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Agri-Food Extracts Effectiveness in Improving Antibacterial and Antiviral Properties of Face Masks: A Proof-of-Concept Study

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The European dependencies for raw materials supply from foreign countries have been unquestionably shown by COVID-19 outbreak and have become particular evident from the slow response to the need for high quality personal protective equipment (PPEs). Among all medical devices, surgical face masks have earned themselves a primary role for the containment of the epidemic. In this context, our work aims at improving the barrier effect of surgical mask by depositing on their external surface a mixture of bioactive compounds, mainly polyphenols, extracted from agronomical sources. The main objective is the integration of the biorefining of agri-food solid wastes with the potential virucidal properties of the polyphenolic extracts for the treatment of PPEs.

Introduction

SARS-CoV-2 infection is the cause of a new disease (COVID-19), for which there is currently no specific drug for its prevention and therapeutic treatment. In the meantime, the principal strategies recommended by World Health Organization (WHO) are social distancing, hand washing, close environments disinfection and the use of masks in order to reduce the main source of transmission, namely the droplets emitted by infected subjects thus guaranteeing the protection of healthy subjects.[1] At the same time, a number of works recently published in the wave of the outbreak are questioning the real effectiveness of these devices in filtering SARS-CoV-2 during coughs by infected patients.[2] The mask is often worn incorrectly (under the nose or loosened) and frequently touched, thereby reducing its protective efficacy both in outgoing-exhalation (protection of others), and in entry-inhalation (protection of the wearer) and increasing the risk of contamination of the user's hands and surfaces. Thus, the improvement of filtering ability and fast virus inactivation of surgical mask is a subject of the outmost importance.

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The demand for these devices is increased significantly in the post-lockdown phases of the fight against COVID-19, which often include the mandatory use of masks for the entire population. In Italy, where the Ministry of Health extended the obligation to wear a mask in close environments as well as outdoors, several regions as Regione Toscana took care of providing a free supply of masks to the citizens.

From a number of studies developed in recent years, the potential pharmacological activity of polyphenols emerged.[3–9] In particular, oleuropein[10] and its derivatives tyrosol and hydroxytyrosol,[11] extracted from olive leaves show antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer activities, antimicrobial activity, antiviral activity, hypolipidemic and hypoglycemic effect, as well as eugenol, the main component of clove buds (family *Myrtaceae*, *Eugenia caryophyllus*).[12–18] A study showed that eugenol inhibits the replication of influenza A virus by interfering with the ERK, p38MAPK and IKK/NF-κB signal pathways.[19]

Various studies in the last ten years have also investigated the antiviral activity of tea polyphenols focusing on *in silico* and *in vitro* activity of epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin, gallocatechin-3-gallate, gallocatechin, catechin gallate and catechin against SARS-CoV-2 main protease.[20–24]

Analysis of SARS-CoV-2 suggests that the novel virus, such as SARS-CoV virus, uses ACE2 receptors to enter cells.[25–27] SARS-CoV-2 has a significant resemblance with SARS-CoV with the percentage resemblance to the genome being 79%.[28,29] There are also studies showing that SARS-CoV and SARS-CoV-2 have nucleotide similarity of 89.1% and nucleotide identity of 80%.[30] The key infection system of SARS-CoV-2 is its trimeric surface spike (S) glycoprotein which mediates the entry into the host cell, and its affinity to the ACE2 receptor is higher than that of SARS-CoV.[31] Protein S, and in particular SARS2-S1B receptor binding domain (338-506 amino acid residues), is characterized by a series of secondary structure loops mainly formed by antiparallel beta planes. It can be reasonably to hypothesize that the interaction of polyphenols with the beta structures of protein S entails conformational changes to prevent the attack of the virus to the host cell.[32,33] A number of molecular docking and dynamic simulation studies aiming at deepening this aspect are currently in progress.[21,22,34,35]

In several applications polyphenols have been used to treat materials in order to give them antiviral properties.[36–38] Thus, the application of polyphenolic compounds on the external surface of disposable face masks is expected to increase their filtering effectiveness inactivating virions, to reduce cross-contamination of operator's hands when the mask is removed, and to extend the lifetime of the mask itself. Catel-Ferreira et al. grafted catechin polyphenol molecules on the surface of non-woven cellulosic fiber filters, obtaining efficient virucidal wipes for elimination of viruses from contaminated surfaces.[36] Two patents have been published in the United States, USA 5888527[37] and USA 5747053[38] for the preparation of masks with antiviral activity, impregnated with polyphenolic tea antioxidants (catechin and theaflavin).

