



## BIOLOGICAL SCIENCES

# Determination of antimicrobial and antioxidant potential of agro-waste peels

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**Abstract:** Natural products from plants are pulling in more interest in exploration due to their therapeutic properties like, mainly because of the drug-resistance in microbes against synthetic drugs. Thus, the present study was designed to assess the antimicrobial and antioxidant actions of some Agro-waste (garlic, ginger, onion, potato) peel extracts. Ginger peel extracts exhibited the highest FRAP ( $0.273 \pm 0.044$  mm Fe<sup>2+</sup> Eq/g dry weight) while garlic peel extracts exhibited the highest TAC ( $0.47 \pm 0.0452$  mm AAE/g dry weight) values among all selections. The antimicrobial activity of the peels was evaluated against different pathogenic bacterial and fungal strains i.e.; *Escherichia coli*, *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Colletotrichum falcatum*, *Fusarium moniliforme*, and *Rhizoctonia solani*. Among all extracts, ginger peel extracts exhibited maximum inhibition against all bacterial strains, while onion peel extracts exhibited zero inhibition against any bacterial strains. All extracts exhibited maximum inhibition against *Staphylococcus aureus* except onion peel extracts. Positive inhibition against all fungal strains was observed for all samples, with maximum inhibition against *Colletotrichum falcatum*. The outcomes of the study, therefore reveal that Agro-wastes have a powerful antimicrobial and antioxidant potency and thus can be used for many medicinal purposes, which will likewise be helpful in waste management and environmental safety.

**Key Words:** antimicrobial, antioxidant potential, drug-resistance, agro-waste peels, garlic peels.

## INTRODUCTION

Vegetable and fruit peel was often thrown aside as waste (agro-wastes) are utilized generally as fertilizer or livestock feed. During the processing of food in industries and household usage, a large amount of agro-wastes is produced. These wastes possess a serious hazard to the environment as they are highly susceptible to microbial spoilage. Thus, they need to be safely disposed of or utilized to get environment beneficial aspects. Many technologies for optimal utilization of agro-wastes in food and non-food materials are established (Khattak

& Rahman 2017). Different scientific studies reveal that different plant parts (pods, fruit rinds, seeds, hulls, latex, fruits, flowers, stalks, leaves, pees, barks, stems, roots etc.) possess many phytochemicals, which exhibit different biological activities (Aref et al. 2010).

A recent research reveals the presence of high fiber and protein contents in papaya peel and seed flours and suggests their use as a substitute of nutrients (Santos et al. 2014). Lately, agro-wastes resulting from industrial food processing have gained much interest of researchers as a rich source of phenolics, therefore special attention has been paid in

developing technologies for isolation and utilization of agro-waste extracts in medicine due to their potential health benefits (Tenore et al. 2012). Different antimicrobial compounds may be kept apart from fruit and vegetable peels, using an efficient extraction medium and method. These peels contain compounds, which may be an appropriate ingredient against microbial spoilage and pathogen attack in the food industry. Illnesses and infections related to mortality induced due to drug-resistance have been an alarming issue.

Fruit rinds and peels are discarded as waste and are often used as bio-fertilizers, but their therapeutic use is a more innovative concept, which is getting more acceptance as they are biodegradable, environment-friendly and an economic source of antimicrobials in inhibition of pathogenic diseases. This is a modern way of waste-management.

The current work focuses on the extraction and assay of antimicrobial components from the peels of fruits and vegetables against infections causing microbes and their qualitative analysis to discover a new source of antimicrobial from waste products.

## MATERIALS AND METHODS

### Collection of sample

Samples of agro-waste (garlic, ginger, potato, onion peels) were collected from the local market and local surroundings of Sargodha. Samples were dried under shade, ground to be able to pass from a 1mm sieve. Prior to further analysis samples were stored in airtight bottles at ambient conditions. All chemicals and reagents used were of HPLC and analytical grade either obtained from Sigma-Aldrich or Merck otherwise specified.

### Extraction of bioactive compounds

Extraction of samples was done with 5 g sample and 50 mL of methanol for 1 hour in a sonication bath (FC, China Model: 230HT) with power supply 50 Hz, AC200-240V, 40 KHz frequency, and 120W power. After filtration, the dried extracts obtained using Rotary Evaporator (IKA Germany, Model: RC 2 B S000) were stored in the dark prior to further analysis.

### TPC (Total Phenolic Content)

TPC (Total Phenolic Content) of agro-waste samples was determined using calorimetric FC reagent (Folin-Ciocalteu) method with some modifications (Kramling & Singleton 1969).

