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COMPARATIVE THE ANTIOXIDANTS CHARACTERISTICS OF ORANGE AND POTATO PEELS EXTRACTS UNDER DIFFERENCES IN PRESSURE AND CONVENTIONAL EXTRACTIONS

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ABSTRACT

This investigation aimed to decrease the extraction time of natural antioxidants and add commercial dimension to plant extracts. Impact the difference in pressure (DE) on antioxidant properties was studied by estimating total phenolic and total flavonoid contents (TPC, and TFC), DPPH˙ scavenging radical activity (IC_{50}), inhibition lipids peroxidation by both TBARs and β-Carotene/Linoleic acid bleaching (βCB) assays, antimicrobial activities, and yield of extracts, comparison with the resulting by conventional extractions (SE). The results showed positive effects of OPE, and PPE on antioxidants and antimicrobial activities, and the extracts of DE were the highest value to both orange and potato peel extracts. However, increase the yield of extracts and TFC by the decrease of ethanolic concentration of both orange and potato peel extracts, TPC, DPPH˙ scavenging radical activity, TBARs, βCB, and antimicrobial activity was increased by the increase of ethanolic concentration, and the extraction by DE was the highest value. The absolute ethanolic potato peel extract by soak extraction method (SE) was the lowest value of yield of extract and TFC (21.38±1.08, and 29.73±1.03; respectively), while absolute ethanolic orange peel extract by extraction method by DE was the highest value of TPC, DPPH˙ scavenging radical activity (IC_{50}), TBARs, and βCB (262.19±1.19, 21.18±1.18, 78.82±0.85, and 83.15±1.15; respectively). Also, the effect of absolute ethanolic orange peel extract by the difference in pressure on antimicrobial activity was the highest.

1.Introduction

In recent years, due to the insufficiency of resources of food, it's become interesting to the utilization of food wastes resulting from several sources and how and/or what is the benefit of re-usage as the feedstock of many products. In general, agricultural and food factories waste used in animals' feeds, the results indicated to roughly one of third of food products lost or wasted globally, which amounts to about 1.3 billion tons per year, approximately, and of course, a huge amount of resources and emission of gas caused by food production are also emission in vain (FAO, 2018). The wastes result from agricultural and food factories considered raw material to many products for it has minerals, pigments, vitamins, other phytochemical compounds have antioxidants and antimicrobial activity, and enough amount of starch.

By-products of food manufactory, such as pomace and peels, represent an abundant source of bioactive compounds. In many cases, these by-products are not used to their potential Besides, the transaction with waste and by-products in a sustainable and environmentally friendly way is becoming a highly important issue in the food manufactory. Due to the European Landfill Directive, the food industry is

obliged to decrease the percentage of waste and by-products going to landfills by 2020 (Kosseva, 2009).

Antioxidants are utilized to elongate shelf-life and preserve the nutritional quality of lipid-containing foods as well as to modulate the consequences of oxidative damage in the human body (Halliwell et al., 1995). The use of synthetic antioxidants such as tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) in maintaining foods is now prohibited or under strict regulation in many countries because of their associated toxic and carcinogenic side effects (Buxiang and Fukuhara, 1997; Jo et al., 2006). The increased demand for natural foods nowadays has bound the food industry to include natural antioxidants in foods. Natural antioxidants have been used instead of synthetic ones to retardation lipid oxidation in foods to improve their quality and nutritional value. Retrogradation of meat lipids can directly affect the color, flavor, texture, nutritive value, and safety of food (Ruiz et al., 1999; Camo et al., 2008; Velasco and Williams, 2011; Mirzadeh, et al. 2020). Consequently, there is solicitude in using naturally occurring antioxidants as food additives. Several natural antioxidants have been added to food preparation and manufacturing and have increased the shelf life and oxidative stability of stored food products (Chen et al., 2008).

Citrus fruits received considerable attention in recent years to consume widely around the world and the potential curative benefits associated with high levels of flavonoids, and antioxidant, anticancer, and anti-inflammatory properties (Benavente-Garcia and Castillo, 2008). A large amount of consumption and processing of citrus fruit results in the generation of a huge quantity of citrus peels which is considered food industrial waste. The results showed Industrial processing increase the value of citrus fruits by producing a wide range of by-products such as pectin, pulp, and flavonoids, etc. (Fakhari et al., 2005).

Potatoes are generally peeled during processing. Potato peels had been proposed as dietary fiber (Arora and Camire, 1994), and a source of natural antioxidants. Polyphenols considered as an important group of antioxidants present in potatoes are largely concentrated in the peel, which has an important role in the defiance mechanism against phytopathogens (Friedman, 1997). Potato peels have therefore been the subject of study in many pharmacology and food industry studies.

The aims of the present investigation were carried out to study the effect of extraction methods by the difference in pressure for different ethanolic concentrations of orange and potato peel extracts on antioxidants activity and antimicrobial activity.

2. Materials and methods

2.1. Materials

2.2.1.Samples

Orange peels (*Citrus xsinensis*) were obtained from private workshops in Mahata Square, Zagazig town, El-Sharkia Governorate, Egypt on 12/2019.

Potato wastes (*Solanum tuberosum*) were obtained from Farm Frites factory on, 10th of Ramadan on 05/2019.

Absolute Ethanol Alcohol, citric acid, Na_2CO_3, sodium hydroxide, phenolphthalein, potassium iodide, sodium thiosulfate, starch, chloroform, acetic acid, and hydrochloric acid were purchased from El-Gomhoria Chemical Company, Zagazig, Egypt .

Folin-ciocalteu reagent, gallic acid, β-carotene, linoleic acid, tween 20, TBA (thiobarbituric acid), phosphatidyl-choline, potassium chloride, iron chloride, TCA (trichloroacetic acid), DPPH (2,2-diphenyl-1-picrylhdrazyl), and butylated hydroxyl anisole (BHA) were purchased from Sigma Chemical Company, Cairo, Egypt.

2.2.Methods

2.2.1.Preparation of Samples

The primary procedures were to separate the parts of the contents of potatoes factories wastes (Water, Starch, and peels). Then, Potato peels (PP) were transferred and washed well with water to urge obviate the remnants of starch

protruding it, then, stacked on trays for a half-hour to urge eliminate excess water before drying. The orange peels (OP) were examined to eliminate the damaged parts and stacked on trays. Both samples were dried in an oven-dryer at 37° C for 48 hr. The samples were flipping once every hour within the first four hours. Then, the dried samples were ground to a fine powder, place in plastic bags, and wrapped with foil, and stored at -20° C until the subsequent procedures.