In these studies, however, pure compounds were used for surface modification. The use of plant-based polyphenols can be identified as an environmentally sustainable and friendly approach to meet the pressing need for highly available sanitizers. Their production can be adapted starting from local biomass waste, thus simplifying the supply of raw materials, and their final yield and exploitation in view of directives and circular economy principles can be significantly increased by advanced extraction technologies (e.g. ultrasounds) although the conventional hydroalcoholic extraction remains the easiest approach to replicate even on a large scale.[39]

This work aimed to test the employment of extracts from three different agri-food resources, known for their content of bioactive molecules with antioxidant, antibacterial and antiviral properties, i.e. cloves, olive leaves and green tea, to modify non-woven tissue and to test their virucidal and antimicrobic properties.

Cloves are the aromatic flower buds of the tree *Syzygium aromaticum*, a plant native native to the Maluku Islands in Indonesia, and mainly used as a spice and in general employed for the essential oil extraction.[40] Clove buds are among the spices with the highest content of total polyphenols,[41] which is for 60–90% eugenol.[42] Moreover, eugenol is generally recognized as a safe and non-mutagenic compound.[43] Clove buds are usually exploited for the production of essential oil resulting in the production of valuable solid and liquid waste, reach of polyphenols.[44] Olive leaves are waste material obtained from olive pruning. Tea is one of most favored drink in all societies and tea waste, named "caffeine dust", is produced at large quantities and typically for energy production and recently as sorbent.[45]

The final goal of this work is the production of a prototypal face mask showing a more effective barrier effect using an easy to implement strategy, that could be extended and replicated promptly with a low effort to different industrial environments. For this reason, the chosen methodology for polyphenols deposition on non-woven tissue was a simple adsorption of solutions or suspensions of the active ingredients on the polymeric fibers. A suitable formulation of this preparation may eventually be used to renew the surface and to extend the use of the mask. An additional application could be the production of filters, treated with polyphenols and inserted into home-made cotton masks to increase the durability, sustainability and efficiency of these devices.

Therefore, by considering the described scenario and the purpose of the research, the results reported in this work are focused on the preparation and characterization of non-woven polypropylene fabric (NWF_PP) treated with polyphenols. NWF_PP samples were directly obtained from facial masks distributed from Regione Toscana during the first phase of the epidemic outbreak. Each extract and NWF_PP treated with the extracts were characterized from the chemical, structural and thermal point of view. The content of active polyphenols in the extracts was determined by reversed phase HPLC. The antibacterial and antiviral properties of the extracts were also evaluated by experimental tests towards NDM-producing *Klebsiella pneumoniae* that can cause different types of health-care-associated infections, including pneumonia and two respiratory transmitted viruses (Human Adenovirus 5-HAdV5 and Human Coronavirus 229E-HCoV229E) following the procedure of UNI EN ISO 18184: 2019 "Determination of antiviral activity of textile products".

Results

HPLC, FT-IR and thermogravimetric analysis of the raw extracts. The individual constituents of the extracts were assessed by HPLC-DAD/FD analysis. HPLC chromatograms of standard compounds (Figures S1–S4) and samples (Figures S5–S7) are shown in ESI. With respect to clove buds, both olive leaves and green tea extracts showed complex chromatograms. The main polyphenols were identified by the comparison of UV absorbance spectra and retention time (t_R) of their corresponding analytical standards, obtaining results are consistent with the literature. Eugenol (t_R 35.09 min) was the main compound in clove buds, while hydroxytyrosol (t_R 15.17 min), tyrosol (t_R 19.11 min) and oleuropein (t_R 30.02 min) were found in olive leaves. In green tea, gallic acid (t_R 11.85 min), pyrocatechol (t_R 14.48 min), catechin (t_R 20.40 min), epicatechin gallate (t_R 22.92 min) and epicatechin (t_R 23.46 min) were identified.

Oleuropein is the main compound found in olive leave extracts obtained using high % of ethanol, as organic solvents tend to deactivate the enzymes responsible for the conversion of oleuropein into other molecules. The high content of oleuropein is also due to the use of dried leaves, where the activity of the enzyme β-glucosidase is inhibited.[46] The intense peak at 16.2 min (Figure S5) could be tentatively assigned to protocatechuic acid, a polyphenolic compounds commonly found in the ethanolic extracts of olive leaves.