The reaction mixture contained 0.5 mL diluted extracts and Gallic acid standards (ranging from 20-500 mg/L) in a volumetric flask having 30 ml dist. H<sub>2</sub>O. The mixture was shaken followed by the addition of 2.5 ml FC reagent. After incubation for 5 min, 7.5 mL of 7% Na<sub>2</sub>CO<sub>3</sub> solution was added followed by immediate dilution to 50 mL with dist. H<sub>2</sub>O. The solution was kept at room temperature for 2 hours. The absorbance of the solution was measured at 760 nm on Perkin-Lambda double beam spectrophotometer. The experiment was repeated thrice, and results were reported as mg GAE/g dry weight (Gallic acid equivalents of dry sample).

### TFC (Total Flavonoid Contents)

TFC (Total Flavonoid Content) of agro-waste extracts was determined using the aluminum chloride colorimetric method with little alterations (Willett 2002). The reaction mixture containing 0.5 mL extracts, 0.1 mL 10% AlCl<sub>3</sub>, 0.1 mL 1 M potassium acetate and 4.3 mL dist. H<sub>2</sub>O was incubated at room temperature for 30 min. After incubation absorbance of the solution was recorded at 415 nm using Quercetin as a standard. The measurements were done in

triplicate and the results were expressed as mg QE/g dry weight (quercetin equivalents of dry sample).

#### **DPPH radical scavenging assay**

The anti-radical potential of sample extracts was estimated by following a reported method with minor modifications (Thaipong et al. 2006). The solution was prepared by diluting 15 ml of stock solution (2.4 mg/ml dry methanol) with 40 ml methanol and stored at 4 °C in the dark before analysis. Sample extracts (10 µl) were added in a 96-well plate, with the addition of 300 µl DPPH solution and 30 µl distilled water. After incubation for half an hour at 30 °C, the absorbance of the reaction mixture was recorded at 517 nm in a microplate reader. Trolox standard solutions (0-3500 µM/mL) were used to plot a linear calibration curve ( $R^2=0.9967$ ). The radical scavenging activities of the extracts were recorded in triplicate and stated as mM Trolox equivalent (TE)/g dry weight.

#### **FRAP (Ferric Reducing Antioxidant Power) assay**

Ferric reducing the antioxidant power of the extracts was determined following a previously reported method with little alterations.

The reagent was prepared by mixing acetate buffer (25 mL, 300mM, pH=3.6) with tripridyltriazine solution (TPTZ, 2.5 mL, 10 mM) and ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 2.5 mL, 20 mM). The working solution was prepared by mixing 10µl of sample extract and 300µl of FRAP working reagent in a 96-well plate. After incubation for 5 minutes at 37°C, absorbance was measured at 593 nm with the help of a microplate reader. Standard solutions of ferrous sulphate were prepared (0-4800 µM/mL) for calibration purposes ( $R^2=0.9974$ ). The measurements were done in triplicate and expressed as mM ferrous equivalent/g dry weight.

#### **ABTS radical cation scavenging assay**

The radical cation scavenging activity of the extracts was determined using an ABTS/TEAC assay (Benzie & Strain 1999). A mixture of ABTS (90mL, 7 mM) and potassium persulfate (10 mL, 2.45 mM) was prepared and left in the dark overnight. The solution was diluted with a phosphate buffer solution (PBS; 5 mM, pH 7.4) to an absorbance of 0.7 at 734 nm. The scavenging activity of extracts was measured by pouring the reaction mixture of 10 µl sample the extracts and 290 µl diluted ABTS solution in 96-well plate. After incubation for 6 minutes at 37°C, absorbance was monitored at 734 nm in a micro plate reader. Trolox standard solution (0-2000 µM) were used to plot a linear calibration curve ( $R^2=0.997$  and the results were reported as Trolox equivalent (TE)/g dry weight of samples.

#### **Total Antioxidant Capacity (TAC)**

Measurement of TAC of peel extracts was carried out following phosphomolybdenum method (Re et al. 1999). An aliquot of 0.1 mL of sample solution (100µg/mL) was mixed with 1 mL of reagent solution (4 mM ammonium molybdate, 0.6 M sulfuric acid and 28 mM sodium phosphate). The reaction mixture was heated in a water bath at 95 °C for 90 min. Samples were cooled to ambient temperature prior to measurement of absorbance at 695 nm against the blank.

#### **Antibacterial activity**

Agar well diffusion method was used to determining the antibacterial activity of agro-waste peel samples (Navarro et al. 1996) against four pathogenic bacterial strains namely *Escherichia coli* (Gram-negative), *Bacillus megaterium*, *Bacillus cereus*, and *Staphylococcus aureus* (Gram-positive) (Figure 4). The sample extracts prepared in methanol were diluted with DMSO (dimethyl sulfoxide) to get the concentration of 15mg/mL.

