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Antimicrobial and antioxidant activities of phytic acid extracted from Wheat Bran

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Abstract

The objective of this study is to investigate the antimicrobial and antioxidant activities of pure phytic acid and phytate extracted from wheat bran. The obtained results showed that, Gram-positive bacteria had more sensitive for phytic acid than Gram- negative bacteria. *Bacillus anthrakoid* and *Staphylococcus aureus* were more sensitive at concentrations of pure phytate 5, 6 %. Inhibition effects in *Escherichia coli O157:H7* and *Staphylococcus aureus* were 20.33 and 38.67 mm, respectively at 6% of pure phytate. Moreover, a moderate inhibition against fungi was observed. Besides the pure phytic acid and phytate extracted from wheat bran have an inhibitory effect on many types of microorganisms. The antioxidant activity of pure phytate increased with increasing the concentration, where, the scavenging activity was 22, 23 and 36% at concentration 1, 3, and 5 mM, respectively. The extracted phytate gave high scavenging activity that reached 90% if compared with pure phytic acid. Aqueous wheat bran extract gave a percentage of free radical scavenging activity reached 36.16%. From the obtained results it can be concluded that phytate extracted from wheat bran has good antimicrobial and antioxidant activity and could be use as natural preservatives.

Keywords:
Antimicrobial, Antioxidant, Phytic acid, phytate, wheat bran

INTRODUCTION

Phytic acid constitutes 1 to 5 % (w w^{-1}, dry weight basis) of most oilseeds, legumes, cereals, nuts, and pollen and represents from 50 to 80 % of the total phosphorus level in seeds. Phosphorus accumulation in developing seeds is higher than that needed for nominal cellular functions. Several plants use the excess phosphorus to synthesize phytic acid (Graf and Eaton, 1990 and Thavarajah *et al*., 2010). Phytic acid may effectively inhibit the growth of foodborne pathogens such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella Typhimurium,* and *Clostridium perfringens* (Zhou *et al*., 2018). Phytic acid has also enhanced the antibacterial effect of nisin against *Listeria monocytogenes* on cabbage and broccoli, significantly reducing bacteria (Bari *et al*., 2005). Phytic acid has(PA) been recognized as a potent antioxidant. It is a potent inhibitor of iron-catalyzed hydroxyl radical formation by chelating the iron required for the generation of hydroxyl radical via the Fenton-type reaction. That PA exhibited an antioxidant effect by suppressing iron (II)-enhanced hydroxyl radical formation induced by1-methyl-4-phenylpyridinium ion (MPP+) in rat striatum. (Graf and Eaton, 1990; Rimbach and Pallauf, 1998 and Obata, 2003). Phytic acid and wheat bran as antioxidants inhibited metmyoglobin formation and stabilized red meat color. In addition, it inhibited lipid peroxidation and degradation of heme pigments caused by cooking and storage. Therefore, they may be useful as additives for meat processing to prevent the off-flavor formation and extension of shelf life (Shatta *et al*., 2005). The phytic acid is an effective antioxidant as it has high chelating capacity of multivalent metal ions such as iron, zinc, and calcium. Phytic acid inactivates the iron's catalytic action and inhibits the hydroxyl radicals ($^{\bullet}OH$) production by forming an iron chelate (Graf and Eaton, 1990). By binding with the six Fe^{3+}coordination sites, phytic acid prevents the reaction between H_2O_2 and chelated Fe^{3+} ion, avoiding iron hydroxyl radical formation. Different examinations have additionally demonstrated the capacity of phytic corrosive to repress lipid oxidation in meat-based foods (Lee and Hendricks,1995 and Park *et al*., 2004). The utilization of phytic corrosive as a reasonable option for the meat industry to further develop industrialized meat items' security and quality, was additionally settled by Stodolak *et al*. (2007) while assessing phytic-corrosive containing arrangements as security extenders for a crude and cooked hamburger and pork meat held under refrigeration, it found that metmyoglobin creation was restrained within the sight of phytic corrosive in crude meat. Therefore, the aim of this study is to investigate the antimicrobial and antioxidant activities of pure phytic acid and phytate extracted from wheat bran.

MATERIALS AND METHODS

Materials

Wheat bran obtained from the mills of Upper Egypt in Sohag Governorate during 2020. Chemicals used in this study were obtained from Pio Chem, Alpha Company, and Sigma – Aldrich (St. Lous, MO, USA).

Microorganisms

The tested microorganisms were summarized in Table (1).

Table (1): Tested microorganisms in the study.

