Antimicrobial Activity of Aqueous Extracts Acquired from the Seeds of Two Apples' Cultivars

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Article History: Submitted: 15.12.2019	Revised: 09.01.2020	Accepted: 14.02.2020
ABSTRACT The pathogenic resistance to many standing antimicrobial agents alarms the urgency to explore original agents especially those derived from a natural provenance for managing this issue. In this study, aqueous extracts were prepared from the seeds of two apples' cultivars, Granny Smith and Red Delicious apples. This preparation was performed via two styles, which are non-serial and serially sorted in the increasing polarity. For each style, the extraction was accomblished by three methods including microwave- and ultrasound- aiding extraction techniques, and kinetic maceration. The microbiological studies were conducted on these extracts utilizing four standard bacterial strains (<i>Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli,</i> and <i>Haemophilus influenzae</i>) and two standard fungal strains (<i>Aspergillus niger</i> and <i>Candida albicans)</i> . These in vitro studies were carried out via a well-documented agar disc diffusion method using ciprofloxacin and nystatin as golden references. The results indicated that these extracts showed an	acceptable antimicrobial activity agains primacy to those extracts acquired from Also, the results revealed that the prefer non-serial one and the favoured techni extraction. It is concluded that these ad as antimicrobial preparations with the p a home remedy for local bacterial and fu Keywords: Extraction, Apple seeds, Gran Antibacterial, Antifungal. Correspondence: Yasser Fakri Mustafa Department of Pharmaceutical Chemistr University of Mosul, Iraq E-mail: Dr.yassermustafa@uomosul.edu DOI: 10.5530/srp.2020.2.56	m Red Delicious apple seeds. rable extraction style was the que was the ultrasound-aiding jueous extracts may be useful ossibility of their application as ngal infections. my Smith, Red Delicious, y, College of Pharmacy

INTRODUCTION

The dawn and quick prevalence of pathogenic resistance to many usable antimicrobial agents ring the alarm in the global health community. This is complicated with the inconvenient use of antimicrobials and the development of pathogenic conservation mechanisms (1). Urban history was recorded many events in which pathogens caused wide spread infections which lead to a high mortality rate (2). To avoid the incidence of such episodes, probing new antimicrobial agents becomes an urgent global desire (3).

Plants may be considered as a rich source of natural products which characterized by their significant biological activities. Vast number of these products have been highlighted because of their antimicrobial activity. Currently, there is a developing trend to utilize natural products in the managment of many human illnesses including those caused by infectious pathogens; this is primarily due to the saftey and efficiency profiles of these products.

The link between the intake of diet rich in fruits and the improvement of human health is widely highlighted in literature. However, the attention is mostly focused on the editable parts of fruits rather than their waste products (4). Among editable fruits, apples are the fourth devoured one worldwide and their consuming is highly advisable because of their richness in beneficial phytochemicals (5).

Red Delicious is an apple cultivar which characterized by its crispy flesh, charming red color, and high nutritional profits (6). In conjunction with the highest amounts of dietary fibers and antioxidants, this apple phenotype is rich in minerals (Iron, Potassium, Calcium) and vitamins (C, A, B6), and poor in sugars and calories (7). Hence, engorging of this apple phenotype is highly advocated in many slimming programs (8).

Granny Smith is a green colored apple cultivar and is usually considered as an icon for a good wellness because

of its significant health benefits and nutritional values (9). As a Red Delicious apple, this cultivar is rich in antioxidants, fibers, vitamins, and minerals. The characteristic differences between these two cultivars are the green one has the more calories, fat, and proteins than the red apple (10).

In general, the ingestion of apple seeds may result in weak harmful effects mainly due to the presence of a humble amount of amygdalin, a cyanogenic glycoside. This amount is comparatively insignificant (0.6 mg/g of dry seeds) and ineligible to cause toxic effects except if a very high amount of seeds has been taken (11).

According to the our view established in December 2019, the efforts to find any data about the antimicrobial activity of the seeds of Red Delicious and Granny Smith apples have failed. This stimulated the authors to initiate this study.

The goal of this work is to test the antibacterial and antifungal activities of aqueous extracts acquired from the seeds of the previously mentioned apple cultivars. The extraction was verified via two extraction styles and each of them were performed in three different techniques.