The concentration of the identified polyphenols in the alcoholic extracts is reported in Table 1.

Table 1. Concentration as mg/g dry weight (DW) of the identified polyphenols found following hydroalcoholic extraction.

HPLC-DAD/FD (mg/g DW)	*Olive leaves*	*Clove buds*	*Green tea*
Oleuropein	5.24	–	–
Hydroxytyrosol	0.022	–	–
Tyrosol	0.0041	–	–
Epicatechin	–	–	1.38
Gallic acid	0.0054	–	2.37
Pyrocatechol	–	–	0.35
Epicatechin gallate	–	–	43.05
Eugenol	–	9.92	–
Catechin	–	–	0.55
Folin-Ciocalteu			
TPC (mg GAE/g DW)	20.42	119.49	101.20

The total content of polyphenols (TPC) evaluated as gallic acid equivalent (GAE) on the basis of the Folin-Ciocalteu test, was almost five times higher in clove buds and green tea with respect to olive leaves.

The antioxidant activity of the extracts was evaluated by measuring the radical scavenging activity assay (DPPH). The results, expressed as % radical scavenging activity (%RSA) show that the antioxidant activity was observed for all the extracts, and depends on the amount of TPC (Figure 2). At a concentration of 900 μg/mL, the scavenging activity of olive leaves, clove buds and green tea was 77.7, 91.4 and 89.5%, respectively. The IC50 of hydroalcoholic extracts of olive leaves, clove buds and green tea was 99.8, 76.5 and 75 μg/mL, respectively. The free radical scavenging activity of the extracts

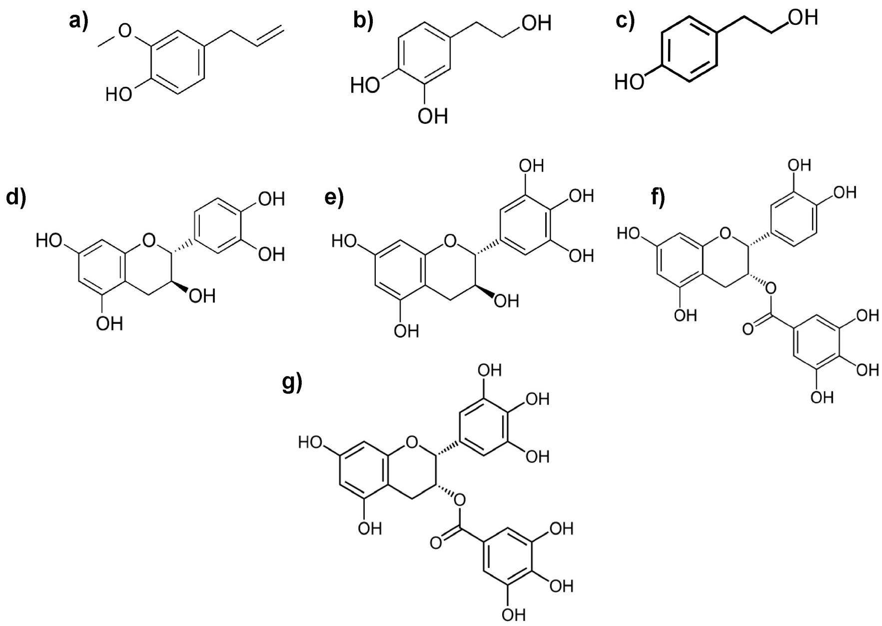

Figure 1. Molecular structure of (a) eugenol, (b) hydroxytyrosol, (c) tyrosol, (d) catechin, (e) epigallocatechin, (f) epicatechin-3-gallate, (g) (−)-epigallocatechin-3-gallate (EPCG).