No	Microorganisms	Source
Gram- negative bacteria		
1	*Escherichia coli O157:H7*	Botany Dept., Fac.of Sci.,Assiut Univ
2	*Klebsiella Pneumoniae*	
3	*Proteus vulgaris*	
4	*Pseudomonas sp*	
Gram- positive bacteria		
1	*Bacillus anthrakoid*	Botany Dept., Fac.of Sci. Assiut Univ
2	*Staphylococcus aureus*	
Fungi		
1	*Aspergillus flavus*	Botany Dept., Fac.of Sci.,
2	*Penicillium duclauxii*	Fac.of Arche., Sohag Univ
3	*Trichoderma sp*	

Methods

Preparation of phytate extract

Phytic acid corrosive was removed from wheat bran as per the strategy portrayed by Canan *et al*. (2011): Briefly 20 grams of wheat bran was blended with 200 mL of 1.0 N HCL and shaken for one hour at room temperature on a stirrer, then its pH was acclimated to 6.2 utilizing 4.0 N NaOH and centrifuged at 3000 rpm for 10 min then the pH of the supernatant was acclimated to 8 with 1.5 M Na_2CO_3 arrangement and stayed for 12 h at room

temperature. After centrifugation at 3000 rpm for 10 min, the supernatant was tapped and the acquired pellet was resuspended in 1.0 N HCL. To eliminate foreign substances and protein denaturation, 10 mL formaldehyde and 0.5-gram celit were added. This suspension was then shaken for 2 h and stayed for 12 h at room temperature. The pre-arranged suspension was then gone through subjective Whatman channel paper No3 and the pH of the filtrate was acclimated to 7 by 1.5 M Na_2CO_3. At last, the framed pellet was recuperated through separating by subjective Whatman filter paper and dried at 60˚C for one day.

Preparation of aqueous wheat bran extract:
Ten grams of each sample was extracted by stirring with 100 ml of distilled water for 1 hour at room temperature. After centrifugation and filtration through Whatman No. 1. This extract was used to perform the required analyzes.

Antibacterial activity
Antibacterial activity of phytic corrosive is not entirely set in stone by the agar dissemination strategy as per the NCCLS (1993).

Antifungal and Yeasts test
Antifungal and yeasts test were completed concurred to ELLOF (1998).

Determination of total antioxidant activity
The free radical scavenging activity of phytic acid and other antioxidants was measured with 2,2 diphenyl- 1- picrylhydrazyl (DPPH) free radical using the method described by Yen and Wu (1999). Sample solution (4 ml) was added to 1 ml, 0.2 mM DPPH in methanol. After reacting for 30 min in the dark, the absorbance was read at 547 nm by a spectrophotometer. Methanol (4 ml) was mixed with 1 ml DPPH and this served as the control. Radical scavenging activity (%) was calculated as follows:

Radical scavenging activity (%) = (1- Absorbance of sample / Absorbance of control) x 100.

Statistical analysis
Data were analyzed by analysis of variance (ANOVA) using a completely randomized factorial design. Basic statistics and ANOVA were performed to test the significance within replication and between treatments (MSTAT-C 1989). The (LSD) tests was used to determine the means at the level of 0.05%.

RESULTS AND DISCUSSION

Antimicrobial activity of phytate and wheat bran

The results shown in Table 2 indicate the antimicrobial activity of pure phytic acid (PA), phytate extract and wheat bran extract at different concentrations. Data showed that, Gram-positive bacteria was more sensitive for PA than Gram-negative bacteria. *Bacillus anthrakoid* was sensitive to pure phytic acid while, *Staphylococcus aureus* was more sensitive to pure phytate at concentrations 5, 6 %. Moreover, no inhibition against fungi was recorded. Besides, it could be noticed that microbial spectra were decreased with increasing the phytic acid concentrations. These results are in the line with those reported by Boukhris *et al*. (2020), they found that minimum inhibitory concentration of PA varied from 0.488 to 0.97 mg/ml for the Gram-positive bacteria that were tested, and was 0.244 mg/ml for the Gram-negative bacteria. Linear and general models were used to further explore the antibacterial effects of PA. The developed models were validated using experimental growth data for *L. monocytogenes, S. aureus and S. Typhimurium*. The antimicrobial activity of phytate extracted from wheat bran at concentration (5 %) was determined and inhibitation zones was recorded in same Table. Data showed that, Gram- positive bacteria was more sensitive than Gram- negative bacteria. *Bacillus anthrakoid* and *Staphylococcus aureus* were sensitive to concentration of phytate extract at 5 % concentration. Data showed that, the inhibition effects on *Escherichia coli O157:H7,* and *Staphylococcus aureus* were 20.33 and 38.67 mm, respectively for 6% concentration of pure phytate. Moreover, results revealed that a moderate inhibition against fungi was observed. The concentration of phytate extract 5 % had no inhibition effects on *Trichoderma sp.* Data revealed that the microbial spectra was decreased with increasing the concentrations of phytate extract and Gram- positive bacteria was more sensitive than Gram- negative bacteria. The antimicrobial