MATERIALS AND METHODS

Solvents, reagents and microbiological cultures employed in this study were acquired from Tokyo Chemical Industry and Sigma-Aldrich. Standard bacterial and fungal strains were gained from Microbiologics°. The fruits were purchased from a regional market and their botany identified by experienced scientists from the College of Agriculture and Forestry/ University of Mosul. The instruments used in the extraction process were shaker water bath (SWBR17 SHEL LAB shaking water bath, USA), ultrasonic water bath (40 kHz, 350 W, Power sonic410, Korea), and household microwave oven (Moulinex - MW Steam 23L, MW531070, France).

Preparation of fruits' extracts

Each single fruit of the studied batches was carefully cleaned by washing with tap water and then with distilled water, and manually sliced into four portions by a sharp steel knife. The acquired seeds were shade-dried at room temperature for 15 days, pulverized by a coffee blender, and sieved to afford a fine powder (12).

The extraction was established via two styles; non-seial and serially arranged in the increasing polarity. In a nonserial style, the seed powder (2 g) was extracted with distilled water (20 ml); while in a serial mode, the powdered seed (20 g) was extracted by n-hexane (20 ml) and the extracted mixture filtered and the residue was then extracted with chloroform (10% w/v). These steps were also applied for the next solvents, methanol and water, in the same way (11).

For each style, the extraction process was achieved by three techniques including kinetic maceration (KM), ultrasound-aiding (UAE) and microwave-aiding (MAE) extraction methods. In the KM, the extract mixture was kinetically macerated at 30°C for 72 hr using a shaker water bath. In the UAE, the extract mixture was sonicated at 30°C for 30 min using an ultrasonic water bath; while in the MAE, the extract mixture was irradiated at 100 W for 5 min using a home microwave oven (11).

MICROBIOLOGICAL ASSAY

Screening the antibacterial activity

The resultant twelve aqueous extracts were tested for their antibacterial activity against the following standard bacterial strains: Klebsiella pneumonia (Kp, ATCC 700603), Pseudomonas aeruginosa (Pa, ATCC 27853), Escherichia coli (Ec, ATCC 25922), and Haemophilus influenzae (Hi, ATCC 49247). This tes was carried out via a well-documented Mueller-Hinton agar disc diffusion method using ciprofloxacin as a golden reference (10 µg/disc), and distilled water as a negative control. Shortly, The test bacteria were pre-cultured in nutrient broth (5 ml) for 16 hr at 37°C and their turbidity was adjusted to 0.5 McFarland standard by normal saline to give a final inoculum of 1.5×10^8 CFU/ml. Discs of 2 mm prepared from Whattman's No. 3 filter papers were steeped with the aqueous extracts of the ratio 10% or 5% (w/v %). Under sterile conditions, a molten agar (20 ml) mixed with precultured bacterial broth (100µl) was poured into a petri plates. upon solidification, the prepared discs were seeded over the agar using sterile forceps in the range of 4 discs for each plate for the prepared extracts. Next to the incubation for 24 hr at 37°C, the zones of bacterial growth inhibition were observed and measured in millimeters via Mitutoyo digital vernier caliper series 500 (USA) (13). The activity index (AI) of the prepared aqueous extracts were calculated via the following mathematic equation: AI = Inhibition zone of extract/ Inhibition zone of reference (14).

Screening the antifungal activity

The antifungal activity of the resultant twelve aqueous extracts was tested against the following standard fungal strains: *Aspergillus niger* (An, ATCC 16888) and *Candida albicans* (Ca, ATCC 10231). The followed procedure was similar to that of examining the antibacterial activity except the nutrient medium for pre-culturing was Potato dextrose broth, the golden reference was nystatin (100 units/disc), culturing medium was Potato dextrose agar and the incubation was performed for 48 hr at 30°C (15).

RESULTS AND DISCUSSION

The accessible and virtual safety of a wide number of natural products support their usage by several ancient and urban cultures to take the benefits of their biological activities (16). The aqueous extracts of many plants are widely employed as a part of the traditional home remedies. This type of extracts can be obtained from different plants' parts including flowers, leaves, fruits, roots, and even seeds (17,18). Since now, this is the first study which considered the antimicrobial activity of the aqueous extracts gained from the seeds of any apple cultivars.

Microbiological investigations

The global hazard of infecious diseases is increasing day by day due to the developed resistance of pathogens to current antimicrobial agents and to the developed defense mechanisms arised over time via these microorganisms. This necissitates the spacious research to find unprecedented antimicrobial agents (19). The antimicrobial activities of many crude plant extracts have been vastly investigated; such plants as mustard, basil, curry, curcumin, cinnamon, and others. Some of these extracts showed a potential antimicrobial activity against many bacterial and fungal pathogenic strains (20).

