

## Evaluation of Antimicrobial and Phytochemical Properties of Agro-Waste and their Potential Applications as Bio-Preservatives and Natural Dyes

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### ABSTRACT

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Around 40% of agro-waste is generated globally every year most of which is not composted due to improper disposal methods, this increases the carbon footprints which in turn causes a great deal of harm to the environment. The aim of this experiment was to evaluate the antimicrobial and phytochemical properties of extracts prepared of lemon peel (*Citrus limon*), onion peel (*Allium cepa*) and potato peels (*Solanum tuberosum*). The project was an attempt to increase sustainability and decrease waste generation by complete utilization of agro-products prior to disposal. The peels were collected from local restaurants and food joints to aid in reduced waste generation. The extracts were prepared using home-based methods; laboratory extraction using Soxhlet apparatus was carried out for lemon peel extract. For the antimicrobial analysis, four organisms were used, two of which were gram positive (*Staphylococcus aureus*, *Bacillus cereus*) and two were gram negative (*Escherichia coli*, *Pseudomonas putida*). *S.aureus* was isolated from a poultry source whereas *E.coli* was isolated from fish drain water. The lemon peel extracts showed a high degree of antimicrobial activity against all the test organisms, whereas the onion peel extracts positively retarded the growth of both the gram-positive test isolates and consecutively, potato peel extracts showed bactericidal effects against *E.coli*. The extracts were analyzed for presence of phytochemicals namely, tannins, phenols, saponins, flavonoids, vitamin C; promising results were obtained proving the importance and medicinal relevance of the extracts. To check for preservative attributes of the extracts, quantitative and qualitative examinations were conducted, wherein, efficacious results were noted for the lemon peel extracts, however, actions of onion peel extracts and a combination of extracts was culminated to be inconclusive due to excess coloration/ pigmentation. Lastly textile/ fabric dyes were yielded from the prepared extracts, and as observed, the color was retained by the cloth after addition of mordants.

**Keywords** : Agro-waste, sustainability, antimicrobial activity, phytochemical analysis, bio-preservatives, textile dyes

## I. INTRODUCTION

The jettison of agro-horticultural peels as a byproduct on a large scale by local or industrial sectors, can in the long run have a negative impact on the environment; this maybe concerning at various economic and environmental levels. Similarly, when household waste is discarded, many a times due to improper disposal methods, inappropriate garbage segregation, etc. it may be detrimental to the environment. Accumulation of such waste may also cause drastic increase in carbon footprints thus aiding in unfavorable and damaging effects like high pollution levels and ozone depletion. The FAO (United Nations Food and Agricultural Organizations) has estimated that the losses and waste in fruits and vegetables are highest and may even reach up to 60% soon if these are not completely utilized.

Lemon, (*Citrus limon*), Onion (*Allium cepa L*) and Potato (*Solanum tuberosum*) are some of the most common fruit and vegetables used in almost every household for the extremely high flavor they add to the delicacy. However, in most kitchens (domestic as well as industrial), the peels of these agro-products are disposed causing substantial loss of essential nutrients. [1] Previously conducted studies have shown that the rinds of these fruit and vegetables are as essential as the pulp itself. [1,2] Throughout the experiment, the peels of lemon, onion and potato have proved to be important antimicrobial agents especially against food borne pathogens that may contaminate food and cause ill health [8, 11, 12]. In addition, they also possess prime phytochemical elements, which made it feasible to test for bio-preservative applications and conclusively due to pigmentation of the extracts, natural textile/ fabric dyes were also yielded.

The micro-organisms selected for the study may cause severe harm to immuno-compromised patients for example, *E.coli* may result in one of the common bacterial infections of the urinary tract, *S.aureus* may contribute in causing skin infections, abscesses, etc. Hence, the extracts prepared may also be used for medicinal formulations like anti-rash creams, ointments, nutraceuticals to treat such infections, etc. Owing to their anti-microbial properties, the agro-waste extracts may also be used as surface disinfectants.