2.2.2.Extraction of the Antioxidants Extracts

Extraction of the antioxidants extracts of dried samples was conducted using two methods (Soak Extraction (SE), and Extraction by the difference in pressure (DE) in several concentrations of ethanolic Solvent (absolute, 70%, and 50% of ethanolic Solvent). 100 g of dried weight of skin and potato skin samples were soaked in 1000 ml of every one of the three concentrations of ethanolic Solvent, separately, in 2000 ml conical flask for 48 hr. on the stirring hotplate at 37° C (Fisher Scientific, Pittsburgh, PA) with a magnetic stirrer (1000 rpm) (the first method), and also the same procedure for less than 6 hr. with decrease the pressure to 0.6 Pa every 30 min within the sample flask during extraction (the second method). The obtained extracts were filtered using paper (Whatman No. 1, England), concentrated employing a rotary evaporator (EYELA, Japan), freeze-dried (Thermo-Electron Corporation-Hot power dry LL300 freeze dryer), and weighed to work out the yield of extracts. Then, stored at frozen temperature until used.

2.2.3.Determination of Total Phenolic Contents (TPC)

The concentration of total phenol content of various antioxidant extracts was measured by a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer), supported a colorimetric oxidation/reduction reaction, that described by Škerget et al. (2005). The oxidizing reagent usage was Folin–Ciocalteu reagent in step with AOAC (2005). 0.5 mL of diluted extract (10 mg in 10 mL solvent) 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with distilled water) and a couple of mL of Na_2CO_3 (75 g/L) were added. The mixture was incubated for five min at 50˚C then cooled to temperature. For an effect sample, 0.5 mL of H_2O was used. The absorbance was measured at 760 nm. Total phenolic content expressed as acid equivalent (GAE g^{-1} of dried extract) was calculated using the subsequent equation supported the calibration curve:

$$y = 0.2269x + 0.4847 \quad (1)$$

$$R^2 = 0.992 \quad (2)$$

where y is that the absorbance and x is that the concentration (mg GAE g^{-1} of dried extract). R^2=Correlation Coefficient.

2.2.4.Determination of Total Flavonoids Contents (TFC)

The content of total flavonoid concentration of different antioxidant extracts was measured according to the method of Ordon et al. (2006) with some modification. 1.5 mL of $AlCl_3$ ethanolic solution (20 g L^{-1}) was added to 0.5 mL of every extract of samples (10 mg in 10 mL solvent) separately and incubated for one hour at room temperature. The absorbance was measured at 420 nm at room temperature and the yellow color indicates the presence of flavonoids. Total flavonoid content expressed as Quercetin equivalent (mg QE g^{-1} of dried extract) was calculated using the following equation based on the calibration curve:

$$y = 0.3033x + 0.6511 \quad (3)$$

$$R^2 = 0.9987 \quad (4)$$

where x is the absorbance and y is the concentration (mg QE g^{-1} of dried extract). R2=Correlation Coefficient.

2.2.5.DPPH˙ Free Radical Scavenging Assay

The ability of different antioxidant extracts to decolorization the purple color of the DPPH˙ solution was measured according to the method of Gulcin et al. (2004). 0.1 ml of each extract (10 mg in 10 mL solvent) was added to 3 mL of 0.1 mM DPPH˙ dissolved in the same solvent to each extract, separately, and measured for two hours every 30 min at room temperature. The control of the assay was prepared according to

usage to negative control from DPPH˙ solution and only solvent without extracts, and the positive control by exchange the extracts by BHA synthetic antioxidants. The absorbance was determined against a negative control at 517 nm for every period, separately .

Percentage of antioxidant activity of DPPH˙ free radical was calculated using the following equation:

$$\text{Inhibition (\%)} = \left(\frac{A_c - A_t}{A_c}\right) \times 100 \qquad (5)$$

where A_c is the absorbance of the negative control and A_t is the absorbance of the sample and/or positive control. IC_{50} is the antioxidant concentration that inhibits the DPPH reaction by 50% under experimental conditions.

2.2.6.β-Carotene/Linoleic Acid Bleaching (βCB) Assay

The ability of various antioxidant extracts and artificial antioxidants (BHA) to stop the bleaching of β-carotene was assessed as described by Kayvan et al. (2007). In brief, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg linolic acid, and 200 mg of tween 20 were placed in a very flask. After removal of the chloroform, 50 mL of water was added, and also the resulting mixture was stirred vigorously. Aliquots (3 ml) of the emulsion were transferred to tubes containing extract or synthetic antioxidants. Immediately after mixing 0.5 mL of extract solution (10mg extract in10 mL solvent), an aliquot from each tube was transferred to a cuvette and therefore the absorbance at 470 nm was recorded (A_0). The remaining samples were placed within the water bath at 50 °C for 120 min, then the absorbance at 470 nm was recorded (A_{120}). An impression without added extract was also analyzed. Antioxidant activity was calculated as follow:

$$\text{A A (\%)} = \left(1 - \frac{(A_{S0} - A_{S120})}{A_{C0} - A_{C120}}\right) \times 100 \qquad (6)$$

where A_{S0} is that the initial absorbance and A_{S120} is that the absorbance at 120 min for samples. while A_{C0} is that the initial absorbance and A_{C120} is that the absorbance at 120 min for negative control.

2.2.7.Thiobarbituric Acid Reactive Substances (TBARs) Assay

The capacity of various antioxidant extracts to inhibit lipid peroxidation was also evaluated by using the modified assay of thiobarbituric acid reactive substances (TBARs) (Gonzalez-Paramas et al., 2004). the tactic relies on the peroxidation of a liposome system (25 mL of fifty mg/ml phosphatidyl-choline in 1.5:1 (v:v) chloroform:ethanol) induced by 200 ml of 1 mM iron chloride containing 300 mM chloride within the presence of the extracts (50 ml). Peroxidation was started by adding ascorbate (125 ml at 0.16 mM) and incubating at 37 °C for twenty-four hr. The reaction was stopped by adding 0.75 ml of a combination 1.5:1 (v:v) of 9.4% TCA in 0.47 N acid (pH 1.5) with 1% TBA and 0.05 ml of BHT (760 mg/l in ethanol). the assembly of TBARS, fundamentally malonaldehyde, as a secondary product of peroxidation, was measured spectrophotometrically at 535 nm after incubation at 95 °C for 60 min.