### Inoculum preparation

Each colony of bacterial strains (24 hours of age) was taken in 20mL of lysogeny broth (L.B) followed by incubation for 24 h with 101 revolutions to obtain a better growth of each strain.

Nutrient Broth medium (Merck-Germany) with composition, meat extract-peptone (12 g/L Agar-agar, 5 g/L Peptone from meat and 3 g/L meat extract) was used for the preparation of growth medium. The turbidity of the strains was optimized using a McFarland 0.5 BaSO<sub>4</sub> turbidity standard until the formation of 10<sup>6</sup> colonies per mL (Koneman et al. 1997). The inoculums were used for culturing the nutrient agar plates.

### Preparation of ager medium

In 1000mL of distilled water 20g of media after sterilization in an autoclave. The medium was cooled at 4-5° C. The medium (30 mL) was solidified in sterile Petri dishes under UV laminar hood (Streamline EN 1822.1 Singapore). Sterilized cork borer was used for the formation of wells after solidification. Then 24h old culture of tested bacterial strains was swabbed in Petri dish with the help of sterilized cotton swab. After swabbing wells were filled with 100µl of each sample and that of DMSO and positive control (chloramphenicol). Afterwards that Petri plates were incubated at 37°C. Inhibition activity of the selected isolates was determined through the diameter of inhibition zone after 24h of incubation.

### Antifungal activity

Agar tube dilution method (Choudhary et al. 1995), was employed for assessment of the antifungal activity of extracts against three fungal strains i.e., *Rhizoctonia solani*, *Fusarium moniliforme* and *Colletotrichum falcatum* (Figure 5). In 10 mL of dimethyl sulfoxide, 150 mg of each extract was added to prepare a sample for antifungal activity. Each fungal strain was grown at 4°C on

sabouraud dextrose agar (MERCK Germany) with the composition of agar 14g/mL, glucose 41g/mL and peptone complex 09 g/mL. For negative control slants without extract were used.

### Procedure of assay

A media having pH 5.6 was prepared. 4m SDA (Merck Germany) was poured in capped test tubes and autoclaved at 121°C for 20 min. Test tubes were placed in UV laminar hood for 15 min after autoclaving. At 50°C the test tubes were cooled and 100 µl of extract was poured in each test tube and kept at room temperature for solidification. Test tubes were inoculated with 4mm piece of 7d old culture of fungus. By addition of no sample in slant a negative control was prepared. At 28°C the test tubes were placed in incubator for 7d and were examined regularly. After 7 days the fungus growth was tested in slant and inhibition was tested with reference to negative control.

## RESULTS AND DISCUSSION

### Polyphenolic contents

For evaluation of total phenolic content (TPC) Folin-Ciocalteu (FC) is most widely used reagent. In this method, a redox reagent is produced, which reacts with polyphenol to produce blue color phosphotungstic-phosphomolybdic complex, which captures the free radicals and thus can be measured spectrophotometrically reactions (Alothman et al. 2009). Therefore, the FC reagent method was employed in this study to evaluate the TPC of agro-waste i.e., potato, garlic, ginger, and onion peel extracts. Significant difference among different peel extracts was observed. TPC was in the range of 20.992-114.135 mg/g of peel extracts. Among all extracts, onion peel exhibited highest TPC (114.135±8.762 mg GAE/g) while lowest for a ginger peel (20.992±4.234 mg GAE/g) as presented in Table I.

**Table I. TPC and TFC of extracts expressed as mg GAE/g and mg QE/g.**

Samples	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Potato Peel	26.306±8.751	1.555±0.568
Garlic Peel	51.662±5.995	7.782±1.258
Ginger Peel	20.992±4.234	2.413±0.885
Onion Peel	114.135±8.762	45.511±11.255

TFC (Total Flavonoid Content) of peel extracts evaluated using a modified calorimetric method and expressed as mg QE/g DW (quercetin equivalent mg/g dry weight of samples), were somehow in agreement with the TPC values. Among all extracts, onion peel exhibited highest TFC (45.511±11.255) while potato peel (1.555±0.568) exhibited the lowest TFC value (Figure 1) (Table I).

### Scavenging activity

Radical scavenging and radical cation scavenging activity of agro-waste peel extracts were determined following modified DPPH radical scavenging and ABTS radical cation scavenging assays. DPPH exists as a free radical in solution and has a maximum absorbance at 517 nm. The decline in absorbance value via antioxidant-radical complex indicates the scavenging ability of the sample extracts (Ichikawa et al. 2003).