This study is mainly aimed at the various properties of and complete exploitation of the rinds of lemon, onion and potato, thus, not only using the peels as a potential nutraceutical but also relieving the burden of unnecessary waste disposal and dumping.

## II. MATERIALS & METHODS

The peels for preparation of extract were obtained from household kitchens across Mumbai, Maharashtra. Peels were also collected from local restaurants and food joints/ trucks around Mumbai. The peels were washed and air-dried overnight and used for further extraction.

Samples used for isolation of microorganisms i.e. chicken breast piece weighing 1.5 g was obtained from a poultry in Borivali, Mumbai and used for isolation of *S.aureus* and sewage sample used for isolation of *E.coli* was obtained from a local sewer of a fish market in Mumbai.

The media used for the experiment was procured from HiMedia. Nutrient agar was used for initial isolation, MacConkey agar media and Salt mannitol agar media

was used for selective, differential isolation of *E.coli* and *S.aureus* respectively. The Muller Hilton agar media was used for antimicrobial analysis. The alcohol (ethanol from alcoholic beverages i.e. vodka) used for preparation of extracts was purchased from local shops in Mumbai, whereas, the hexane used for the process of Soxhlet extraction was procured from Labogens.

#### A. Isolation of test organisms:

For isolation of *S.aureus* (isolate 1) 1.5 g of meat sample was minced and suspended in 5 ml sterile saline. [3, 4] Whereas, for isolation of *E.coli* (isolate 2) sewage water was used [5]. The respective samples were serially diluted (10-fold dilutions). The last 3 dilutions ( $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) were inoculated by the spread plate method on nutrient agar and incubated at 37°C for 24 hours. Following which, the suspected colonies were picked and a culture suspension of respective organisms was prepared and used for further isolation and identification.

#### B. Identification of the isolates:

Characterization and identification of the isolates was carried out by gram staining and performing a series of biochemical tests (indole, methyl red, Voges-Proskauer, citrate tests). The isolates were confirmed by streaking on selective media i.e. Mannitol salt agar for *S.aureus* and MacConkey agar for *E.coli*. These isolates were used as gram-positive and gram-negative test organisms for antimicrobial evaluation of the peel extracts. [4,5]

#### C. Preparation of extracts from the peels:

50 g of peels (onion [OP], lemon [LP] & potato [PP]) were soaked in the respective solvents, water for aqueous extract (Aq) and ethanol for alcoholic extract (Alc) for 48 hours [9, 10, 13]. Following which the solution was boiled for 10 minutes and the extracts were strained and stored in sterile flask in dark conditions until further use. The Soxhlet extract of

lemon peels (Sox) was prepared using hexane as the solvent. The temperature was set at 60°C and the Soxhlet was run for 5 hours [6]. The extract was kept in a rotary shaker to evaporate any excess solvent and stored in a sterile flask in dark conditions until further use.

#### D. Antimicrobial analysis of the prepared extracts:

Antimicrobial activities of the extracts were evaluated by a combination of pour plate and agar well diffusion method, microbial test cultures of *E.coli*, *S.aureus*, *P.putida* and *B.cereus* were used for the same. Plates were prepared by inoculating, 0.5ml of each culture in 50ml of Mueller-Hinton agar media (40° C) and mixed by simple inversion. Wells were punched with the help of a cork borer after solidification of the media, to which the extracts and antibiotics (for positive control) were added. The zone of microbial growth inhibition for bacteria was measured after incubation at 37°C for 24 hours and the clear zones of inhibition if observed around the wells were measured in cm. [8, 11, 13]

The antibiotics used as positive control were of 10 mg/ml concentration. Ampicillin was used against *E.coli* and *B.cereus*. Vancomycin against *S.aureus*, and kanamycin against *P.putida*

#### E. Phytochemical tests:

The extracts were evaluated for the presence of the following phytochemicals: [14, 15, 16]

**Test for Tannins:** 2-3 drops of FeCl<sub>3</sub> was added to 2 ml of extract.

**Test for Flavonoids:** To 1.5 ml of AlCl<sub>3</sub>, 2.5 ml of extract was added.

**Test for Phenols:** 1 ml of extract with 5 ml of Folin-Ciocalteu reagent and 4 ml of sodium carbonate solution was mixed and left to stand in a water bath for 30 minutes at 40°C.