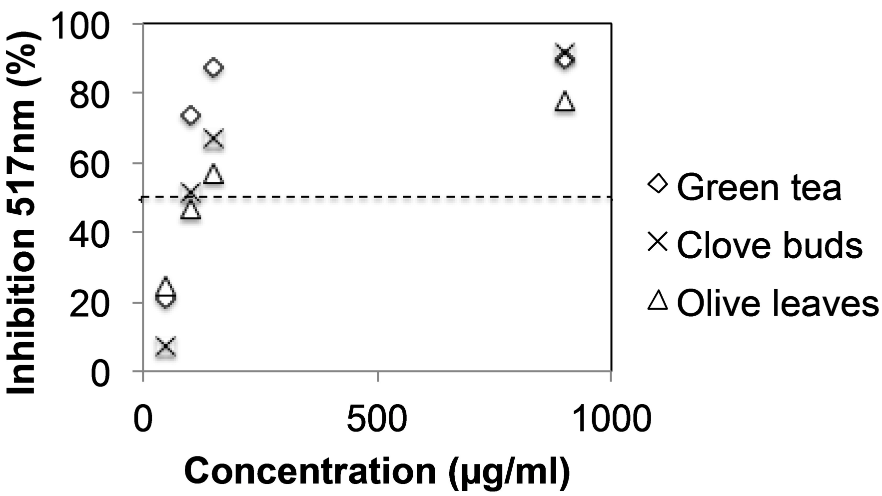

Figure 2. Determination of DPPH radical scavenging activity for the studied plant extracts.

was in the following order: green tea≅clove buds > olive leaves.

Figure 3 shows representative transmission FTIR spectra of the hydroalcoholic raw extracts of clove buds, olive leaves and green tea.

FTIR spectra of the three extracts show absorptions typical of polyphenols (Figure 2). Spectra show the characteristic high intense band (located between 3200 and 3700 cm^{-1}) of phenolic O–H stretching mode, and the unresolved absorptions at 2933–2852 cm^{-1} of asymmetrical and symmetrical stretching vibrations of CH_2 and CH_3 groups. The band at 1715 cm^{-1} is characteristic of C=O stretching of ketones which combine with more than one ring. The band at 1624–1643 cm^{-1} is typical of aromatic carbonyl group belonging to quinine and C=C stretching in aromatic group. The absorption peaks at 1606 cm^{-1} could be attributed to the presence of C–O stretching in carboxyl coupled to the amide linkage in amide I. The band at 1527 cm^{-1}, which is characteristic of amide II, arises, as a result of the N–H stretching modes of vibration in the amide linkage. The intensity of the band at 1560 cm^{-1} was increased by the contribution of the asymmetric COO^{-} stretching vibration, resulting from the carboxylate groups of the acetate ion. The band at 1513 cm^{-1} in clove buds spectrum (3 A) can be associated with C–C aromatic rings vibrations of eugenol, as well as the less intense peak at 1637 cm^{-1}. The symmetric COO^{-} stretching band can also give a contribution to the band at 1406 cm^{-1}. The bands at 1452 and 1396–1402 cm^{-1} are assigned to the methylene scissoring vibrations; the intense band at 1070–1076 cm^{-1} is due to the C–O stretching and C–N stretching vibrations of aliphatic amines.

TGA analysis of the three dried extracts (green tea, cloves and olive leaves) was carried out under nitrogen and the weight loss curves and DTG signals (Figure 4 and Figure S8 (a) in ESI) showed complex paths with three main recognizable steps. The thermograms of cloves and olive leaves extracts showed similar degradation temperatures and profiles, even if the associated mass losses were different. The degradation steps of green tea extract were shifted to higher temperature, probably owing to the different polyphenolic composition and consequently thermal stability (Figure S8). The first step from 50 °C to 180 °C was probably due to the loss of volatiles and absorbed water, especially in the case of green tea extract, or of loosely bonded water as for clove extract. Instead, the olive leaves extract resulted fairly stable until 120 °C and no water loss was detected. The non-oxidative degradation of polyphenols started at about 120–130 °C and it was particularly effective for cloves extract containing large amount of eugenol, which was more volatile than the other polyphenols: for clove