activity of aqueous wheat bran extract was also determined and the inhabitation zones illustrated in Table (2). From these date it can be conceded that, pure phytic acid and phytate extracted from wheat bran have an inhibitory effect on many types of microorganisms. The inhibition zones varied according to pure phytate the concentration degree and the highest inhibition zones were at a concentration 5% and 6%. The finding showed that effect of phytate extract on fungi, was moderate but was better than the effect of phytic acid, which did not show any effect on fungi.

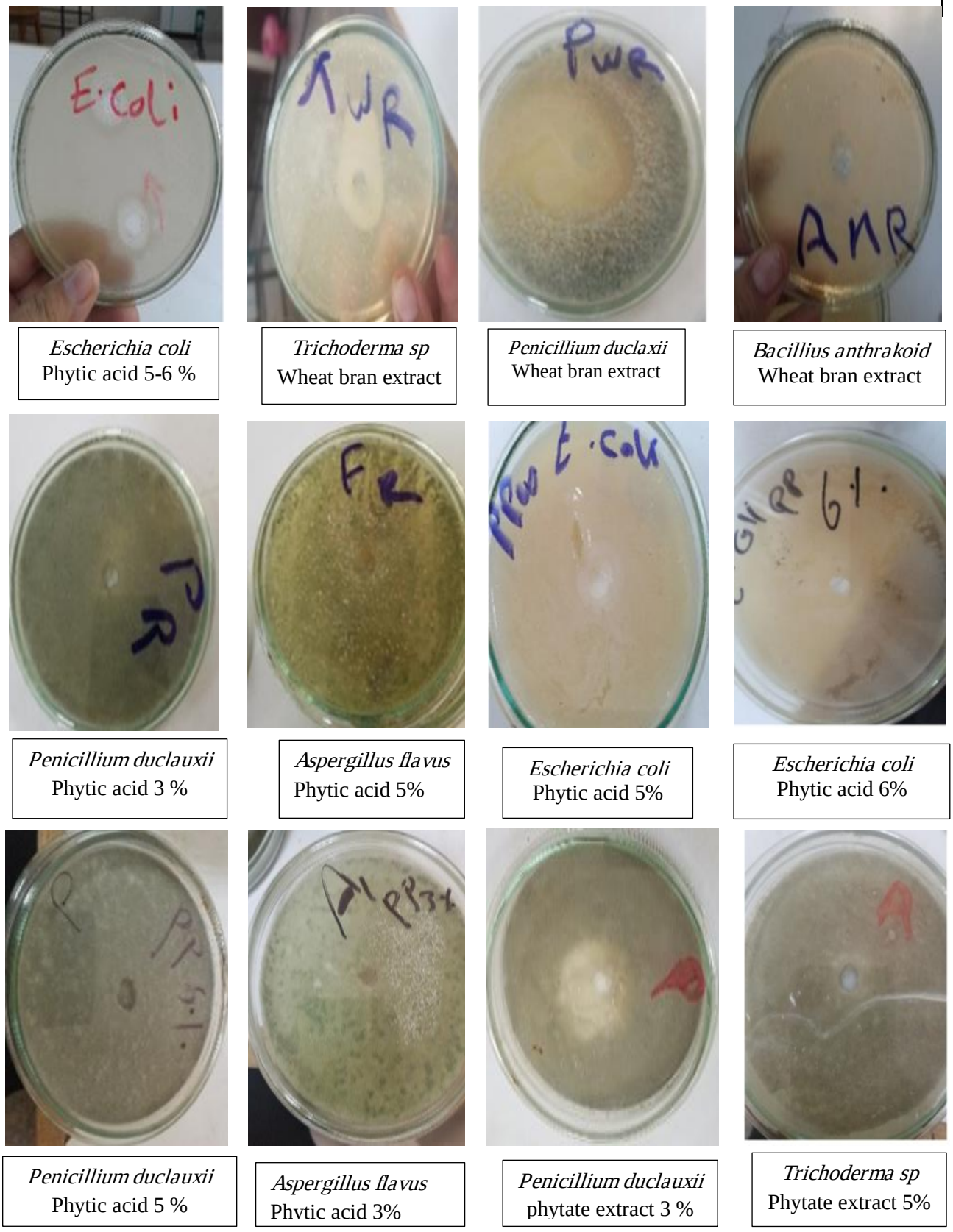
Antioxidant activity of phytic acid, phytate extract and wheat bran

