava* fruit extract showed weak antiproliferative effect aganist breast (MCF-7) carcinoma cell line (Garcia-Solis *et al.,* 2009) [18], the methanolic extract of banana peel showed an inhibition of cell growth of prostate cancer cells which was due to an antiandrogen effect (Akamine *et al.,* 2009) [3] and wasn't as a result of cell cytotoxic effect as in case of our study. From these three species cultivated in Egypt only *P. guajava* leaves were studied for its oil composition that revealed that hydrocarbons composition were higher than oxygenated constituents (61.92%, 33.93%, respectively), (Soliman *et al.,* 2016) [38], in contrast to our result for guava pulp oil that revealed the oxygenated compounds (88.45%) were prominent than hydrocarbons(5.34%).

The antibacterial and antifungal activities of banana, cantaloupe, guava oils may be due to the high percentage constituents of eugenol, α-cadinol, hydrocinnamyl acetate, respectively (Bennis *et al.,* 2004; Ho *et al.*, 2011; Aziz *et al.*, 2013) [7,20,5]. The cytotoxic activities of the three oils may be also due to the high concentration of butylated hydroxyl

toluene which was in accordance with previous data (Ohno *et al.*, 1984) [33].

The prepared banana pectin gels were clear, homogenous and pale brown in color. F6 formulation containing 0.5% carbopol 934 showed the best antimicrobial activity and it has been significantly shown to enhance wound healing compared to panthenol. The stimulation of healing may be due to the immune-modulatory activity of pectin.

Table 10: Wound contraction in rats treated with the prepared pectin gels

Time (days)	% of Wound Contraction		
	Control (group I)	Banana Pectin gels (group II)	Panthenol (group III)
3	3.900±0.1517	9.60±1.265	7.40±2.015
6	14.04± 3.382	26.77± 3.361	18.72± 6.811
9	20.28±3.670*	35.74±2.684*	29.14±5.794
12	46.96±4.546	81.15±1.090*	58.40±1.833*#
15	66.80±4.465	90.33±0.918*	76.66±2.059*#
18	75.92±3.882	94.50±0.846*	88.00±2.145*

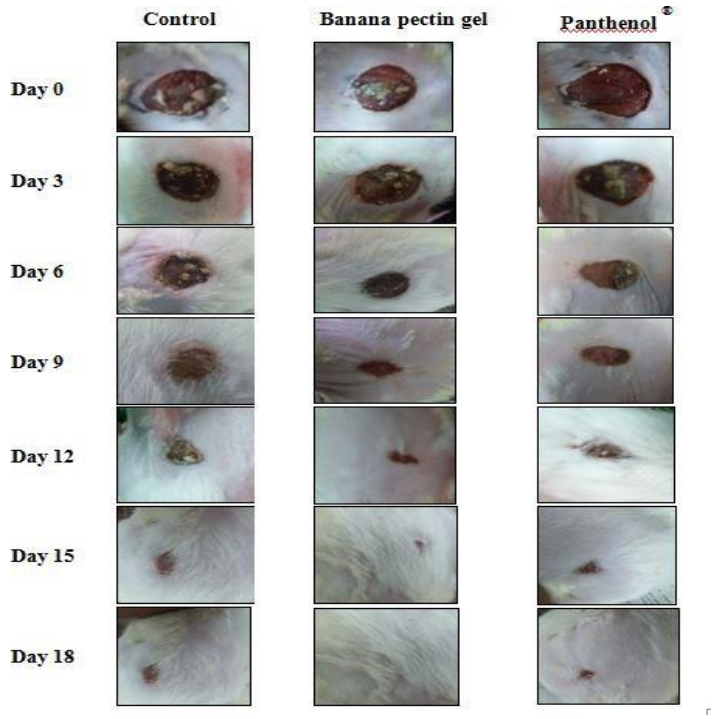

Fig 1: Photographs of macroscopic appearance of wound excised from rats that were untreated (control), treated with pectin gel and treated with panthenol® emulgel

4. Conclusion

This research dealt with the study of essential oils, pectins composition, antimicrobial and cytotoxic activities of the peels of both *Musa paradisicae* var. s*apientum* (banana), *Cucumis melo* L. (cantaloupe) and pulp of *Psidium guajava* L. (guava) which were reported for the first time. Also the present chemical and pharmaceutical evaluation of the banana pectin formulations revealed that F6 formulation has been significantly shown to enhance wound healing compared to panthenol, so it is highly recommended to give importance of the unusable wastes of plants for their economical and medicinal uses.

Authors' Contribution

AMK, RML contributed in collecting plants samples, undergoing the chemical and biological laboratory work, analysis, interpretation of the data and drafted the paper. MET, EGH designed the study, supervised the work, contributed in interpretation of the data and writing the manuscript. MHS contributed in designing and preparing gel formulation. AMGG contributed in carrying out in vivo evaluation of the formula.

All authors have read the final manuscript and approved the submission.

Conflict of interest

The authors declare no conflicts of interest

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