Antibacterial activity

In general, the tested extracts showed a promising antibacterial activity versus the test bacteria with a supremacy attainded to the extracts of the highest percentage of a phytochemical content (i.e. 10%) (21). Specifically, the results displayed in Tables 1-8 indicated that the best antibacterial activity versus Kp can be refered equally to G2 and R2 (AI= 0.32) while the best activity aganist Pa can be assigned to R2 (AI= 0.39) followed by G2 (AI= 0.38). For Ec, R2 (AI= 0.41) pursued by G2 (AI= 0.40) revealed the preferable activity; whereas for Hi, the prime activity can be connected to R2 (AI= 0.50) supervened by G2 (AI= 0.49).

Table 1: The means of the bacterial growth inhibition zone (mm) \pm SD (n=3) for the reference compound and the aqueous extracts (10%) acquired from the Granny Smith apple seeds.

Bacteria	Reference	G1	G2	G3	G4	G5	G6
Кр	31.72	7.37	10.09	8.86	7.05	9.60	8.49
	± 1.00	±	±	±	±	±	±
		0.94	0.71	0.66	0.95	0.56	0.63
Pa	35.89	13.36	13.57	11.70	13.06	13.01	11.12
	± 0.78	±1.10	±	±	±	±	±
			1.14	1.22	1.03	1.16	1.05

Ec	32.77	12.74	13.10	12.05	12.30	12.69	11.68
	± 0.89	±	±	±	±	±	±
		1.44	0.96	0.52	1.61	0.67	0.71
Hi	27.80	10.84	13.55	9.68	10.38	13.13	9.30
	± 1.23	±	±	±	±	±	±
		1.25	1.37	0.62	1.20	1.15	0.51

G: Granny Smith apple, G1: aqueous extract obtained from KM set on non-serial style, G2: aqueous extract obtained from UAE set on non-serial style, G3: aqueous extract obtained from MAE set on non-serial style, G4: aqueous extract obtained from KM set on serial style, G5: aqueous extract obtained from UAE set on serial style, G6: aqueous extract obtained from MAE set on serial style.

exti	extracts (5%) acquired from the Granny Smith apple seeds.							
Bacteria	Reference	G1	G2	G3	G4	G5	G6	
Кр	31.72	5.99	8.33	5.63	5.61	7.88	5.26	
	± 1.00	±	±	±	±	±	±	
		0.81	0.84	0.51	0.76	0.76	0.46	
Pa	35.89	8.73	11.36	8.69	8.37	10.87	8.19	
	± 0.78	±	±	±	±	±	±	
		0.85	1.21	0.56	0.76	1.11	0.43	
Ec	32.77	9.48	10.03	9.27	9.14	9.57	8.84	
	± 0.89	±	±	±	±	±	±	
		1.06	0.69	0.58	1.09	0.65	0.64	
Hi	27.80	7.94	9.75	7.23	7.59	9.17	6.92	
	± 1.23	±	±	±	±	±	±	
		1.09	1.51	1.25	1.10	1.26	1.36	

Table 2: The means of the bacterial growth inhibition zone (mm) \pm SD (n=3) for the reference compound and the aqueous extracts (5%) acquired from the Granny Smith apple seeds.

Table 3: The values of AI for the aqueous extracts (10%) acquired from the Granny Smith apple seeds versus the test bacteria.

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Bacteria	G1	G2	G3	G4	G5	G6
Кр	0.23	0.32	0.30	0.22	0.30	0.26
Pa	0.37	0.38	0.33	0.36	0.36	0.31
Ec	0.39	0.40	0.37	0.38	0.39	0.36
Hi	0.39	0.49	0.35	0.37	0.47	0.33

Table 4: The values of AI for the aqueous extracts (5%) acquired from the Granny Smith apple seeds versus the test bacteria.

Bacteria	G1	G2	G3	G4	G5	G6
Кр	0.19	0.26	0.18	0.18	0.25	0.17
Pa	0.24	0.32	0.24	0.23	0.30	0.23
Ec	0.29	0.31	0.28	0.28	0.29	0.27
Hi	0.29	0.35	0.26	0.27	0.33	0.25

Table 5: The means of the bacterial inhibition zone (mm) \pm SD (n=3) for the reference compound and the aqueous extracts (10%) acquired from the Red Delicious apple seeds.