A control without the extracts (with the various solvents employed in the extractions) was wont to evaluate the phosphatidylcholine peroxidation as inhibition ratio (IP, %):

$$\text{IP (\%)} = \left(1 - \frac{A_t}{A_t^0}\right) \times 100 \qquad (7)$$

where A_t and A_t^0 are extracted and control absorbance after incubation for 60 min. The repetition variance of the procedure was always <10%.

2.2.8.Antimicrobial activity

2.2.8.1.Antibacterial activity

The antibacterial activity was estimated according to Bayer et al. (1966) and Akl et al. (2020). Discs of filter paper were saturated with 30 µL of different antioxidant extract (800 µg/mL) and placed on Petri dishes containing agar media contaminated with pathogenic bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), and incubated for 24 h at 37 °C. Then, measured the inhibition zone diameters

(mm). A disc saturated with distilled water was a negative control, and levofloxacin was a positive control.

2.2.8.2.Minimum inhibitory concentration (MIC)

The effect of the different antioxidant extracts on the visible turbidity of tubes contaminated with pathogenic bacteria before and after incubation was measured according to Andrews (2001). 30 µL of different antioxidant extract at different concentrations of (0, 200, 400, 800, and 1000 µg/mL) was incubated with broth media contaminated with pathogenic bacteria, then, observed the turbidity of tubes before and after incubation. The MIC was the lowest concentration exhibiting a clear zone on Muller–Hinton agar (MHA) plates according to Reda et al. (2020) and Sheiha et al.(2020) .

2.2.8.3.Bacterial growth curve (turbidity test)

The bacterial growth assays were estimated according to El-Saadony et al. (2020). 30 µL of the different antioxidant extract (800 µg/mL) was added to tubes containing 100 µL of tested pathogenic bacteria and 10 mL nutrient broth. Then, incubation at 37 °C for 6 h intervals of (0–24 h). The turbidity of tubes was measured at 600 nm and compared with distilled water as a negative control and levofloxacin as a positive control.

2.2.8.4.Antifungal activity

The inhibition of fungal growth of different antioxidant extracts was evaluated against four fungal species; *Aspergillus niger, Aspergillus ochraceus, Penicillium citrinum,* and *Fusarium oxysporum*, according to Elgorban et al. (2016) and El-Saadony et al. (2019). Five-millimeter discs of filter paper were saturated with 50 µL of different antioxidant extract (800 µg/mL) and applied on both sides of potato dextrose agar (PDA) plates. Carefully, the disc of mycelia was picked from the edge of fungal cultures and placed in each Petri dish center, then incubated at 28 °C for 3–5 days. The fungal mycelium's radial growth was measured by a ruler (cm/4 days). The PDA plates with 50 µL distilled water were a negative control, and Difenoconazole (800 µg/mL) was a positive control.

The minimum fungal concentration (MFC) was estimated according to Alizadeh et al. (2014). 50 µL of different antioxidant extract (0, 200, 400, and 800 µg/mL) was added to different PDA tubes containing fungi. Then, incubation at 28 °C for 48–72 h. The least concentration of the different antioxidant extract that removes fungal growth was considered as MFC.

2.2.9.Statistical Analysis

The tests were done in triplicate according to Steele and Torrie (1996), and the data were analyzed using the means, standard deviation by Microsoft Office Excel (2016), Paired sample t-test, and one-way ANOVA variance analysis by IBM SPSS version 25.0 software (SPSS Inc., Chicago, IL, USA) at the level of probability of ($P \leq 0.05$).

3.Results and discussions

Extraction of the antioxidant extracts depended on soak the fine ground powder of samples in extraction solvents where exposure huge area of a sample to extraction solvent leads to facilitation and increase efficiency extraction operation. While the magnetic stirrer's rotational speed expands the field of exposure area of the sample to extraction solvents and facilitates extracting the phytochemical compounds (the main source of the antioxidant act) to the solution of extraction. A difference in pressure in the sample flask increases cell wall permeability which gives more facilitates extracting in little time.

3.1.Total Phenols (TPC) and Total Flavonoids (TFC) Contents

Phenolic and flavonoid compounds are derived from compounds of the secondary metabolism of plants which have the ability to scavenger free radicals, protect food elements during the food processing chain, prolong the shelf-life of food products, and protect organs of the human body from oxidative stress (Granato et al., 2018). So, the effectiveness of the antioxidant activity of phenolic and flavonoid compounds was a very important incentive to determine the total contents of phenolic and flavonoid compounds. The concentration of phenolic and flavonoid

compounds of different extracts was expressed as mg Gallic acid (GAE) and Quercetin (QE); respectively per g of dried extracts (Table 1). The results showed a significant mean difference ($P \leq 0.05$) between the soak and extraction by difference in pressure methods, a positive effect to the difference in pressure methods on the concentration of phenolic and flavonoid compounds. The data also shows a significant mean difference ($P \leq 0.05$) between some samples and no significant mean difference ($P \leq 0.05$) between other samples of orange peels and potato peels of the different extracts for soak and extraction by difference in pressure methods, and only a significant mean difference ($P \leq 0.05$) between the samples of orange peels and potato peels of the different extracts for extraction method by the difference in pressure.

For the soak extraction (SE) method, orange peel extracts showed a higher concentration value of total phenolic contents than potato peel extracts for all different extracts. The absolute ethanolic extracts of orange peels (OPE_{100}) shows the highest concentration value of total phenolic compounds followed by 70% ethanolic extracts of orange peels (OPE_{70}) then 50% ethanolic extracts of orange peels (OPE_{50}) (231.43±1.43, 220.19±1.09, and 218.47±1.07 mg GAE g^{-1} of dried extract; respectively), while the lowest concentration value of total phenolic compounds was 50% ethanolic extracts of potato peels (PPE_{50}) (180.72±1.02 mg GAE g^{-1} of dried extract). Also, orange peel extracts showed a higher concentration value of total phenolic contents than potato peel extracts for all different extracts of the extraction method by the difference in pressure (DE). The absolute ethanolic extracts of orange peels (OPE_{100}) shows the highest concentration value of total phenolic compounds followed by 70% ethanolic extracts of orange peels (OPE_{70}) then 50% ethanolic extracts of orange peels (OPE_{50}) (262.19±1.19, 247.58±0.58, and 232.41±0.41 mg GAE g^{-1} of dried extract; respectively), while the lowest concentration value of total phenolic compounds was 50% ethanolic extracts of potato peels (PPE_{50}) (191.19±1.19 mg GAE g^{-1} of dried extract).