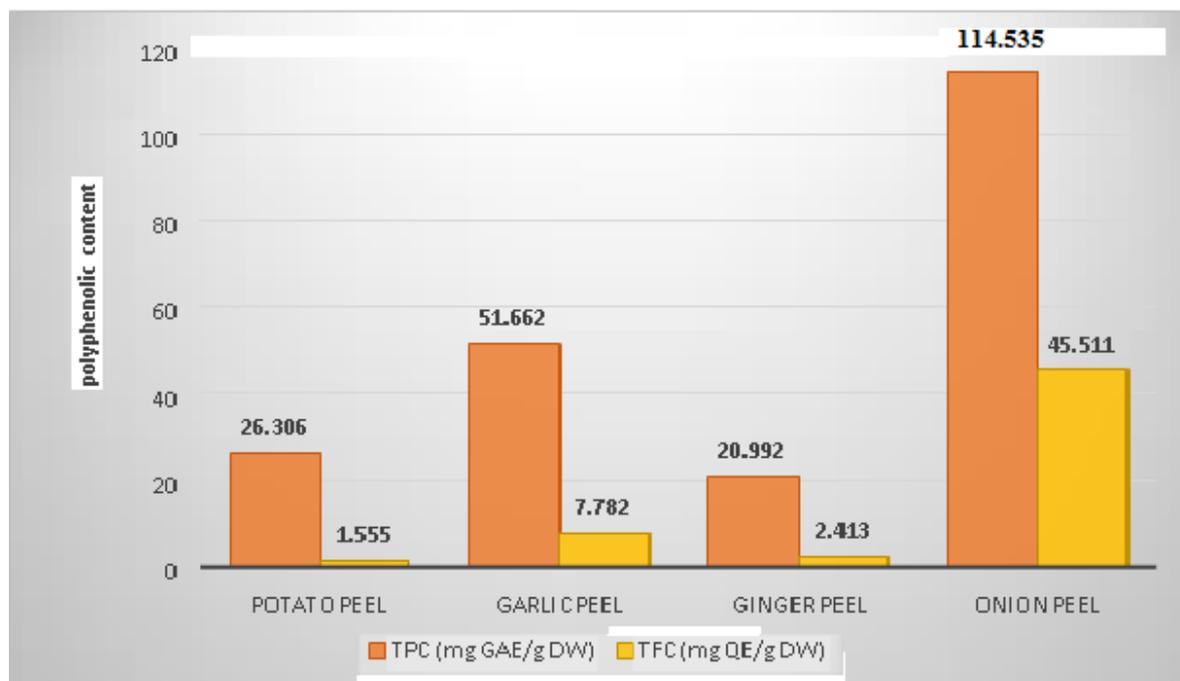
Antioxidant activity was measured following a modified DPPH assay using Trolox as a standard. Results were presented as  $\mu\text{M}$  Trolox equivalent fresh mass. The results shown in table II express the values of antioxidant activity exhibited in all extracts  $\mu\text{M}$  TE/g fresh mass. The radical scavenging potential exhibited by the peel extracts was in the order of 0.068±0.014, 0.093±0.059, 0.197±0.082 and 0.314±0.108 mM TE/g fresh mass for garlic, onion, potato and ginger

peel extracts respectively (Figure 2). As evident from the results (Table II), ginger peel extracts exhibited the highest antioxidant ability among all samples.

Another method employed to measure the scavenging potential of peel extracts was ABTS radical cation scavenging activity. The ABTS<sup>•+</sup> scavenging potential for peel extracts was in the order of 0.151±0.064, 0.231±0.169, 0.469±0.167 and 0.601±0.225 mM TE/g dry weight of garlic, onion, potato, and ginger peel extracts respectively (Table III). Although lower in TPC and TFC values among all extracts, ginger peel exhibited highest scavenging potential in terms of both DPPH<sup>•</sup> and ABTS<sup>•+</sup> scavenging assays.

### Reducing activity

Literature reports are evident that bioactive compounds responsible for antioxidant activity exhibit reducing power (Siddhuraju et al. 2002). The high reducing power of the bioactive compounds is their characteristic property to reduce higher oxidation state to a lower oxidation state. In the present study, reducing the power of peel extracts was evaluated using FRAP (ferric reducing antioxidant power) and TAC (total antioxidant capacity by phosphor-molybdenum) methods (Figure 3). Ferric reducing antioxidant power is linearly associated with the molar concentration of antioxidants and



**Figure 1.** Comparison among peel extracts in terms of TPC (mg GAE/g DW) and TFC (mgQE/g DW).

is reproducible. One of the disadvantages with FRAP reagent is that it does not react readily with some antioxidant like glutathione (Guo et al. 2003). The FRAP values of peel extracts varied from 0.058 to 0.273 mM Fe<sup>2+</sup> Eq/g DW. Among all extracts, ginger peel exhibited the highest and onion peel exhibited lowest FRAP value. Among all extracts, garlic peel extracts (0.47±0.0452 mM AAE/g DW) exhibited highest, while ginger peel (0.097±0.0486 mM AAE/g DW) exhibited lowest reducing capacity in terms of TAC. The highest TAC value for garlic peel indicates that it contains as much quantity of antioxidant compound as equal to ascorbic acid to reduce the oxidant in the reaction matrix (Ouattara et al. 2011). No correlation was found between the polyphenolic contents and reducing the power of the extracts.