Bacteria	Reference	R1	R2	R3	R4	R5	R6
Кр	31.72	7.63	10.30	9.05	7.27	9.76	8.85
	± 1.00	±	±	±	±	±	±
		0.96	0.85	0.66	0.94	0.58	0.75
Pa	35.89	13.60	13.97	12.10	13.37	13.31	11.45
	± 0.78	±	±	±	±	±	±
		1.13	1.01	0.90	0.89	1.20	1.02
Ec	32.77	13.01	13.39	12.25	12.53	12.90	11.88
	± 0.89	±	±	±	±	±	±
		1.38	0.95	0.55	1.61	0.67	0.72
Hi	27.80	11.06	13.80	9.89	10.55	13.40	9.63
	± 1.23	±	±	±	±	±	±
		1.24	13.80	0.68	1.15	1.20	0.40

R: Red Delicious apple, R1: aqueous extract obtained from KM set on non-serial style, R2: aqueous extract obtained from UAE set on non-serial style, R3: aqueous extract obtained from MAE set on non-serial style, R4: aqueous

extract obtained from KM set on serial style, R5: aqueous extract obtained from UAE set on serial style, R6: aqueous extract obtained from MAE set on serial style.

Table 6: The means of the bacterial inhibition zone (mm) \pm SD (<i>n</i> =3) for the reference compound and the aqueous extracts
(5%) acquired from the Red Delicious apple seeds.

Bacteria	Reference	R1	R2	R3	R4	R5	R6
Кр	31.72	6.20	8.43	5.65	5.74	8.00	5.37
	± 1.00	±	±	±	±	±	±
		0.81	0.83	0.62	0.62	0.81	0.45
Pa	35.89	8.90	11.52	8.83	8.53	11.07	8.23
	± 0.78	±	±	±	±	±	±
		0.63	1.14	0.53	0.80	1.05	0.49
Ec	32.77	9.66	10.07	9.36	9.17	9.78	9.06
	± 0.89	±	±	±	±	±	±
		1.13	0.68	0.59	1.16	0.74	0.54
Hi	27.80	8.16	9.89	7.35	7.76	9.40	7.03
	± 1.23	±	±	±	±	±	±
		1.12	1.52	1.11	1.08	1.18	1.43

Table 7: The values of AI for the aqueous extracts (10%) acquired from the Red Delicious apple seeds versus the test bacteria.

Bacteria	R1	R2	R3	R4	R5	R6
Кр	0.24	0.32	0.29	0.23	0.31	0.28
Pa	0.38	0.39	0.34	0.37	0.37	0.32
Ec	0.40	0.41	0.37	0.38	0.39	0.36
Hi	0.40	0.50	0.36	0.38	0.48	0.35

Table 8: The values of AI for the aqueous extracts (5%) acquired from the Red Delicious apple seeds versus the test bacteria.

Bacteria	R1	R2	R3	R4	R5	R6
Кр	0.20	0.27	0.18	0.18	0.25	0.17
Pa	0.25	0.32	0.25	0.24	0.31	0.23
Ec	0.29	0.31	0.29	0.28	0.30	0.28
Hi	0.29	0.36	0.26	0.28	0.34	0.25

Antifungal activity

Commonly, the tested extracts showed a bright antifungal activity against the test fungi with a dominance assigned to the extracts of the towering percentage of a phytochemical content (i.e. 10%) (22). Particularly, the results presented in Tables 9-16 revealed that the best

antifungal activity against An can be attributed to R2 (AI= 0.64) followed equally by G2 and R5 (AI= 0.63) while the best activity against Ca can be attained to R2 (AI= 0.47) supervened by G2 (AI= 0.46).

Table 9: The means of the fungal growth inhibition zone (mm) \pm SD ($n=3$) for the reference compound and the aqueou	us
extracts (10%) acquired from the Grappy Smith apple seeds	

extracts (10%) acquired from the Granny siniti apple seeds.								
Fungi	Reference	G1	G2	G3	G4	G5	G6	
An	12.85	5.90	8.11	7.22	6.30	7.94	7.77	
	± 0.98	±	±	±	±	±	±	
		0.45	0.80	0.77	1.02	0.49	0.78	
Са	18.98	7.61	8.74	8.06	6.96	8.17	7.81	
	± 0.69	±	±	±	±	±	±	
		0.87	0.59	0.50	0.52	0.58	0.46	

Table 10: The means of the fungal growth inhibition zone (mm) \pm SD (*n*=3) for the reference compound and the aqueous extracts (5%) acquired from the Granny Smith apple seeds.