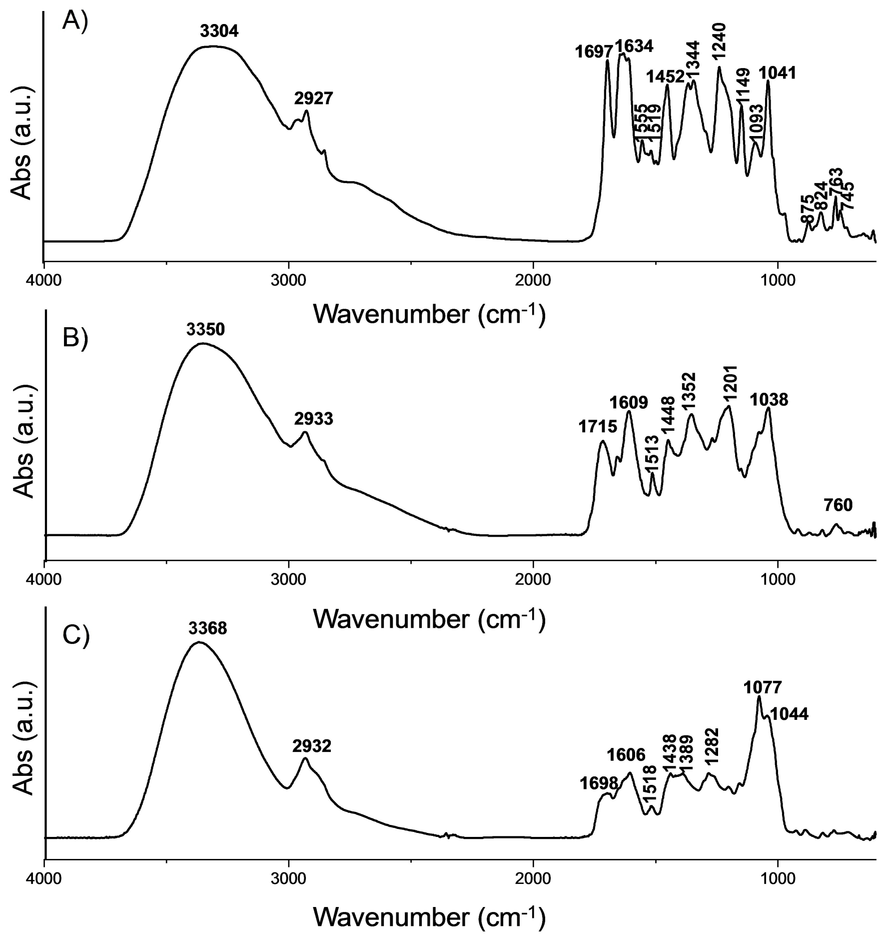

Figure 3. Transmission FTIR spectra of hydroalcoholic extract of A) green tea, B) clove buds, C) olive leaves.

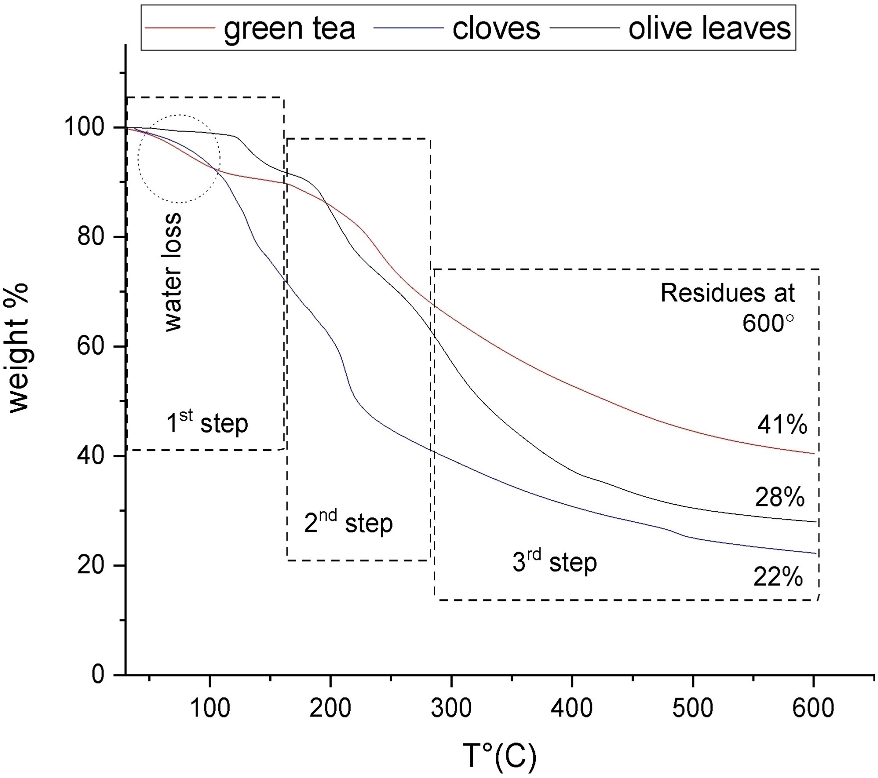

Figure 4. TG curves of green tea, cloves and olive leaves extracts (analysis carried out in N_2 atmosphere). In the figure it is reported the percentage of residues at 600 °C for each extract.

bud extract the mass loss at 200 °C was about 35 % while for green tea and olive leaves extracts the mass loss was about 15 %. The second step from 180 to 280–300 °C was related to the main degradation of all polyphenols. In the case of the green tea extract, it was shifted to higher temperature accordingly to the chemical structure of the most abundant compounds of green tree extracts which are of the catechin family, that was reasonably more thermally stable than eugenol, hydroxytyrosol and tyrosol.