For the concentration of total flavonoid contents, 50% ethanolic extracts of orange peel (OPE_{50}) showed the highest concentration value of total flavonoid contents followed by 50% ethanolic extracts of potato peel (PPE_{50}) then 70% ethanolic extracts of potato peel (PPE70) (82.39±1.09, 81.48±1.08, and 61.42±1.42 mg QE g^{-1} of dried extract; respectively), and absolute ethanolic of potato peel extracts (PPE_{100}) was the lowest concentration value of total flavonoid contents (29.73±1.03 mg QE g^{-1} of dried extract), of soak extraction (SE) method. While the highest concentration value of total flavonoid contents of the extraction method by the difference in pressure (DE) was 50% ethanolic extracts of orange peel (OPE_{50}) followed by 50% ethanolic extracts of potato peel (PPE_{50}) then 70% ethanolic extracts of orange peel (OPE_{70}) (86.28±1.28, 83.43±1.03, and 78.16±1.16 mg QE g^{-1} of dried extract; respectively), and absolute ethanolic of potato peel extracts (PPE_{100}) was the lowest concentration value of total flavonoid contents (36.87±0.87 mg QE g^{-1} of dried extract).

The increase of the total flavonoid contents came with a decrease in the concentration of ethanolic solvent, an increase of total phenolic contents with an increase in the concentration of ethanolic solvent, and the highest efficiency was the extracts of the extraction method by the difference in pressure. The results agreed with the results reported by Brahmi et al. (2012), and Rosa et al. (2019), although the comparison is highly difficult because of the different extraction conditions used.

Table 1. The contents of total phenol (TPC), total flavonoids compounds (TFC), and yield of different antioxidants extracts

		TPC (concentration mg GAE g^{-1} of dried extract)		TFC (concentration mg QE g^{-1} of dried extract)		Yield of Extracts (g/100g of dried materials)	
		SE	DE	SE	DE	SE	DE
OP	E_{100}	231.43±1.43^{a}	262.19±1.19^{a}	35.26±1.06^{d}	49.15±1.15^{e}	27.24±2.24^{ab}	29.15±1.15^{ab}
	E_{70}	220.19±1.09^{b}	247.58±0.58^{b}	54.51±1.1^{c}	78.16±1.16^{c}	28.51±1.01^{a}	30.57±0.5^{ab}
	E_{50}	218.47±1.07^{b}	232.41±0.41^{c}	82.39±1.09^{a}	86.28±1.28^{a}	30.19±1.09^{a}	32.49±1.4^{a}
PP	E_{100}	186.73±1.03^{c}	207.43±1.03^{d}	29.73±1.03^{e}	36.87±0.87^{f}	21.38±1.08^{c}	22.42±1.02^{c}
	E_{70}	181.27±1.07^{d}	198.45±0.45^{e}	61.42±1.42^{b}	70.42±0.42^{d}	25.6±1.2^{b}	27.04±1.04^{b}
	E_{50}	180.72±1.02^{d}	191.19±1.19^{f}	81.48±1.08^{a}	83.43±1.03^{a}	29.4±1.2^{a}	31.07±1^{a}

Values mean ±SD; n = 3. Different letters in the same column indicate significant differences (P≤ 0.05).

Table 2. Assays of antioxidants activity of different antioxidants extracts

		IC_{50} (µg mL^{-1})		% of inhibition lipid peroxidation			
				β -Carotene		TBARs	
		SE	DE	SE	DE	SE	DE
OP	E_{100}	22.53±0.53^{c}	21.18±1.18^{c}	81.52±0.52^{a}	83.15±1.15^{a}	77.04±1.04^{b}	78.82±0.85^{b}
	E_{70}	26.49±1.4^{bc}	24.19±1.1^{b}	76.35±1.35^{b}	78.51±0.51^{b}	70.21±1.2^{c}	72.89±0.89^{d}
	E_{50}	29.45±0.45^{a}	27.94±0.94^{a}	69.23±1.2^{d}	71.54±0.54^{c}	69.31±1.31^{c}	71.62±0.62^{d}
PP	E_{100}	24.61±0.61^{c}	22.97±0.97^{c}	78.85±0.85^{ab}	80.94±0.94^{a}	82.02±1^{a}	87.83±0.83^{a}
	E_{70}	27.83±0.83^{b}	25.37±1.3^{ab}	73.26±1.2^{c}	77.82±0.82^{b}	77.25±1.2^{b}	79.42±1.02^{b}
	E_{50}	29.03±1.03^{a}	27.49±1.4^{a}	69.36±1.3^{d}	73.04±1.04^{c}	70.15±1.15^{c}	76.12±1.1^{c}

Values mean ±SD; n = 3. Different letters in the same column indicate significant differences (P≤ 0.05).

Table 3. Anti-bacterial activity of different antioxidants extracts

Anti-Bacterial Activity									
		Staphylococcus aureus		***Bacillus cereus***		***Escherichia coli***		***Pseudomonas aeruginosa***	
		SE	**DE**	**SE**	**DE**	**SE**	**DE**	**SE**	**DE**
OP	**E_{100}**	30±0.5^{b}	32±0.2^{b}	26±0.4^{b}	29±0.5^{a}	24±0.4^{b}	26±0.1^{b}	28±0.9^{b}	28±0.4^{b}
	E_{70}	25±0.1^{c}	27±0.5^{d}	20±0.5^{c}	25±0.7^{b}	20±0.6^{c}	22±0.5^{c}	24±1^{d}	26±0.5^{c}
	E_{50}	20±1^{d}	24±0.5^{e}	17±1^{d}	20±0.3^{c}	15±0.5^{e}	18±0.6^{e}	19±0.8^{e}	21±1^{d}
PP	**E_{100}**	29±0.5^{b}	30±0.5^{c}	26±0.4^{b}	29±0.5^{a}	21±1^{c}	25±0.4^{b}	26±0.3^{c}	28±0.5^{b}
	E_{70}	25±1^{c}	26±0.3^{d}	21±0.4^{c}	25±0.5^{b}	18±0.7^{d}	20±0.5^{d}	23±1^{d}	26±0.5^{c}
	E_{50}	19±0.5^{d}	23±1^{e}	16±0.4^{d}	19±0.5^{c}	15±0.4^{e}	17±0.6^{e}	20±0.5^{e}	22±0.5^{d}
Cont.	**PC**	34±0.2^{a}		30±0.1^{a}		28±0.3^{a}		31±0.7^{a}	
	NC	ND		ND		ND		ND	

Values mean ±SD; n = 3. Different letters in the same column indicate significant differences (P≤ 0.05).