**Test for Saponins:** 2.5 ml of extract was supplemented to 10 ml of distilled water followed by vigorous shaking.

**Test for Vitamin C:** 1g of DCPIP was dissolved (dichlorophenolindolphenol) in 100 ml of distilled water. 1.5 ml of this solution was then added to a test tube followed by drop-wise addition of the extracts until a color change was observed.

**F. Bio-preservative efficacy tests:**

1) Qualitative evaluation of bio-preservative activity: Preservation properties of the extracts were evaluated by coating 1cm cubed pieces of apple and tomato with the extracts and maintaining appropriate controls (positive control coated with of vinegar and negative control without any extracts). The coated pieces were maintained for a period of 7 days at 12°C -18°C and changes were observed. [18]

2) Quantitative evaluation of bio-preservative activity:

To a test tube, 1ml of nutrient broth (NB) along with 1 ml of extract was added. These tubes were then inoculated with *S.aureus*, *B.cereus*, *E.coli* and *P.putida*. The optical density (O.D.) of the suspension was measured at 540 nm prior to incubation. Post incubation, at 37°C for 24hours, the O.D. of the tubes containing the mixture were measured again and the differences were noted. A negative control with 2 ml of nutrient broth and a positive control with 1 ml nutrient broth and 1 ml of known preservative (vinegar) were maintained for reference and comparison. [15, 17]

**G. Potential of the extracts as textile/fabric dye:**

A piece of white cotton cloth 2.5x2.5 cm was boiled in a mixture of water and sodium chloride (38%) for 5 minutes and dipped in cold water to help dye absorption. Following which, the piece was soaked in the prepared peel extracts for 5hours. Alum (potassium Aluminum sulfate,  $KAl(SO_4)_2 \cdot 12H_2O$ ) at a 15% concentration, was used as mordant to increase color retention and the cloth was soaked again overnight and allowed to dry for 2 hours. [19, 20] The cloth was then washed to check if it retained color. A negative control

as a plain white cloth was maintained along with a positive control that was an already dyed cloth.

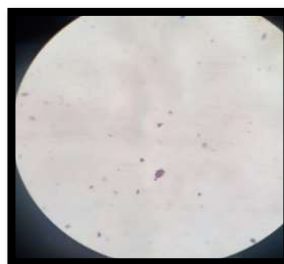
**III. RESULT & DISCUSSION**

**A. Isolation and characterization of isolates:**

Colonies on the two nutrient agar plates, 24 hours post inoculation, were observed and studied. The round, golden – yellow colonies and the large, thick greyish white colonies were characterized to be *S.aureus* and *E.coli* respectively.

**B. Identification of the isolates:**

Gram positive cocci were observed on gram staining (figure 1a), whereas streaking on Mannitol Salt agar helped in presumptive verification as colonies observed were yellow while turning the media acidic as well (figure 1b). Positive results on catalase, coagulase, nitrate reduction and urease tests and the fermentation of glucose, mannitol and lactose sugars helped in validating the identification of isolate 1 as *Staphylococcus aureus*.



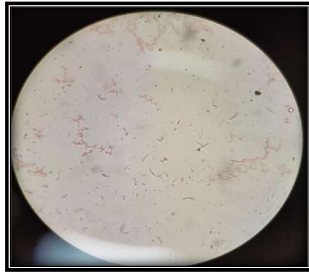
**Fig 1a-** Gram positive cocci observed under the microscope on gram staining of isolate 1.



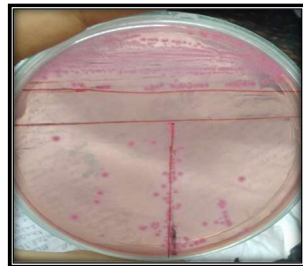
**Fig 1b-** Colonies observed on T – streaking isolate 1 on mannitol salt agar.