This general trend in the degradation path is also reflected in the consecutive endothermic and exothermic profiles of DTA curves (Figure S8b in ESI) in agreement with data concerning several different plants extracts.[47,48] The first and the second steps accounted for a total mass loss at 280 °C of about 35–37 % for green tea and olive leaves extracts and of about 60 % for cloves extract. Finally, from 300 to 600 °C the samples were thoroughly degraded by pyrolysis of resistant aromatic species and eventually lignin and/or fibers, especially for olive leaves and green tea extracts. This final degradation step left different residual contents at 600 °C as reported in the Figure 4.

ATR-FTIR spectroscopy of plant extract-treated NWF_PP. ATR-FTIR analysis revealed that all the three layers of the surgical mask provided by the regional administration (Regione Toscana) were NWF_PP, showing the characteristic PP peaks at 2950–2916– 2839 cm^{-1} (CH stretching), 1455 cm^{-1} (CH_2 deformation), 1375 cm^{-1} (symmetric CH_3 deformation), and 1165–998–974–841 cm^{-1} (isotactic PP band).

ATR-FTIR analysis was repeated after deposition of each hydroalcoholic extract (clove, olive leaves, green tea), and the related spectra are shown in Figure 5. The absorption-free regions of PP spectrum allow the clear identification of the characteristic signals of the extracts, the O–H stretching band at 3330–3360 cm^{-1}, the C=O stretching, aromatic ring deformation and aromatic C=C stretching region at 1714–1634–1606–1514 cm^{-1} and the C–O stretching, C–N stretching band at 1201–1036 cm^{-1}.

Signals intensity for clove buds and green tea extracts are generally higher than olive leaves, which is in agreement with the results of TPC analysis.

TGA analysis of plant extract-treated NWF_PP. A comparative TGA of NWF_PP face masks was performed in order to estimate the degradation temperatures and the content of the plant extracts deposited onto the surface. Figure 6 shows the comparison of TG curves of neat NWF_PP face mask and face masks treated with green tea, clove bud and olive leave extracts. In the inlet the temperature at weight loss of 5 %, 10 %, the temperature at maximum rate of weight loss (inflection point) and the residual percentage at 600 °C are reported. NWF_PP samples after impregnation with the alcoholic solutions of the plant extracts clearly confirmed a significant presence of bioactive molecules on the surface of the non-woven fabric. Most phenolic compounds once supported on the non-woven fabric showed degradation steps at higher temperatures than dry extracts. In particular, the cloves