Phytic acid, phytate extract and aqueous wheat bran extract were subjected to the antioxidant activity using 2,2-diphenyl-picrylhydrazyl (DPPH) radical scavenging assay. Results in Table 3 and Fig. 2 showed that, the free radical scavenging activity of phytic acid at (1, 3 and 5mM) concentrations were 22.23, 33.60 and 36, respectively. These means that the antioxidant activity of pure phytate increased with increasing in phytate concentration, these results are in agreement with those obtained by Skarin *et al* (2005) they reported that, phytic acid had low radical scavenging effect, which was in the range of 10.1 to 41.0%. Ahn *et al*. (2004) reported that the irradiation dose was positively correlated with the DPPH radical-scavenging effects. Phytic acid has antioxidant functions by virtue of forming a unique iron chelate and it suppresses the iron-catalyzed oxidative reactions, serving as a potent antioxidant function in the preservation of seeds (Graf and Eaton, 1990).The same mechanism, dietary phytic acid may lower the incidence of colonic cancer and protect against other inflammatory bowel diseases. However, the free radical-scavenging activity of phytic acid has not yet been reported. A concentration effect of phytic acid was also observed, and radical-scavenging capacity increased with increasing concentration. Phytate extracted from wheat bran gave the free radical scavenging activity 16.70, 81 and 90.33% at concentrations 1, 3 and 5%, respectively. Phytate extract from wheat bran gave a high scavenging activity that reached 90% compared with pure phytic acid. On the other hand, aqueous wheat bran extract gave a free radical scavenging activity reached 36.16%. These results are in the line with those reported by Iqbal *et al.* (2007), they found that the remaining amount as percentage of DPPH radical at 5 min after initiation of the reaction were 24, 30, 38,43, and 48% for five wheat varieties bran. Free radical and radical cation scavenging activity were comparable to previous findings for wheat bran of different varieties from USA (Yu *et al.*, 2003). Li *et al.* (2007) reported that the DPPH scavenging activity of purple wheat bran extract was 63.17%. From this result it can be concluded that pure phytic acid as well as phytate extracted from wheat bran have an important role as natural antioxidants that can be used to preserving many food items.

Table (2) Diameter of inhibition zones (mm) of phytic acid, phytate extract, and wheat bran extract against some selected microorganisms:

Concentration (mg/ml) Microorganism	Diameter of inhibition zones (mm)					
	PA 1%	PA 3%	PA 5%	PA 6%	PE 5%	WBE
Escherichia coli O157:H7	N.d	N.d	12.67	20.33	20.33	16.33
Klebsiella Pneumoniae	N.d	N.d	30.67	33.00	15.67	N.d
Proteus vulgaris	N.d	N.d	N.d	N.d	19.67	N.d
Pseudomonas sp	N.d	N.d	30.67	35.00	17.00	N.d
Bacillus anthrakoid	24.33	24.00	31.67	20.33	17.33	N.d
Staphylococcus aureus	N.d	N.d	31.00	38.67	23.33	20.67
Aspergillus flavus	N.d	N.d	N.d	N.d	20.00	22.00
Penicillium duclauxii	N.d	N.d	N.d	N.d	35.00	49.33
Trichoderma sp	N.d	N.d	N.d	N.d	N.d	23.67
LSD 5%	4.29	3.03	3.59	9.36	10.52	3.20

PA=phytic acid, PE= phytate extract, WBE= wheat bran extract, N.d= Not detected



Escherichia coli Phytic acid 5-6 %

Trichoderma sp Wheat bran extract

Penicillium duclaxii Wheat bran extract

Bacillius anthrakoid Wheat bran extract

Penicillium duclauxii Phytic acid 3 %

Aspergillus flavus Phytic acid 5%

Escherichia coli Phytic acid 5%

Escherichia coli Phytic acid 6%

Penicillium duclauxii Phytic acid 5 %

Aspergillus flavus Phvtic acid 3%

Penicillium duclauxii phytate extract 3 %

Trichoderma sp Phytate extract 5%

Fig (1): Antimicrobial activity of phytate against certain types of microorganisms.

Table (3): Antioxidant activity of phytic acid, phytate extract, and wheat bran.

