PHENOLIC COMPOUNDS CONTENT, ANTIOXIDANT, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF RED ONIONS SKIN

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ABSTRACT

This study was aimed to search and identify potentially active antibacterial, anti-oxidants, and antifungal compounds in red onion skin. Phenolic-rich onion extract was obtained from red onion skin extracted with methanol 70% for 2 h. The extract's antioxidant, antibacterial and antifungal activities were determined. The extract demonstrated the maximum DPPH radical scavenging activity (96% ±3.8) at 2000 g / mL, according to the findings of this study. Anthocyanin concentration, total soluble solids (TSS), total phenolics (TPCs), and, total flavonoids (TFs) in tested sample can all be linked to antioxidant ability. For all microorganisms tested, the minimum inhibitory concentration (MIC) of the tested sample was 200 g/mL. A red onion skin methanolic extract obtained ultimate suppression at 1000 g/mL after 7 days of incubation at 25 °C, equating to a 77% linear growth reduction of *Fusarium graminearum*. As a result, the outer skin of the red onion could be employed in nutritional fortification or nutraceutical preparations to improve health outcomes.

Keywords: Onion waste, Flavonoids, Biological activities

محتوى المركبات الفينوليه، ومضادات الأكسدة، والفعاليه المضادة للبكتيريا والفطريات لقشرة البصل الأحمر

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المستخلص

تهدف الدراسة الى البحث والتعرف على مضادات البكتيريا ومضادات الأكسدة والمركبات المضادة للفطريات التي يحتمل أن تكون نشطة في قشرة البصل الأحمر. تم الحصول على مستخلص البصل الغني بالفينول من قشر البصل الأحمر المستخلص بميثانول 70٪ لمدة ساعتين. تم تحديد نشاط المستخلص المضاد للأكسدة والبكتيريا والفطريات. أظهر المستخلص الحد الأقصى لنشاط الكسح الجذري لـ DPPH (96٪ ± 3.8) عند 2000 ميكروجرام/ مل، وفقًا لنتائج هذه الدراسة. يمكن ربط كل من تركيز الأنثوسيانين والمركبات الذائبة الكلية والمركبات الفينولية ومركبات الفلافونويد في العينة المختبرة بقدرتها المضادة للاكسدة. كان الحد الأدنى للتركيز المثبط (MIC) للعينة المختبرة 200 ميكروجرام / مل. أظهر المستخلص تأثير قاتل للفطر عند تركيز 1000 ميكروجرام / مل بعد 7 أيام من الحضانة عند 25 درجة مئوية، وهو ما يعادل انخفاض نمو خطي بنسبة 77 ٪ من *Fusarium graminearum*. نتيجة لذلك، يمكن استعمال القشرة الخارجية للبصل الأحمر في تدعيم المنتجات الغذائية وذلك لتحسين الصحة العامة.

الكلمات المفتاحية: مخلفات البصل، الفلافونويدات، الأنشطة البيولوجية

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NTRODUCTION

Since ancient times, onions have been one of the most popular global vegetable crops (5, 30, 33). Onions have antioxidant, antibacterial, antifungal, antitumor, and anti-inflammatory properties (10) due to their high phytonutrient content, which includes flavonoids and anthocyanins, as well as other health benefits (6, 12). With an annual production of almost 47 million tonnes, onion production has increased by at least 25% in the last 20 years, making it the second most significant horticultural crop (9, 18). More waste has resulted from the rising demand for processed onions. As a result, sustainable agricultural techniques and solutions for recovering vital natural resources are required to deal with the large output of food ingredients and trash (34). The onion skin is rapidly eliminated before processing and sale because it is not edible. It is, however, unsuitable for fodder or landfill disposal due to its composition and odour, and so accounts for the majority of onion processing waste. As a result, valuing onion peel as a "food by-product" is required for long-term Allium processing. Food processing waste generally contains valuable molecules that can be used as useful additives in the food, cosmetics, and pharmaceutical industries (14, 16, 20). Antibiotics' antibacterial potency has been eroding in recent decades as organisms evolve and develop resistance to them. So, novel antimicrobial agents from natural sources are urgently required (23, 24). The regular metabolism of the human body produces reactive oxygen species, which can cause oxidative damage to all of the body's important cellular components and has been linked to a range of disorders, including hypertension, diabetes, cancer, and ageing. Antioxidants have been shown to improve human health. As a result, there is a great demand for new antioxidants produced from natural sources (7, 23). Food protection and waste are major concerns around the world. One-third of all food provided for human consumption worldwide is lost or polluted. One of the most significant causes of this major loss may be postharvest diseases and pollution. Fungi-related post-harvest losses of perishables account for 10% to 50% of total losses worldwide. To combat postharvest diseases and their related rots, synthetic chemical fungicides are still commonly used. However, increased public concern about food safety and human health has had an impact (1, 22). This study's principal objective is to search and identify potentially active antibacterial, anti-oxidants, and antifungal compounds in red onion skin.