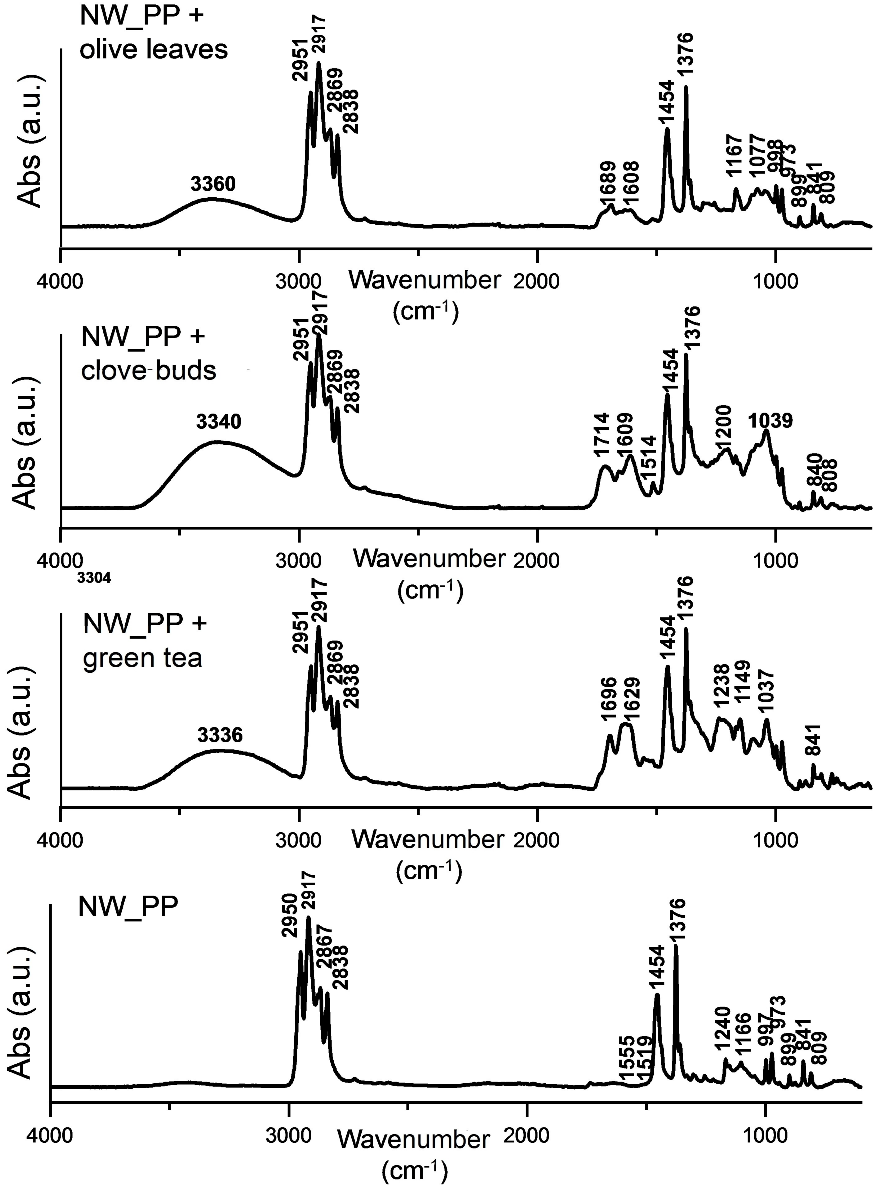

Figure 5. Comparison of ATR-FTIR spectra of NWF_PP face mask before and after deposition of three different hydroalcoholic plant extracts.

extract seemed to be stabilized once deposited likely due to physical entrapment in NWF_PP. The main step due to NWF_PP degradation was practically unchanged (see Figure S9).

On the basis of the residue content at 600 °C, the quantity of plant extract deposited on the surface of NWF_PP masks was roughly determined by comparing TGA residue of plant extracts with that of neat NWF_PP mask. By assuming that in plant-treated NWF_PP samples the PP and plant extract parts degrade independently during the thermal treatment, for each treated face mask the residue of plant-treated NWF_PP samples can be calculated as the percentage combination of plant extract and neat NWF_PP residues (values are shown in the inset of Figure 6). The analysis confirmed the high content of bioactive molecules, particularly for the sample treated with clove extract, in accordance with the previous spectroscopic results. The TGA determination was carried out onto N = 3 replicates and the standard variations was about 3–4% probably because of the non-homogeneous covering of the sample due to the non-optimized impregnation procedure (Figure S10).

Oxidative-Induction Time (OIT) and Oxidative-Onset Temperature (OOT) measurements. OIT and OOT results showed that the impregnation of plant extracts onto NWF_PP remarkably increased the oxidation resistance of all materials (Figure 7 (a) and (b)).

The OIT measurements carried out at 180 °C evidenced that non treated NWF_PP oxidized less than one minute after the switch to oxygen, while the same substrate treated with cloves and olive leaves extracts did not show any oxidative degradation, even after more than 60 min. The OIT value of the NWF_