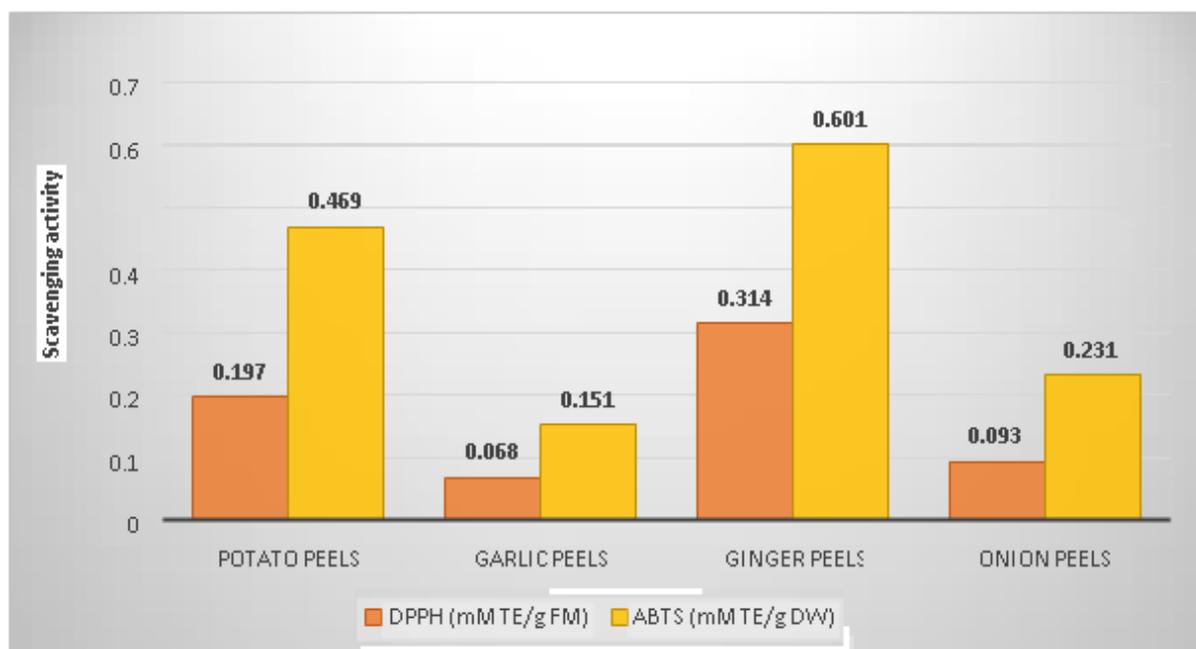
### Antibacterial activity

Antibacterial activity of peel extracts was determined following agar well diffusion method.

Inhibition exhibited by samples against one Gram Negative (*Escherichia coli*) and three Gram Positive (*Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*) bacterial strains was evaluated in Petri plates along control having no drug. Ginger peels exhibited the strongest inhibition against all the bacterial strains while onion peels did not show any inhibition against bacterial strains. No inhibition was found against *Bacillus cereus* by garlic peels while it exhibited slightly positive inhibition against *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus aureus* (Figure 4). Potato peels exhibited strong positive inhibition against *Staphylococcus aureus*, while no inhibition was found against *Bacillus* however, medium positive inhibition was exhibited by potato peel extracts against *Escherichia coli*, *Bacillus megaterium* (Table IV).

**Table II. Scavenging activities of peel extracts.**

Samples	DPPH (mM TE/g FM)	ABTS (mM TE/g DW)
Potato Peels	0.197±0.082	0.469±0.167
Garlic Peels	0.068±0.014	0.151±0.064
Ginger Peels	0.314±0.108	0.601±0.225
Onion Peels	0.093±0.059	0.231±0.169



**Figure 2. Comparison among peel extracts in terms of scavenging activity.**

**Table III. Reducing power of peel extracts.**

Samples	FRAP (mM Fe <sup>2+</sup> Eq/g DW)	TAC (mM AAE/g DW)
Potato Peels	0.101±0.062	0.275±0.08
Garlic Peels	0.098±0.036	0.47±0.0452
Ginger Peels	0.273±0.044	0.097±0.0486
Onion Peels	0.058±0.028	0.185±0.0791