Table 4. Anti- fungal activity of different antioxidants extracts

Anti-Fungal Activity									
		Aspergillus niger		***Aspergillus ochraceus***		***Penicillium citrinum***		***Fusarium oxysporum***	
		SE	**DE**	**SE**	**DE**	**SE**	**DE**	**SE**	**DE**
OP	**E_{100}**	1.7±0.2^{c}	1.4±0.3^{c}	1.8±0.2^{c}	1.5±0.5^{b}	1.6±0.2^{d}	1.4±0.2^{d}	2±0.5^{c}	1.8±0.2^{c}
	E_{70}	1.9±0.1^{b}	1.8±0.2^{ab}	2.3±0.3^{b}	1.9±0.1^{ab}	2±0.5^{c}	1.8±0.2^{c}	2.6±0.2^{b}	2.2±0.2^{b}
	E_{50}	2.3±0.3^{ab}	2.1±0.1^{b}	2.8±0.2^{a}	2.5±0.5^{a}	2.6±0.2^{ab}	2.1±0.1^{ab}	3±1^{a}	2.9±0.1^{a}
PP	**E_{100}**	1.9±0.3^{b}	1.5±0.1^{c}	2±0.5^{c}	1.6±0.2^{b}	1.9±0.1^{c}	1.7±0.2^{c}	2.1±0.1^{c}	1.9±0.1^{c}
	E_{70}	2.3±0.3^{ab}	1.9±0.2^{b}	2.4±0.4^{b}	2.1±0.1^{c}	2.5±0.2^{b}	2±0.1^{ab}	2.8±0.2^{ab}	2.4±0.4^{b}
	E_{50}	2.9±0.1^{a}	2.6±0.2^{a}	2.9±0.1^{a}	2.7±0.2^{a}	2.9±0.1^{a}	2.8±0.2^{a}	2.3±0.3^{a}	2.9±0.1^{a}
Cont.	**PC**	1.2±0.2^{d}		1.1±0.1^{d}		1.2±0.2^{d}		1.6±0.2^{d}	
	NC	9		9		9		9	

Values mean ±SD; n = 3. Different letters in the same column indicate significant differences (P≤ 0.05).

3.2.The Antioxidants Activity of Different Antioxidants Extracts

The antioxidants activity of different antioxidant extracts was determined by some of the different methods; DPPH˙ scavenging radical activity, β-Carotene/Linoleic acid bleaching (βCB) assay, and thiobarbituric acid reactive substances (TBARs) assay and the results shown in table 2. The results showed a significant mean difference (P≤ 0.05) between both extraction methods (soak and extraction by the difference in pressure) for all the methods of antioxidants activity assays, a positive effect to the extraction method by the difference in pressure. The data also shows a significant mean difference (P≤ 0.05) between some samples and no significant mean difference (P≤ 0.05) between other samples of the different orange and potato peel extracts for both different methods of extraction.

DPPH˙ scavenging radical activity was used as a wide model to evaluate the scavenging radical activity of the natural antioxidant extracts. The antioxidant extracts are able to reduce the DPPH˙ free radical and change the color of the solution from purple to yellow, in the non-radical situation (Shen et al., 2016). Data in fig. 1 shows the increase in IC_{50} with the decrease of ethanolic concentration solvent for both orange and potato peel extracts of both soak and extraction methods by the difference in pressure. Absolute ethanolic extracts give more stabilization of DPPH˙ scavenging radical activity more than 70% ethanolic extracts more than 50% ethanolic extracts for both orange and potato peel in both soak and extraction methods by the difference in pressure. The absolute ethanolic of orange peel extracts (OPE_{100}) give the most stabilization of DPPH˙ scavenging radical activity followed by absolute ethanolic of potato peel extracts (PPE_{100}) then 70% ethanolic of orange peel extracts (OPE_{70}) (22.53±0.53, 24.61±0.61, and 26.49±1.4 µg ml^{-1}; respectively), while 50% ethanolic of orange peel extracts (OPE_{50}) gives the lowest stabilization of DPPH˙ scavenging radical activity (29.45±0.45 µg ml^{-1}) of soak extraction method. Also, absolute ethanolic of orange peel extracts (OPE_{100}) give the most stabilization of DPPH˙ scavenging radical activity followed by absolute ethanolic of potato peel extracts (PPE_{100}) then 70% ethanolic of orange peel extracts (OPE_{70}) (21.18±1.18, 22.97±0.97, and 24.19±1.1 µg ml^{-1}; respectively) and 50% ethanolic of orange peel extracts (OPE_{50}) gives the lowest stabilization of DPPH˙ scavenging radical activity (27.49±0.49 µg ml^{-1}) for extraction method by the difference in pressure.

Determination of the antioxidant activity of the different extracts on inhibition lipid peroxidation by β-Carotene/Linoleic acid bleaching was dependent on the activities of lipid radicals as auto-oxidation products of linoleic acid which attack double bonds of β-carotene, and the ability of the antioxidative substance to protect β-carotene (yellowish-orange colour) (Zhang et al., 2015). In both orange and potato peel extracts, absolute ethanolic extracts give a high value of β-Carotene/Linoleic acid bleaching (βCB) assay more than 70% ethanolic extracts more than 50% extracts for both extraction methods (fig. 2). For the soak extraction method, both orange and potato peel extracts give approximate and nested of a significant and non-significant mean difference (P≤ 0.05) value of β-Carotene/Linoleic acid bleaching (βCB) assay in all of the ethanolic concentrations. Data shows no significant mean difference (P≤ 0.05) between absolute ethanolic of orange peel (OPE_{100}) and absolute ethanolic potato extract (PPE_{100}) (81.52±0.52, and 78.85±0.85% percentage; respectively), also no significant mean difference (P≤ 0.05) between the lowest value of β-Carotene/Linoleic acid bleaching (βCB) assay of both 50% ethanolic orange peel extract (OPE_{50}) and 50% ethanolic potato extract (PPE_{50}) (69.23±1.2, and 69.36±1.3% percentage; respectively), while 70% ethanolic concentration of both orange and potato peel give significant mean difference (P≤ 0.05) (76.35±1.35, and 73.26±1.2% percentage; respectively). Whilst, no significant mean difference (P≤ 0.05) between orange and potato peel in the same concentration of ethanolic solvent for extraction method by the difference

in pressure (83.15±1.15, and 80.94±0.94% percentage of absolute ethanolic orange peel extract (OPE_{100}) and absolute ethanolic potato peel extract (PPE_{100}); respectively, 78.51±0.51, and 77.82±0.82% percentage of 70% ethanolic orange peel extract (OPE_{70}) and 70% ethanolic potato peel extract (PPE_{70}); respectively, and 71.54±0.54, and 73.04±1.04% percentage of 50% ethanolic orange peel extract (OPE_{50}) and 50% ethanolic potato peel extract (PPE_{50}); respectively).