MATERIALS AND METHODS

Phenolic rich extract preparation and chemical characterization: Sample preparation: The previously mentioned technique (26) was used to obtain the phenolic-rich onion extract (3). A neighbourhood store provided fresh red onions (*Allium cepa* L.). Fresh onions' outer shell, as well as the leaves, were tightly secluded. In 250 mL of 70% methanol, 100 g onion skin was combined and homogenised; the homogenate was agitated for 2 hours before being filtered through Whatman No. 2 filter paper. Vacuum evaporation at 45°C in a BüCHI-water bathB-480 evaporator separated the methanol from the extract, which was followed by lyophilization in a freeze dryer (Thermo-electron Corporation–Heto power dry LL 300 Freeze drier).

Estimation of total phenolic compounds (TPCs): TPCs were determined in the onion skin methanolic extract (1 mg/mL) using a Foline-Ciocalteu reagent (diluted with water 1:10, V/V) as previously described (19), 5 mL diluted Foline–Ciocalteu reagent plus 4 mL sodium carbonate (75 g/L) plus one millilitre of the sample The tubes were vortexed for 15 seconds before being left at room temperature for 30 minutes before being tested for absorbance at 765 nm. The standard curve was created using gallic acid at various doses (50-1000 mg/ml). The same steps were done for gallic acid as described for the unknown samples.

Total flavonoids (TFs) estimation

The TFs were estimated according to **Wang et al. (39)**, which entailed combining 2 mL of 20 g/L $AlCl_3$ ethanol reagent with 1 mL of the extract (1 mg/mL) for 1 hour and measuring the colour absorbance at 420 nm. To produce the standard curve, several amounts of quercetin (50-1000 mg/ml) were utilised.

Estimation of total soluble solids (TSS): According to **Horwitz and Latimer (15)** the TSS were estimated.

Anthocyanin determination

The total anthocyanin content in red skin onion methanolic extract were estimated according to **Du and Francis (11)**, where 100 mL of the methanol was added to a five ml of the filtered extract. The colour intensity was recorded at 535 nm using a spectrophotometer. The total content of anthocyanins assigned to cyanidin-3-glucoside was calculated using the following formula:

$$\boldsymbol{Total\ anthocyanin}\ (\boldsymbol{mg/100g}) = ((\boldsymbol{Absorbance\ x\ diluted\ factor})) / (\boldsymbol{sample\ weight\ x\ 5.99})\boldsymbol{x\ 100}$$

Antioxidant activity estimation (DPPH-assay): The antioxidant activity of red skin onion methanolic extract at different concentrations (0-2 mg/ml) was estimated by removing the purple-colored of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution as described by **Ramadan, Osman (28)**. One hundred microliters from each concentration from red onion methanolic extract (0-2 mg/ml) dissolved in methanol were added to 2 mL of DPPH 0.1 mM. After the incubation time (30 min) at room temperature, the absorbance was recorded at 517 nm against a control (DPPH only). The free radical DPPH's antioxidant activity (%) was calculated as follows:

$$\%\ \boldsymbol{inhibition} = [(\boldsymbol{Absorbance\ of\ Control} - \boldsymbol{Absorbance\ of\ Sample}) / (\boldsymbol{Absorbance\ of\ Control})]\mathbf{x100}$$

Antibacterial activity evaluation

2.3.1. Determination of the minimum inhibitory concentration (MIC)

The Kirby-Bauer disk-diffusion method was used to determine the MIC values of red skin onion methanolic extract at different concentrations (0-2 mg/ml) against Gram positive bacteria (*B. subtills* and *S. aureus*) and Gram negative bacteria (*E. coli* and *P. aeruginosa*) **(38)**. The bacterial suspension was swabbed over the surface of Oxoid agar (brain heart infusion) plates. Then, 6-mm diameter filter paper discs were soaked in red skin onion methanolic extract at various concentrations (0-2 mg/ml deionized water) and placed on an agar surface with appropriate spacing between them. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibitory zones (mm) was measured using a millimetre ruler (2).

Examination using transmission electron microscopy (TEM): Before TEM examination, *S. aureus* and *P. aeruginosa* (10^8 CFU/mL) in peptone buffer solution were treated to 200 g/mL red onion skin methanolic extract for 4 hours at 37 °C **(4)**.