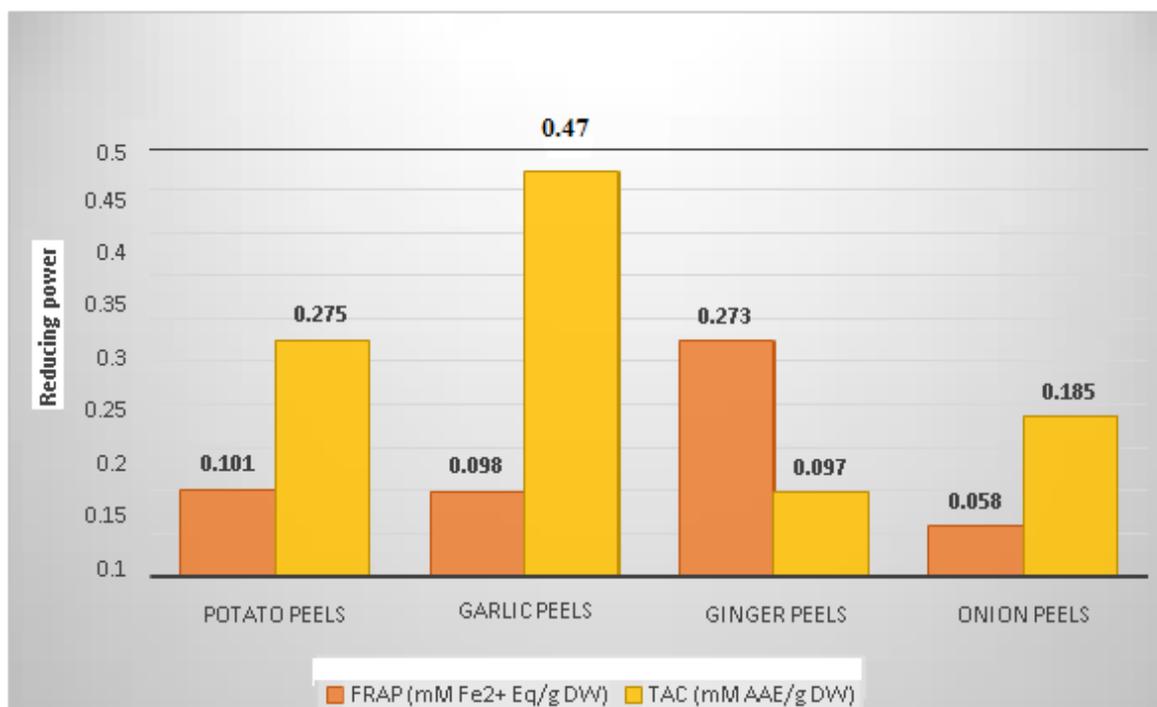


Figure 3. Comparison among peel extracts in terms of reducing power.