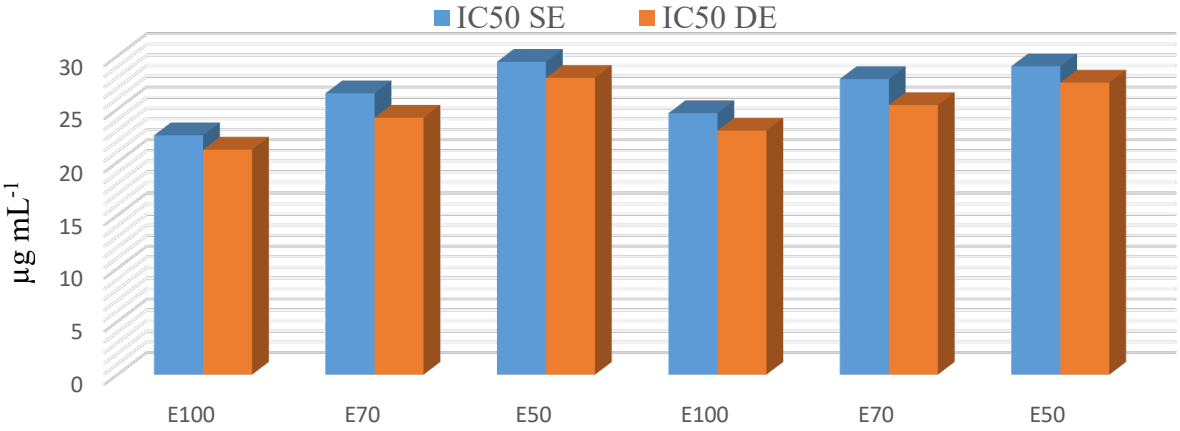

Figure 1. DPPH˙ Free Radical Assay (IC_{50})

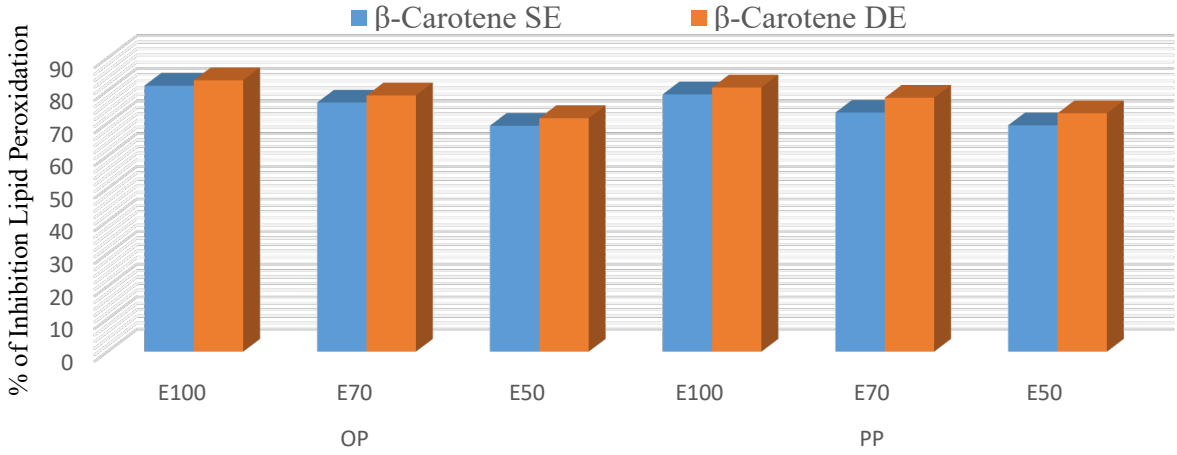

Figure 2. Inhibition Lipid Peroxidation; β-Carotene/Linoleic acid Bleaching (βCB) Assay

Determination of TBARs value was widely used to estimate the antioxidant activity and the ability of extracts to inhibit lipid peroxidation (Yim et al., 2013). For both soak and extraction method by the difference in pressure, absolute ethanolic extracts give the value of inhibiting lipid peroxidation more than 70% ethanolic extracts more than 50% ethanolic extracts for both orange and potato peel, and potato peel give the highest value per every type of ethanolic concentration separately (fig. 3). Absolute ethanolic potato peel extract (PPE_{100}) gives the highest value of thiobarbituric acid reactive substances (TBARS) assay followed by 70% ethanolic potato peel extract (PPE_{70}) with no significant mean difference ($P \leq 0.05$) between absolute ethanolic of orange peel extract (OPE_{100}) and 70% ethanolic potato peel extract (PPE_{70}), and no significant mean difference ($P \leq 0.05$) between 50% ethanolic potato peel extract (PPE_{50}) and both 70% and 50% ethanolic orange peel extracts (OPE_{70} and OPE_{50}) (82.02±1, 77.25±1.2, 77.04±1.04, 70.21±1.2, 70.15±1.15, and 69.31±1.31% percentage; respectively) for soak extraction method. While, absolute ethanolic potato peel extract (PPE_{100}) gives the highest value followed by 70% ethanolic potato peel extract (PPE_{70}) with no significant mean

difference (P≤ 0.05) between 70% ethanolic potato peel extract (PPE_{70}) and absolute ethanolic orange peel extract (OPE_{100}), and no significant mean difference (P≤ 0.05) between 70% ethanolic orange peel extract (OPE_{70}) and 50% ethanolic orange peel extract (OPE_{50}) (87.83±0.83, 79.42±1.02, 78.82±0.85, 76.12±1.1, 72.89±0.89, and 71.62±0.62% percentage; respectively) for extraction method by the difference in pressure.