Antifungal activity evaluation

Linear growth reduction estimation: The fungal strain (*Fusarium graminearum*) used in this study was obtained from Princess Nourah bint Abdulrahman University's Department of Microbiology, College of Science. The effect of skin red onion methanolic extract at different concentrations (0, 250, 500, and 1000 microgram per millilitre) was tested on the linear growth of *Fusarium graminearum* using PDA media as described in (21). Linear growth reduction was calculated as follow:

$$\textbf{Linear growth reduction}\ (\%) = \frac{[\textbf{Control growth} - \textbf{Treatment growth}]}{[\textbf{Control growth}]\mathbf{x100}}$$

Scanning electron microscopy (SEM)

The comparison of *Fusarium graminearum* treated with red onion skin methanolic extract at 400 g/mL to the control (untreated) was done using SEM as described by (37).

RESULTS AND DISCUSSION

Chemical characterization of phenolic rich extract: The results of TPCs, TFs, anthocyanins, and TSS of skin red onion methanolic extract are presented in **Table 1**. TPC was recorded 612.5 ± 15 µg / mL. Onions contain a variety of phenolic compounds, mostly flavonols, as well as anthocyanins in red varieties (6, 27). The TPC content of red onion skin extract was virtually identical. Another research discovered that the TPC content of red onion skin ranged between 63.62 and 208.42 mg GAE/g, depending on the solvent used to extract them (36). Ethanol and methanol are commonly used in the food industry to produce bioactive compounds since they have no adverse effects on food products or humans (39). Alcohol (ethanol or methanol) as a solvent increases extraction levels more than other organic solvents, according to a report (13). TF was recorded 430.2 ± 10 µg / mL. The same results of TFC was reported by (21). TFC levels in red onion skin were found

to be higher according to (36). Anthocyanin was recorded 7 ± 0.005 mg / 100 gm extract, and TSS was recorded 0.5% ±0.002. Anthocyanins account for around 10% of red onions' total flavonoid content (31).

Table 1. Chemical characteristics of skin red onion phenolic rich extract

Chemical constituents	Concentrations
TPCs (µg / mL)	**612.5 ± 15**
TFs (µg / mL)	**430.2 ± 10**
Anthocyanin (mg / 100 gm extract)	7 ± **0.005**
TSS %	**0.5 ±0.002**

TPCs: total phenolic contents, TFs: total flavonoid contents, TSS: total soluble solids

Antioxidant activity

The DPPH assay was used to estimate antioxidant activity. The idea behind this assay is to donate hydrogen atoms to neutralise DPPH's purple radicals, so that when the absorbance is measured, DPPH's colour is reduced to pale yellow or colourless. Since the antioxidant activity is higher, the samples' capacity to reduce free radicals is also higher (17). The DPPH test was used to determine the antioxidant activity (inhibition %) of red onion skin methanolic extract at different concentrations (100, 200, 500, 1000, 1500, and 2000 µg / mL) as presented in **Fig. 1.** The radical scavenging effect increases with higher concentrations. The extract demonstrated the maximum DPPH radical scavenging activity (96% ±3.8) at 2000 µg / mL. The amount of anthocyanin, TSS, TPCs, and, TFs in tested sample can all be linked to antioxidant ability. These compounds serve as free radical scavengers during the oxidation reaction (35). The extract's antioxidant capabilities are measured in SC_{50} µg / mL (The concentration of the sample that scavenges 50% of the radicals). The SC_{50} value of the methanolic extract of red onion skin was found to be 520 µg / mL. A high amount of antioxidant activity is indicated by low SC_{50} values. (19). The outer skin of onions showed the similar pattern of antioxidant activity (28, 33).

Antibacterial activity

The antibacterial activity of red onion skin methanolic extract against Gram positive bacteria (*B. subtills* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) was determined using the Kirby-Bauer disk-diffusion method at various concentrations (100, 200, 500, 1000, and 2000 g / mL), as shown in **Fig. 2** and **Table 2.**

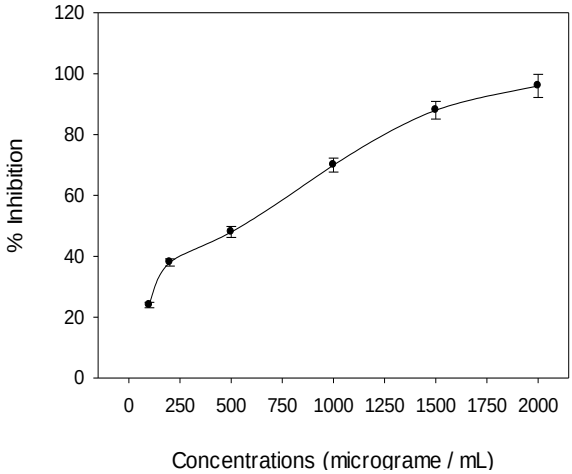

Fig. 1. The DPPH assay was used to determine the antioxidant activity (percent inhibition) of red onion skin methanolic extract at various concentrations (100, 200, 500, 1000, 1500, and 2000 g / mL).