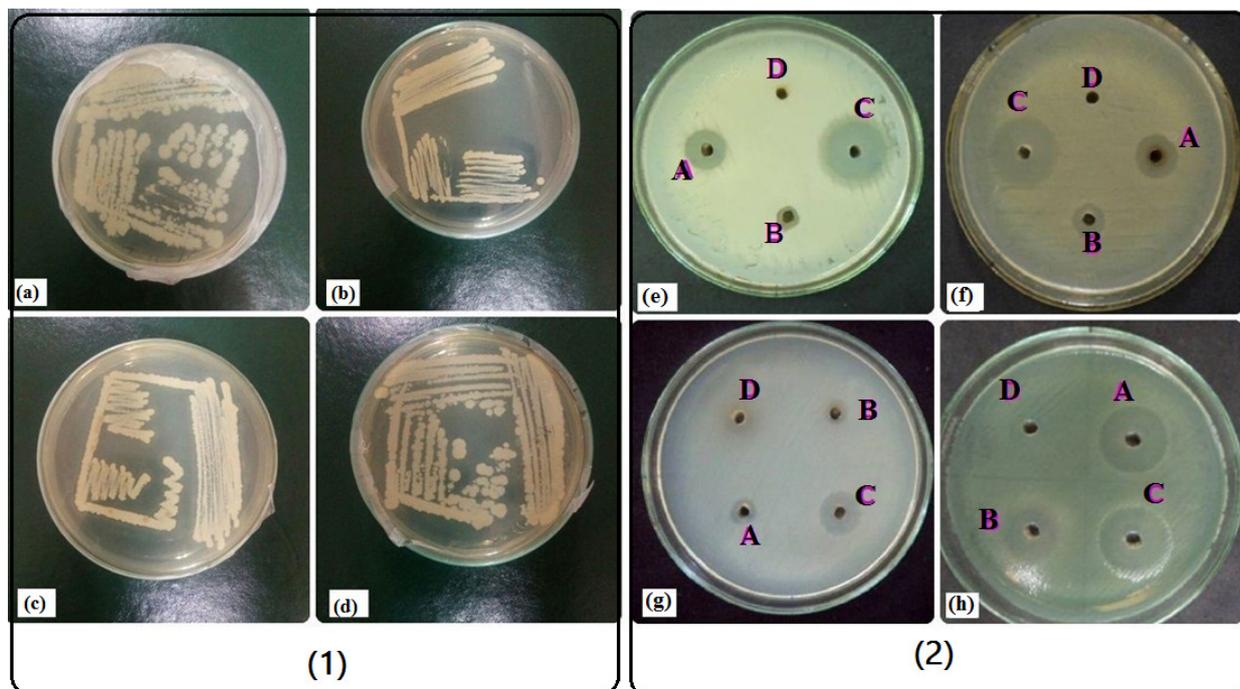
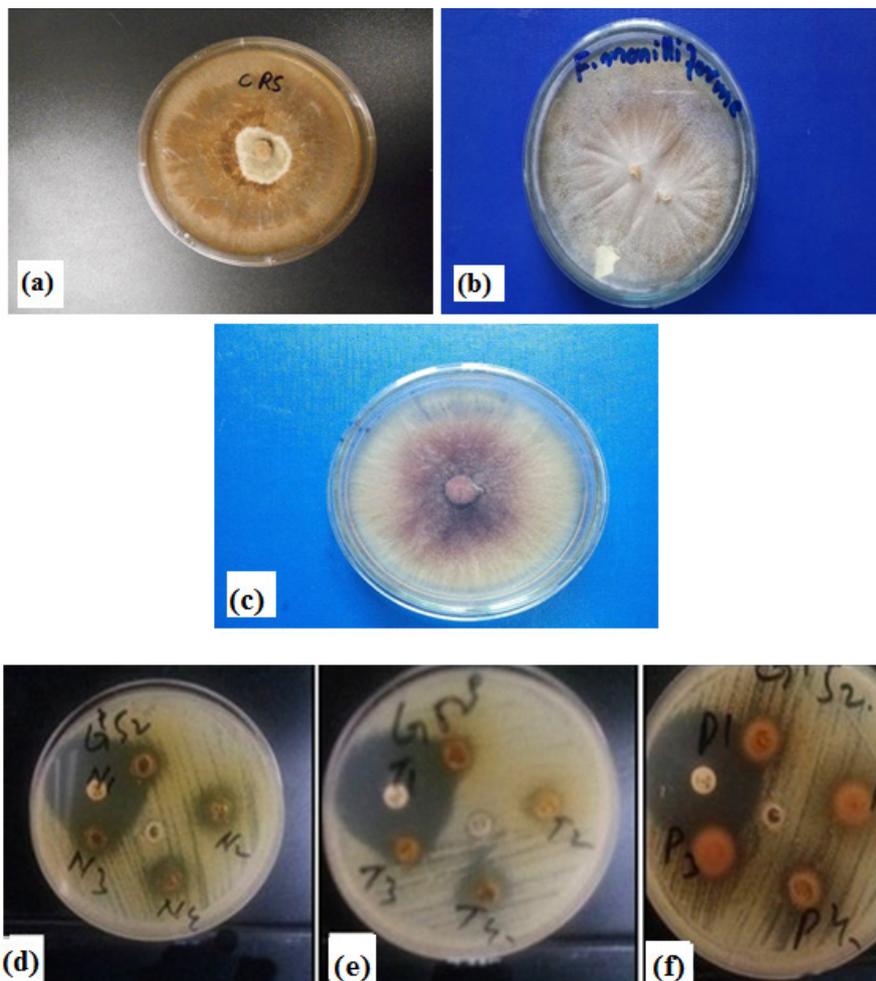


Figure 4. (1) Bacterial Strains (a) *Escherichia coli*, (b) *Bacillus megaterium*, (c) *Bacillus cereus* (d) *Staphylococcus aureus* (2) - Antibacterial activity of extracts against bacterial strains (e) *Escherichia coli*, (f) *Bacillus megaterium*, (g) *Bacillus cereus*, (h) *Staphylococcus aureus*.



**Figure 5.** Three fungal strains (a) *Rhizoctonia solani*, (b) *Fusarium moniliforme*, (c) *Colletotrichum falcatum* (d) Antifungal activity Against *Rhizoctonia solani*, (N1) Potato peels, (N2) Garlic peels, (N3) Ginger peels, (N4) Onion peels); (e) Antifungal activity against *Fusarium moniliforme* (T1) Potato peels (T2), Garlic peels (T3), Ginger peels (T4), Onion peels); (f) Antifungal activity against *Colletotrichum falcatum*, (P1) Potato peels, (P2)Garlic peels, (P3) Ginger peels,(P4) Onion peels).