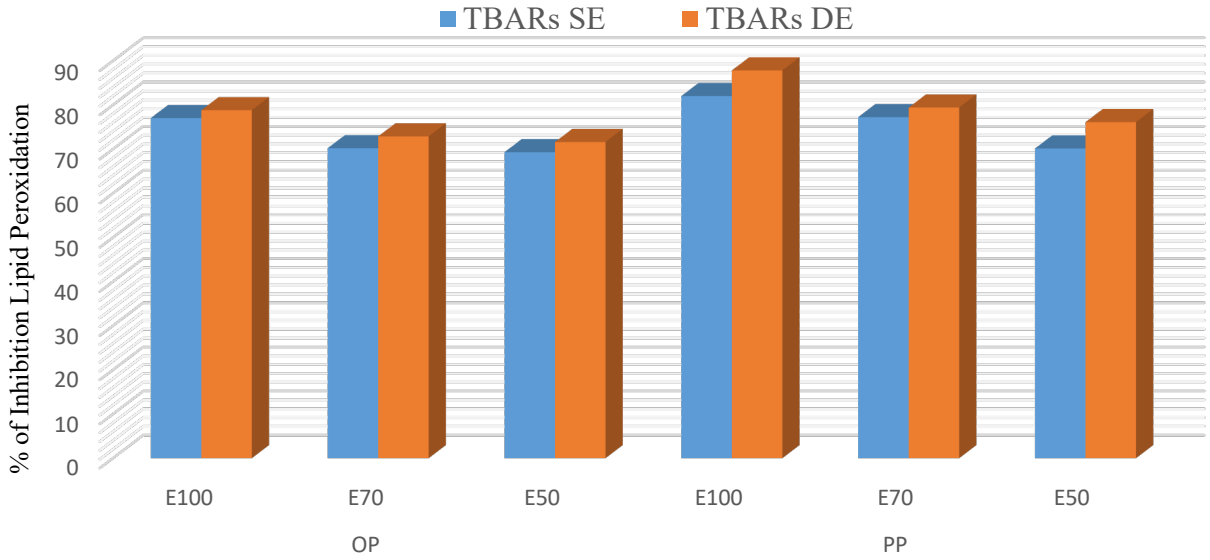

Figure 3. Inhibition Lipid Peroxidation; thiobarbituric acid reactive substances (TBARs) Assay

The increase of antioxidant activity by an increase in ethanolic solvents concentration came compatible with the increase of total phenolic and the highest efficiency of the extracts of the extraction method by the difference in pressure, and the results agreed with the results reported by Rosa et al. (2019), although the different extraction conditions used.

3.3.Antimicrobial activity

The effect of different orange and potato peel extracts on microbial activity was measured by different methods. The ability of different orange and potato peel extracts to inhibit bacterial zone and restrain fungal growth was estimated and the results are shown in tables 3 and 4, and fig. 4. The minimum bacterial and fungal inhibitory concentration was in (600 µg mL^{-1}) for all microbial growth in absolute and 70% ethanolic orange and potato peel extracts, and (800 µg mL^{-1}) for almost microbial growth in 50% ethanolic orange and potato peel extracts in both soak and extraction method by the difference in pressure.

The results showed significant mean differences (P≤ 0.05) between both extraction methods for all samples, the positive effect was for the extraction method by the difference in pressure, no significant mean differences (P≤ 0.05) between orange and potato peel extracts for the same ethanolic concentration of only gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, and *Bacillus cereus* ATCC 11778) in soak extraction method, no significant differences (P≤ 0.05) between positive control and both absolute orange and potato peel extracts, and no significant differences (P≤ 0.05) between orange and potato peel extracts for the same ethanolic concentration of only gram-negative bacteria (*Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) in the extraction method by the difference in pressure (Table 3). Positive control gives the highest value for inhibition of bacterial activities for all bacteria followed by absolute ethanolic extracts, then 70% ethanolic extracts, then 50% ethanolic extracts for both extraction methods.