The second isolate showed gram negative rods on gram staining (figure 2a) and showed lactose fermenting colonies with pink color as well as dark pink precipitates around the border when plated on MacConkey agar media (figure 2b). Positive catalase, methyl red, indole, citrate utilization and triple sugar iron tests, while also fermenting the sugars, xylose,

sucrose, maltose, glucose, mannitol and lactose, thus substantiated the identification of isolate 2 as *Escherichia coli*.



**Fig 2a-** Gram negative rods observed under the microscope on gram staining of isolate 2



**Fig 2b-** Colonies observed on T – streaking isolate 2 on MacConkey agar.

**C. Antimicrobial Assay of the extracts:**

Antimicrobial assay of the prepared extracts was carried using agar well diffusion method and was compared to the activity of standard antibiotics and maintaining appropriate controls. The results of the

antimicrobial assays showed that lemon peel extracts have the highest antimicrobial activity among the extracts and the onion peel extracts are more active against gram positive organisms than gram negative organisms. The extracts of potato peel showed inconclusive antimicrobial activity, this may be owing to that fact that part of the extraction procedure was performed outside a controlled environment. (zone sizes in cm mentioned in the graphical representation i.e. graph 1).

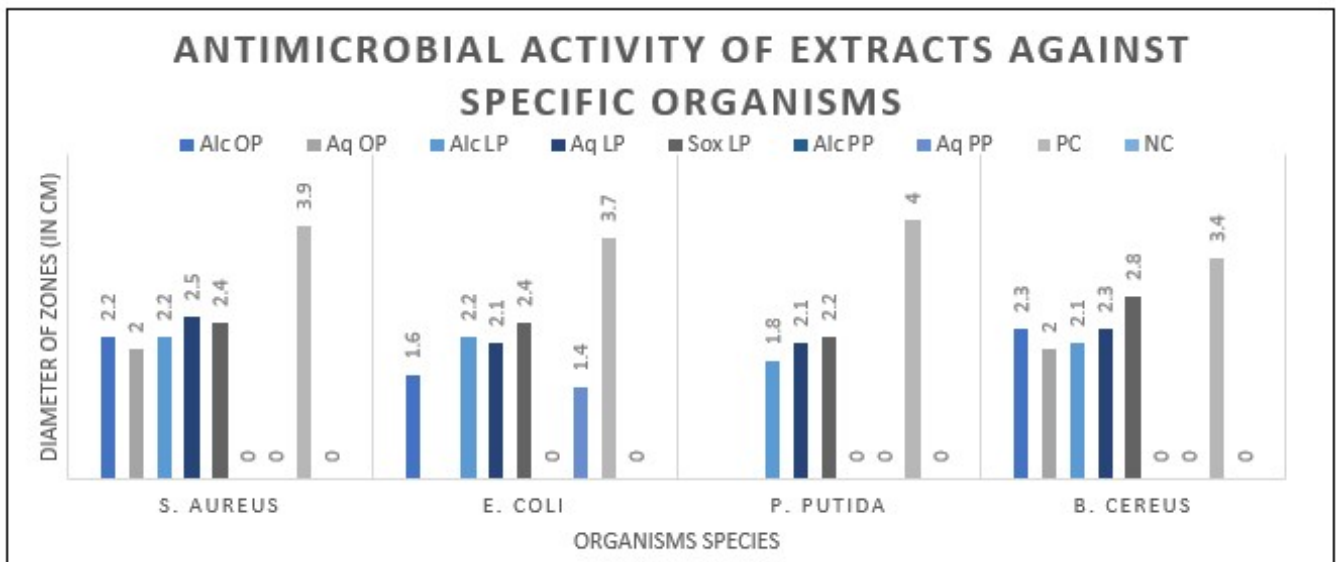
The below mentioned sequences are the antimicrobial activities of different extracts in descending order (from most active against a specific organism to least active).