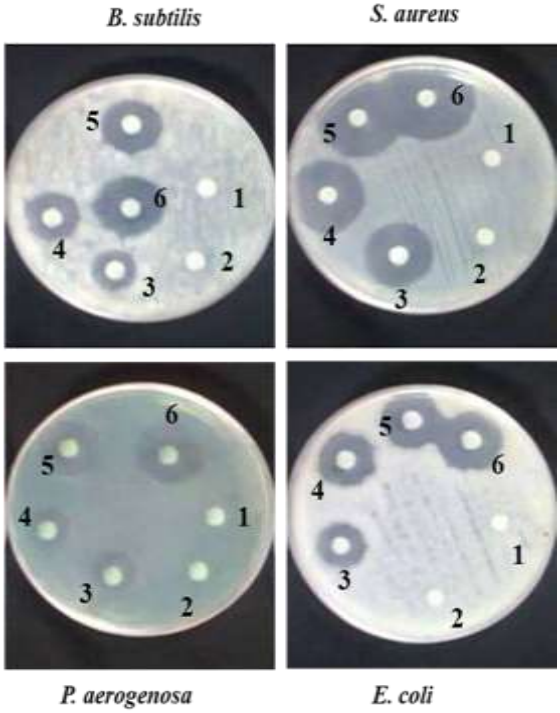

Fig. 2. Antibacterial activity of red onion skin methanolic extract against Gram positive bacteria (*B. subtills* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) at different concentrations (1: 0 µg / mL, 2: 100 µg / mL, 3: 200 µg / mL, 4: 500 µg / Ml

Table 2. The Kirby-Bauer disk-diffusion method was used to test the antibacterial activity of red onion skin methanolic extract against Gram positive bacteria (*B. subtills* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) at different doses (100, 200, 500, 1000, and 2000 µg / mL).

Microorganisms	Inhibition zone diameter (mm; µg / mL)					
	0	100	200	500	1000	2000
	Gram positive bacteria					
B. subtills	0±.00	0±.00	19±.11	24±.19	24±.16	29±.27
S. aureus	0±.00	0±.00	36±.32	39±.82	42±.43	44±.36
	Gram-negative bacteria					
E. coli	0±.00	0±.00	12±.16	19±.19	20±.13	22±.21
P. aeruginosa	0±.00	0±.00	18±.17	19±.14	21±.12	24±.19

The antibacterial activity of red onion skin methanolic extract improved when the concentrations were increased against the bacteria tested. For all microorganisms examined, the MIC of the tested sample was 200 µg/mL. When the antibacterial activity of skin and edible onion bulb extracts was examined, it was revealed that the skin extract (5 µg/100 mL) of red onion inhibited 83.3% *Bacillus cereus* growth, whereas the edible part extract inhibited only 63.3% (32). Onion phenols and flavonoids are thought to have antimicrobial properties, and it's generally known that the onion skin contains more bioactive components than the edible bulb (8). Red onion skin methanolic extract treated bacteria display different signs of cellular deformation in TEM images (Fig. 3), suggesting that red onion skin methanolic extract has a direct disrupting effect on the cell wall and cell membrane.

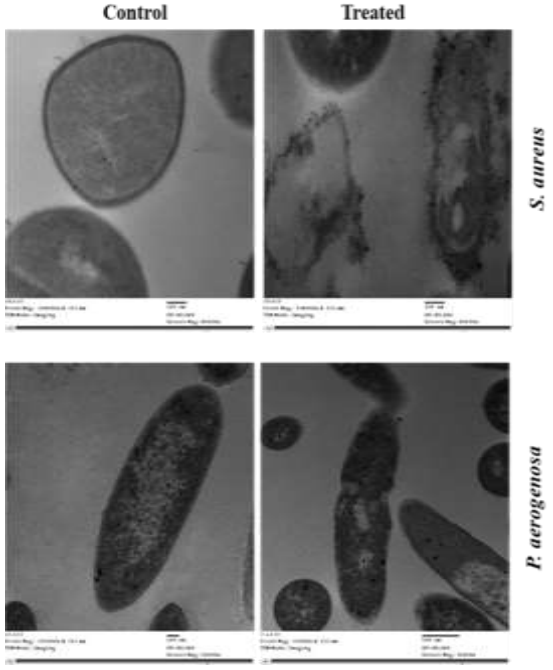

Fig. 3. Transmission electron microscopy (TEM) of *S. aureus* and *P. aeruginosa* at 37 °C for 4 hours after treatment with 200 g/mL of red onion skin methanolic extract