CAL	extracts (570) acquired if off the Oranny Similar apple secus.								
Fungi	Reference	G1	G2	G3	G4	G5	G6		
An	12.85	3.12	4.40	3.51	2.98	4.52	3.48		
	± 0.98	±	±	±	±	±	±		
		0.58	0.53	0.64	0.51	0.48	0.44		

C)a	18.98	3.79	5.49	4.30	4.24	4.01	4.18
		± 0.69	±	±	±	±	±	±
			0.28	0.43	0.58	0.69	0.34	0.61

Table 11: The values of AI for the aqueous extracts (10%) acquired from the Granny Smith apple seeds versus the test fungi.

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Fungi	G1	G2	G3	G4	G5	G6
An	0.46	0.63	0.56	0.49	0.62	0.60
Са	0.40	0.46	0.42	0.37	0.43	0.41

Table 12: The values of AI for the aqueous extracts (5%) acquired from the Granny Smith apple seeds versus the test fungi.

Fungi	G1	G2	G3	G4	G5	G6
An	0.24	0.34	0.27	0.23	0.35	0.27
Са	0.20	0.29	0.23	0.22	0.21	0.22

Table 13: The means of the fungal inhibition zone (mm) \pm SD (n=3) for the reference compound and the aqueous extracts (10%) acquired from the Red Delicious apple seeds.

	(10%) acquired from the red Denelous apple seeds.								
Fungi	Reference	R1	R2	R3	R4	R5	R6		
An	12.85	5.86	8.26	7.34	6.55	8.04	7.88		
	± 0.98	±	±	±	±	±	±		
		0.33	0.77	0.74	0.97	0.45	0.27		
Са	18.98	7.74	8.85	8.20	7.07	8.24	8.11		
	± 0.69	±	±	±	±	±	±		
		0.87	0.59	0.50	0.52	0.55	0.72		

Table 14: The means of the fungal inhibition zone (mm) \pm SD (n=3) for the reference compound and the aqueous extracts (5%) acquired from the Red Delicious apple seeds.

Fungi	Reference	R1	R2	R3	R4	R5	R6	
An	12.85	3.18	4.45	3.56	3.14	4.58	3.53	
	± 0.98	±	±	±	±	±	±	
		0.58	0.55	0.61	0.41	0.40	0.51	
Са	18.98	4.02	5.57	4.35	4.41	4.11	4.22	
	± 0.69	±	±	±	±	±	±	
		0.29	0.45	0.53	0.74	0.33	0.66	

Table 15: The values of AI for the aqueous extracts (10%) acquired from the Red Delicious apple seeds versus the test fungi.

Fungi	R1	R2	R3	R4	R5	R6
An	0.46	0.64	0.57	0.51	0.63	0.61
Са	0.41	0.47	0.43	0.37	0.43	0.43

Table 16: The values of AI for the aqueous extracts (5%) acquired from the Red Delicious apple seeds versus the test fungi.

Fungi	R1	R2	R3	R4	R5	R6
An	0.25	0.35	0.28	0.24	0.36	0.27
Са	0.21	0.29	0.23	0.23	0.22	0.22

Based on the results showed in Tables 1-16, it is proposed that the prepared aqueous extracts may be useful as antimicrobial preparations with the probability of their utilization as a residence medication for topical bacterial and fungal infections.

CONCLUSION

This work reported the success in the aqueous extraction of seeds belong to two apple cultivars, Granny Smith and Red Delicious apples, via three techniques and two styles. Microbiological studies on the resultant extracts revealed the following conclusions: the extracts showed an encouriging antimicrobial activity versus the test microorganisms. The style achieved the best extraction for the phytochemicals with antimicrobial activity was a nonserial one. The extracts acquired by UAE showed the best antimicrobial activity. The antifungal activity of the extracts was better than their antibacterial activity for the test pathogens. Finally, the aqueous extracts from Red Delicious apple seeds showed a better antimicrobial activity than that from Granny Smith apple seeds.

ACKNOWLEDGEMENTS

The authors are very grateful to the University of Mosul/College of Pharmacy for their provided facilities, which helped to improve the quality of this work.

CONFLICT OF INTEREST

There are no conflicts of interest.

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