*S.aureus:* Aq LP > Sox LP > Alc OP > Alc LP > Aq OP

*E.coli:* Sox LP > Alc LP > Aq LP > Alc OP > Aq PP

*B.cereus:* Sox LP > Alc OP > Aq LP > Alc LP > Aq OP

*P.putida:* Sox LP > Aq LP > Alc LP



**Graph 1 :** Graphical representation of antimicrobial activity against specific organisms.

**A. Phytochemical analysis:**

Phytochemical tests	Alc OP	Aq OP	Alc LP	Aq LP	Sox LP	Alc PP	Aq PP



<b>Phenols</b>	+	+	+	+	+	-	+
<b>Tannins</b>	+	+	+	+	-	-	+
<b>Flavonoids</b>	+	+	+	+	+	+	-
<b>Saponins</b>	+	+	+	+	+	+	+
<b>Vitamin C</b>	+	+	+	+	+	+	-
<b>Key:</b>	+		Presence of phytochemical in the given extract				
	-		Absence of phytochemical in the given extract				

**Table 1** : Results of the phytochemical analysis of agro- peel extracts

Based on the phytochemical analysis, identical phytochemical constituents were observed in Aqueous and Alcoholic extracts of Onion and Lemon peel. The red onion peel is purplish-red in colour and contains quercetin 4'-O-β-glucopyranoside as the major flavonoid in the peels which can be developed as complementary and alternative healthcare products. [15]

Tannins that were observed in extracts apart from Soxhlet extract of lemon peel and alcoholic extract of potato peel are reported to have antibacterial activity, anti-carcinogenic property against human melanoma cells, anti-oxidant property and anti-helminthic activity[14, 16]. Saponins are glycosides with triterpenoid steroidal aglycones and display bioactive properties, such as antimicrobial activity and were seen to be present in every extract, whereas polyphenols and flavonoids inhibit lipid oxidation and prevent cellular membrane damage and were noted in all extracts except aqueous extract of potato peels [14,15].

**D. Bio-preservative efficacy tests:**

1) Qualitative/ observatory bio-preservative test:

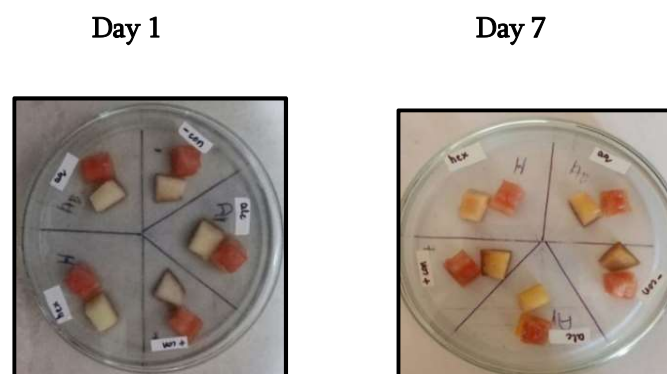
The bio-preservative potential of the prepared extracts was studied by coating fruit pieces with the extracts and observing the changes throughout a week. (Refer to figure 3 to observe changes occurred.)

**Day 1:** All fruit pieces were properly maintained and no signs of spoilage were observed.

**Day 4:** The pieces not coated with the extract (control) started to oxidize (turn brown) and the extract-coated pieces showed no signs of spoilage.

**Day 7:** Fungal growth was observed in the negative control pieces of tomato and apple. The apple piece coated with alcoholic extract had turned considerably brown indicating oxidation. The apple piece coated with Soxhlet extract showed no signs of spoilage. The pieces of tomato coated with aqueous and alcoholic extracts were properly preserved.

The findings of this study suggest that organic peel extracts could have potential applications in the food industry as natural preservatives. The odor and taste imparted by these extracts can be masked with further organoleptic studies.



**Figure 3-** Qualitative bio-preservative test i.e. effect of extracts coating on fruits.

2) Quantitative bio-preservative test:

The optical density (O.D.) was measured using a colorimeter, at a wavelength of 540nm on day 1 (day of inoculation) and day 2 (24 hours after incubation of prepared inoculated mixture).