Samples	Antioxidant activity
Pure phytic acid 1mM	22.23
Pure phytic acid 3mM	33.60
Pure phytic acid 5mM	36.00
Phytate extract 1%	16.17
Phytate extract 3%	81.30
Phytate extract 5%	90.33
Wheat bran	36.13
LSD 5%	3.28

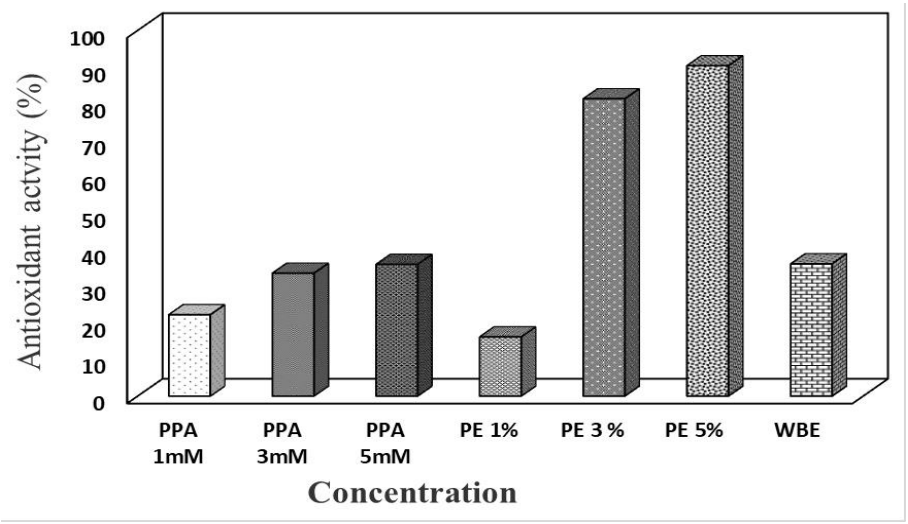


Fig (2): Antioxidant activity of phytic acid, phytate extract, and wheat bran Extract. PPA_1= pure phytic acid 1mM, PPA_3 = pure phytic acid 3mM, PPA_5 = pure phytic acid 5mM, PE_1 = phytate extract 1%, PE_3 = phytate extract 3%, PE_5 = phytate extract 5%, WBE= wheat bran extract

CONCLUSION

The obtained results showed that, Gram-positive bacteria had more sensitive for phytic acid than Gram- negative bacteria. A moderate inhibition against fungi was observed. The results indicated that pure phytic acid and phytate extracted from wheat bran have an inhibitory effect on many types of microorganisms. Phytate extracted from wheat bran gave a high scavenging activity that reached 90% compared with pure phytic acid. Aqueous wheat bran extract gave a percentage of free radical scavenging activity reached 36.16%. It can be concluded that phytate extracted from wheat bran have antimicrobial and antioxidant activity with the possibility of using them as natural preservatives

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الملخص العربي

النشاط المضاد للأكسدة والميكروبات لحمض الفيتك المستخلص من ردة القمح

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الهدف من هذه الدراسة هو التعرف على النشاط المضاد للميكروبات والأكسدة لحامض الفيتك النقي والمستخلص من ردة القمح. حيث أظهرت النتائج أن البكتيريا الموجبة الجرام كانت أكثر حساسية لحامض الفيتك من البكتريا السالبة لجرام. وكانت بكتريا *Bacillus anthrakoid* و*Staphylococcus aureus* أكثر حساسية من لحمض الفيتك النقي عند تركيزات 5، 6٪. وكانت تأثيرات التثبيط بكتريا القولون *Escherichia coli* O157: H7 والمكورات العنقودية الذهبية 20.33 و38.67 مم على التوالي بتركيز 6٪ من الفيتات النقي. علاوة على ذلك، أظهرت النتائج وجود تثبيط معتدل للفطريات. اضافة إلى ذلك وجد أن حامض الفيتك النقي والفيتات المستخلصة من ردة القمح لهما تأثير مثبط على العديد من الكائنات الحية الدقيقة. زاد النشاط المضاد للأكسدة للفيتات النقية بزيادة تركيز الفيتات حيث كان النشاط المضاد للأكسدة 22 و23 و36٪ بتركيزات 1 و3 و5 مل مول على التوالي. أعطى حامض الفيتك المستخلص من ردة القمح نشاط مضاد للأكسدة عالي وصل إلى 90٪ مقارنة بحامض الفيتك النقي.قد أعطى مستخلص ردة القمح نسبة نشاط مضاد الأكسدة وصلت 36.16٪. من النتائج المتحصل عليها تتضح أن الفيتات المستخلصة من ردة القمح لها نشاط مضاد للميكروبات والأكسدة و يمكن استخدامها كمواد حافظة طبيعية.