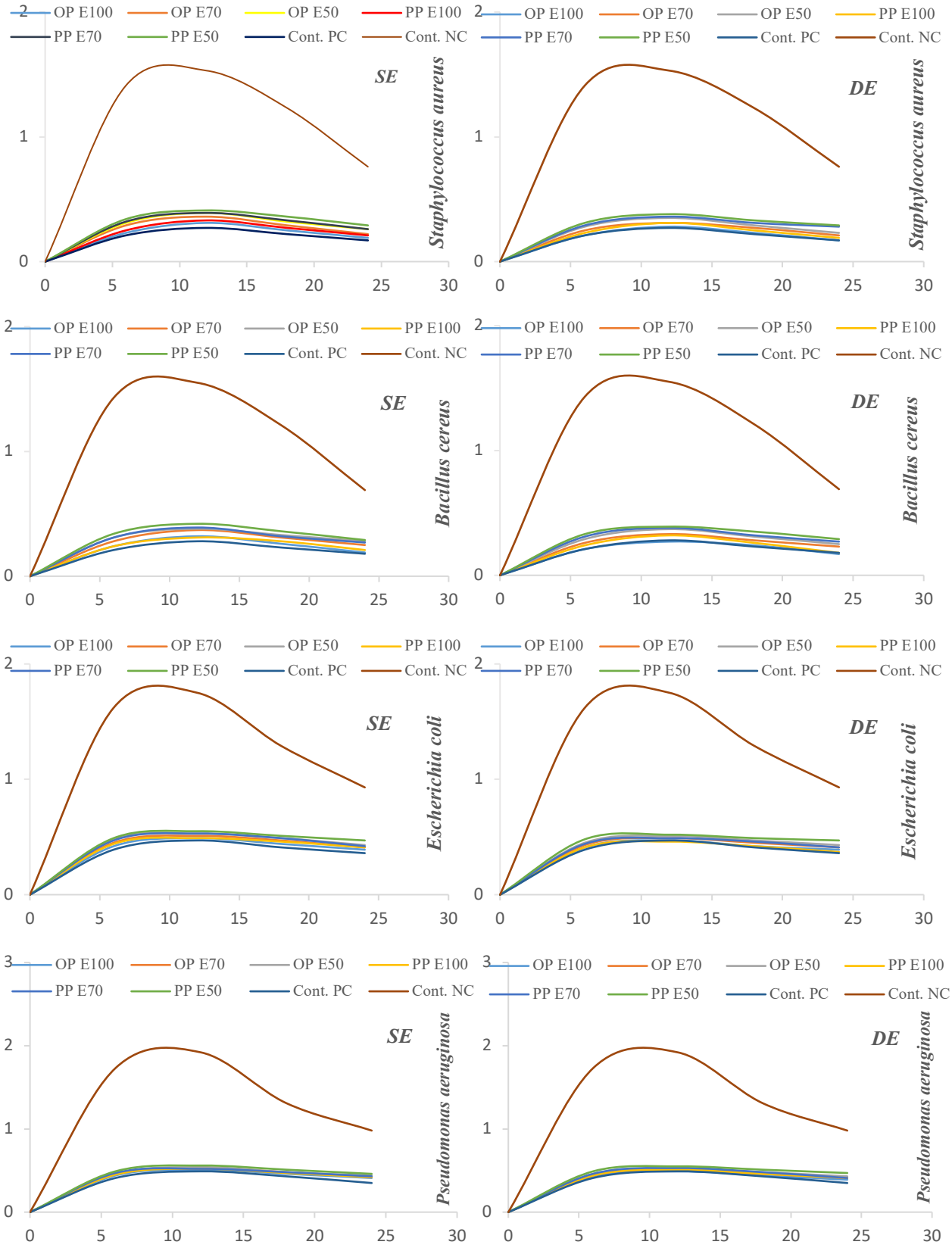

Figure 4. Growth curve of gram-positive and gram-negative bacteria in the presence of MIC of different orange and potato peel extracts (800 µg mL^{-1}).

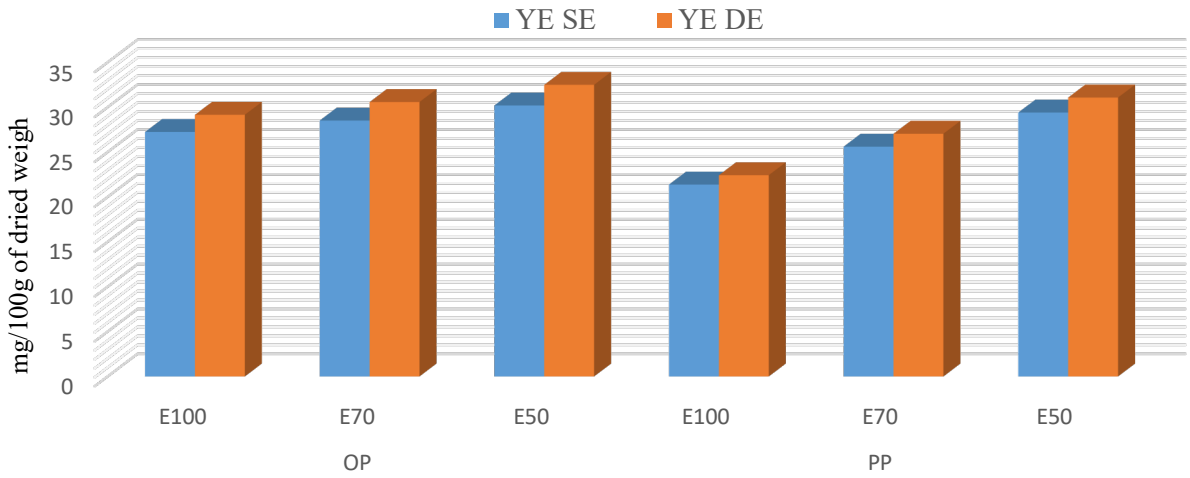

Figure 5. Yield of Extracts of Different Antioxidants Extracts

Table 4 showed fungal radial growth (cm) affected by different orange and potato peel extracts for the four fungal strains: *Aspergillus niger, Aspergillus ochraceus, Penicillium citrinum,* and *Fusarium oxysporum*. The extracts reduced the diameter colony of fungi from 9.0 cm for negative control to (1.4:2 cm) in the highest inhibition of fungal activities for different orange and potato peel extracts. The absolute ethanolic extracts give the highest value for inhibition of fungal activities for all fungal strains, followed by 70% ethanolic extracts, then 50% ethanolic extracts for both extraction methods, and positive control was more active against fungal activities than different orange and potato peel extracts .

The antimicrobial activities of different orange and potato peel extracts may originate from its high contents of phenolic compounds and flavonoids following Abdel-Shafi et al. (2019). The different orange and potato peel extracts were effective against the pathogenic bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), and *fungal strains: Aspergillus niger, Aspergillus ochraceus, Penicillium citrinum,* and *Fusarium oxysporum*. The results indicated that gram-negative bacteria were more resistant than gram-positive ones, probably because of their more sophisticated membranes. The phenolics might alter the permeability and rigidity of the cell wall by inhibiting the cell wall enzymes.

3.4.The yield of Extract of Different Antioxidants Extracts

The yield of extracts resulted from different ethanolic concentrations of different methods of extraction was measured and the result shown in fig. 5. Data shows different amounts of extracts value between the different concentrations of ethanol, a significant mean difference ($P \leq 0.05$) between the soak and extraction method by the difference in pressure, a positive effect for the extraction method by the difference in pressure. In both orange and potato peel, 50% ethanolic extracts give the high amount value of yield extracts more than 70% ethanolic extracts more than absolute ethanolic extracts for both extraction methods. 50% ethanolic orange peel extract (OPE_{50}) shows the highest value of yield extracts followed by 50% ethanolic potato peel extract (PPE_{50}) then 70% ethanolic orange peel extract (OPE_{70}) (OPE_{50}) (30.19±1.09, 29.4±1.2, and 28.51±1.01 mg/100g of dried weight; respectively), while absolute ethanolic potato peel extract (PPE_{100}) gives the lowest value of yield extracts (21.38±1.08) for soak extraction method. Also, 50% ethanolic orange peel extracts (OPE_{50}) show the highest value of yield extracts followed by 50% ethanolic potato peel extracts (PPE_{50}) then 70% ethanolic orange peel extracts (OPE_{70}) (32.49±1.4, 31.7±1, and

30.57±0.5 mg/100g of dried weight; respectively), and absolute ethanolic of potato peel (PPE_{100}) gives the lowest value of yield extracts (22.42±1.02) for extraction method by the difference in pressure. These results are in agreement with the study of El-Naggar et al. (2017).

4.Conclusion

This study was executed to determine the impact of the difference in pressure on the antioxidants and antimicrobial activities for different concentrations of ethanolic of orang and potato peel extracts. The extraction method by the difference in pressure showed enhanced the antioxidants and antimicrobial activities with observed increases in the yield of extracts. The increase of the antioxidants and antimicrobial activities extraction by the differences in pressure method may be due to the increase of phenolic compounds concentration. We suggest that more studies on the effect of pressure on the antioxidants and antimicrobial activities for natural extracts and never use only soaking to the extraction of natural antioxidant extracts.

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