The excessive coloration/ pigmentation of the onion peel extracts and a combination of onion and lemon

peel extracts affected the O.D. readings leading to inconclusive results.

The lemon peel extracts showed bacteriostatic to mild bactericidal activity as evident by the O.D readings (as mentioned in table 3) suggesting their use as preservatives on a large scale.

		NB + Alc LP	NB + Aq LP	NB + Sox LP	NB (NC)	Vinegar + NB (PC)	Distilled water (standard)
Day 1	<i>S.aureus</i>	0.83	0.73	0.64	0.04	0.02	0.0
	<i>E.coli</i>	0.83	0.75	0.59	0.03	0.03	0.0
	<i>P.putida</i>	0.87	0.72	0.52	0.03	0.02	0.0
	<i>B.cereus</i>	0.88	0.59	0.63	0.03	0.04	0.0
Day 2	<i>S.aureus</i>	0.81	0.75	0.54	0.09	0.03	0.0
	<i>E.coli</i>	0.81	0.79	0.46	0.11	0.04	0.0
	<i>P.putida</i>	0.88	0.60	0.46	0.33	0.03	0.0
	<i>B.cereus</i>	0.88	0.66	0.60	0.24	0.02	0.0

**Table 3 :** Optical density of the prepared mixtures before and after a 24-hour incubation for quantitative analysis of bio-preservative activity

**A. Potential of the extracts as textile/ fabric dye:**

Lemon peel extract: The colors of the fabric showed a gradient effect; aqueous extract of lemon peel showed the highest intensity of color while not fading even during or after washing. The alcoholic extract of lemon peel gave a lighter shade of yellow. Whereas the Soxhlet extract of lemon peel gave a lime-yellow dye.

Onion peel extract: Neither of the colors of dye faded after washing. The alcoholic extract of onion peel gave a dark wood brown color while the aqueous extract gave a lighter more vibrant shade of brown.

Potato peel extracts: The colors very slightly faded after a thorough wash. The alcoholic extract of potato peel gave an off-white creamish color appearance

whereas, the aqueous extract of potato peel gave a more neutral, nude/ skin color.

The positive and negative controls maintained were unaffected and remained stable throughout.

**IV. CONCLUSION**

Agro-wastes cannot be exhaustively minimized but can be reused to a certain extent. Hence, this study was an attempt to progressively deploy these wastes. This study was aimed at utilizing lemon, onion and potato peels which are a major part of agro-wastes in a salutary manner. The extracts of onion, potato and lemon peel were prepared using suitable aqueous and alcoholic solvents. The antimicrobial activity of the extracts was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and

*Pseudomonas putida*. On comparing the zones of inhibitions of the lemon, onion and potato peel extracts it was interpreted that the extracts of lemon peel showed prominent antimicrobial activity against all the organisms as compared to the extracts of onion and potato peels. The results from the phytochemical tests performed supported the fact that the presence of phytochemicals in the peels enhances the antimicrobial and bio-preservative activity of the extracts.

The bio-preservative activity of the prepared extracts was studied and it showed that the pieces coated with extracts showed delayed signs of spoilage as compared to the negative control which, along with the quantitative bio-preservative test suggested that the extracts could have potential as bio-preservatives in the food industry. In addition, an effort was also made to utilize waste material as an efficient natural colorant which could reduce the cost as well as harmful effects of chemical dyes.

The results obtained in the study proved the significance and medical relevance of these extracts and suggested their potential as commercial antimicrobial, bio-preservative and dyeing agents. With extensive research these extracts could be further used as antimicrobial agents (antibiotics) against emerging resistant microbial strains.

**ABBREVIATIONS**

Codes	Names
Alc OP	Alcoholic onion peel extract
Aq OP	Aqueous onion peel extract
Alc LP	Alcoholic lemon peel extract
Aq LP	Aqueous lemon peel extract
Sox LP	Soxhlet extract of lemon peel
Alc PP	Alcoholic potato peel extract
Aq PP	Aqueous potato peel extract
PC	Positive control
NC	Negative control

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