**Table IV.** Antibacterial activity of peel extracts against four bacterial strains.

S. No	Sample Names	<i>Escherichia coli</i>	<i>Bacillus megaterium</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>
1	Potato peels	++	++	-	+++
2	Garlic peels	+	+	-	++
3	Ginger peels	+++	+++	++	+++
4	Onion peels	-	-	-	-

+++ = Strongly Positive Inhibition, ++ Medium Positive Inhibition, + Slightly Positive Inhibition, - No Inhibition.

### Antifungal activity

Agro-waste (peel) extracts were investigated to determine their antifungal activity against three plant pathogenic fungal strains (*Colletotrichum falcatum*, *Fusarium moniliforme* and *Rhizoctonia solani*) (Figure 5). According to the results shown in Table V potato, garlic and ginger peel extracts exhibited strong positive inhibition against *Colletotrichum falcatum* while, medium inhibition was observed by onion peels against *Colletotrichum falcatum*. Medium positive inhibition was observed in case of ginger, garlic and potato peel extracts against *Fusarium moniliforme*, however, onion peels exhibited slight positive inhibition against *Fusarium moniliforme*. Onion and garlic peels exhibited medium positive inhibition against *Rhizoctonia solani* while, potato and ginger peels exhibited slightly positive inhibition against *Rhizoctonia solani*. Inhibition against fungal strains (*Rhizoctonia solani*, *Fusarium moniliforme*, *Colletotrichum falcatum*) was observed in Petri plates along with the control having no drug. Peel extracts exhibit great

potential as antimicrobial agents against pathogenic microbe, thus, possess potential in the pharmaceutical industry. The synergistic effect of plant extract and antibiotic association against resistant pathogens leads to innovative choices for the treatment of infectious diseases. This effect enhances the therapeutic ability of antibiotic, which itself is no longer effective during medical treatment. Ginger peels and garlic peels exhibited highest antibacterial and antifungal activities as reported in previous studies (Elizabeth et al. 2013).

### CONCLUSION

The samples examined in this study may be used as low-cost natural antimicrobials and antioxidants. It is predicted that the use of natural products as therapeutic agents probably does not induce resistance in microorganisms. Further isolation and purification of active components of these agro-wastes are required to be used in food and pharmaceutical industries. Thus, garlic peels, as evident from its highest antioxidant

**Table V. Antifungal activity of extracts against three fungal strains examined in experiment.**

S. No	Sample Names	<i>Rhizoctonia solani</i>	<i>Fusarium moniliforme</i>	<i>Colletotrichum falcatum</i>
1	Potato peels	+	++	+++
2	Garlic peels	++	++	+++
3	Ginger peels	+	++	+++
4	Onion peels	++	+	++

+++ = Strongly Positive Inhibition, ++ Medium Positive Inhibition, + Slightly Positive.

Inhibition, - Negative Inhibition.

Inhibition against fungal strains (*Rhizoctonia solani*, *Fusarium moniliforme*, *Colletotrichumfalcatum*) was observed in petri plates along with the control having no drug.

and antimicrobial activities, could be utilized as an economic way against pathogenic diseases and has been proposed as a novel ingredient for this purpose as well as to reduce the problem of multi-drug resistant pathogenic bacteria. This study has unlocked the opportunity of the use of agro-waste in drug development for the treatment of various infectious diseases. These are fresh, natural and economic sources, which can be employed in the prevention of disorders induced by pathogenic microbes. Thus, this research opens insights for future utilization of the agro-waste for medicinal uses. The study recommends that selective extraction of agro-waste, by an appropriate solvent, is vital for obtaining fractions with high biological activity.

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**How to cite**

NAQVI SAZ, IRFAN A, ZAHOR AF, ZAFAR M, MARIA A, CHAND AJ & ASHFAQ S. 2020. Determination of antimicrobial and antioxidant potential of agro-waste peels. *An Acad Bras Cienc* 92: e20181103. DOI 10.1590/0001-3765202020181103.

*Manuscript received on October 23, 2018;  
accepted for publication on January 8, 2019*

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All authors contributed to the research work. Syeda Andleeb Zahra Naqvi, Ali Irfan, performed experimental work. Anum Maria, Amar Jeet Chand, Shomaila Ashfaq did sample collection and paper write up. Syeda Andleeb Zahra Naqvi, Ameer Fawad Zahoor and Muhammad Zafar supervised the work.