Antifungal activity

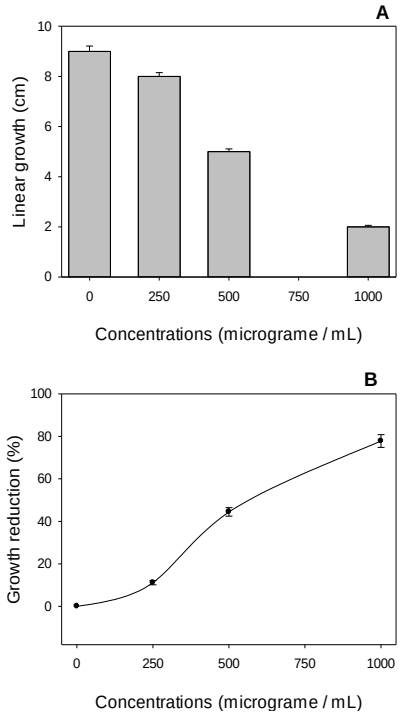

Fig. 4. *Fusarium graminearum* growth as expressed in (A) linear growth (cm) and (B) growth reduction (%) on potato dextrose agar medium petri dishes (C) after incubation at 25 ºC for 7 days with red onion skin methanolic extract at different concentrations (0, 250, 500 and 1000 µg/mL).

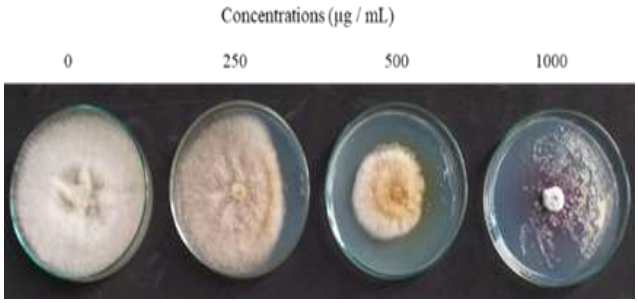

Fig. 5. *Fusarium graminearum* growth on potato dextrose agar medium petri dishes after incubation at 25 ºC for 7 days with red onion skin methanolic extract at different concentrations (0, 250, 500 and 1000 µg/mL).

After incubation at 25 °C for 7 days with red onion skin methanolic extract at different concentrations (0, 250, 500, and 1000 µg/mL), *Fusarium graminearum* growth was measured in (Fig. 4A) linear growth (cm) and (Fig. 4B) growth reduction (%) on potato dextrose agar medium petri dishes (Fig. 5). The methanolic extract of red onion skin inhibited mycelium growth over a wide concentration range (250–1000 µg/mL). After 7 days of incubation at 25 °C, a red onion skin methanolic extract achieved ultimate inhibition at 1000 µg/mL, corresponding to a 77% linear growth reduction. Fig. 6 shows SEM photos of *Fusarium graminearum* treated with red onion skin methanolic extract at 500 µg/mL. Model hyphae with clearly intact walls were found in the control fungus. At the concentration used, extract has completely destabilized and malformed this model. Antifungal activity is attributed to onion anthocyanin, phenols and flavonoids.

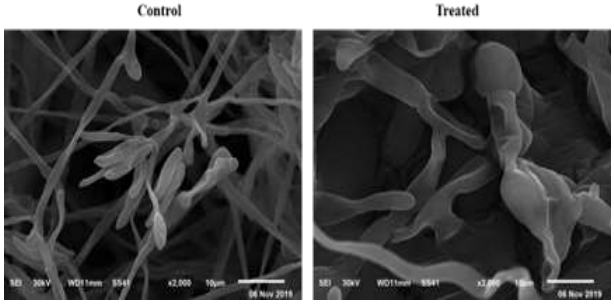

Fig. 6. Scanning electron microscopy (SEM) of *Fusarium graminearum* growth on potato dextrose agar medium petri dishes after incubation at 25 ºC for 7 days with red onion skin methanolic extract at 500 µg/Ml

CONCLUSIONS

Based on the findings, red onion skin methanolic extract, which is high in phenolic compounds and anthocyanins, may be used as an antioxidant, antibacterial, and antifungal agent. It can be used as a clean, natural product effectively and successfully. It can be made at a low cost.

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