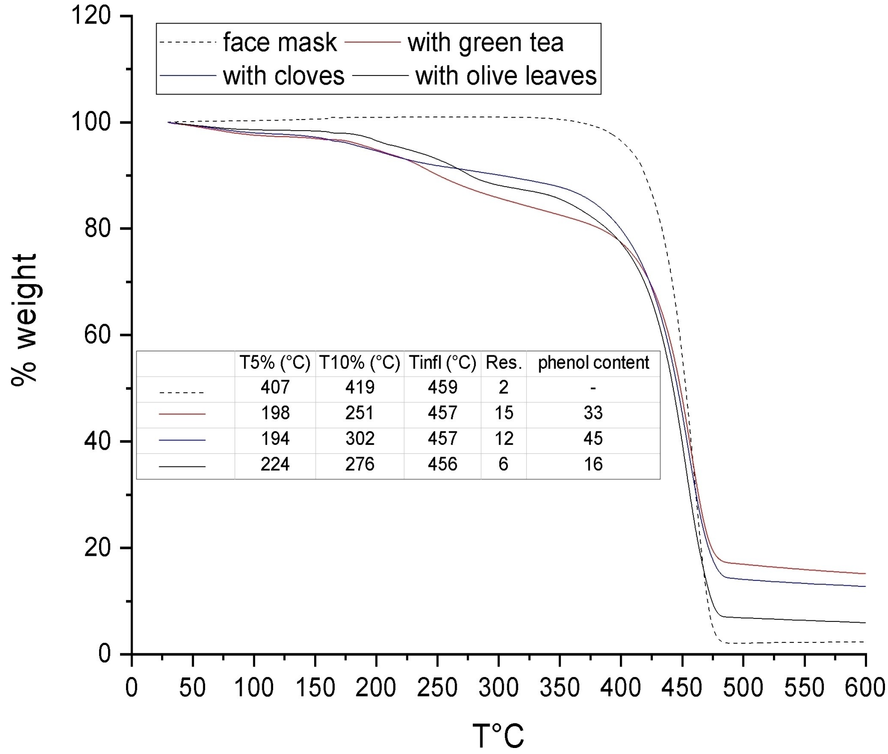

Figure 6. TG curves of NWF_PP face mask and face mask treated with green tea, cloves and olive leaves, in the inset the data concerning: the temperature at weight loss of 5%, 10% and the temperature at maximum rate of weight loss (inflection point); the residual percentage at 600 °C.

PP sample treated with green tea extract was 67 min. The analyses carried out in dynamic mode (OOT) confirmed the general trend: the untreated face mask support showed an onset at about 200 °C, while a consistent shift towards higher temperature of about 40 °C was observed for the fabric treated with green tea extract in agreement with the OIT measurements and with the literature related to stabilized PP samples,[49] also with polyphenolic extracts.[50] Interestingly, OOT measurements did not evidence oxidative degradation in NWF_PP samples treated with cloves and olive leaves extracts up to over 300 °C.

Evaluation of the antibacterial and antiviral activity. OIT and OOT measurements clearly showed that, despite the results of the Folin-Ciocalteu and DPPH assays, clove buds and olive leaves extracts had the most significant antioxidant properties. Catechins, which are the most abundant polyphenols in green tea extract, are characterized by a low redox potential and the tendency to autoxidation accompanied by the formation of active forms of oxygen.[51] We can hypothesize that, after deposition on the surface of face masks and the exposure to the atmosphere, the bioactive compounds of green tea undergo extensive autoxidation losing their potential biological activity.

On the basis of these results, clove buds and olive leaves extracts were selected for preliminary biological tests. Table 2 summarizes the results of virucidal tests performed on HAdV5 and HuCoV229E. Olive leave extract revealed a virucidal effect on both viruses with a mean of one log and 90% reduction. Clove bud extract was more effective on HAdV5 than on HuCoV229E, which resulted more resistant. No cytotoxic effect was revealed by polyphenol solutions.

Table 3 summarized the results of bactericidal test. Olive leaves and clove buds revealed to have a low bactericidal activity against a multidrug resistant microorganism as NDM-producing *K. pneumoniae*, with mean of 0.7 log and 68% reduction.

Table 2. Results of virucidal tests performed on HAdV5 and HuCoV229E using clove bud and olive leave extract.

	Initial virus titer	Titer after test ($TCID_{50} \pm SD$)	Log10 Reduction	Percentage Reduction
Clove buds extract				
HAdV5	$5.40\times10^4 \pm 1.60\times10^4$	$2.33\times10^3 \pm 2.10\times10^3$	1.36	95.60%
HuCoV229E	$7.77\times10^3 \pm 6.61\times10^3$	$5.27\times10^3 \pm 0.91\times10^3$	0.16	32.2%
Olive leaves e×tract				
HAdV5	$5.40\times10^4 \pm 1.60\times10^4$	$4.58\times10^3 \pm 3.33\times10^3$	1.07	91.50%
HuCoV229E	$7.77\times10^3 \pm 6.61\times10^3$	$8.43\times10^2 \pm 0$	0.96	89.15%

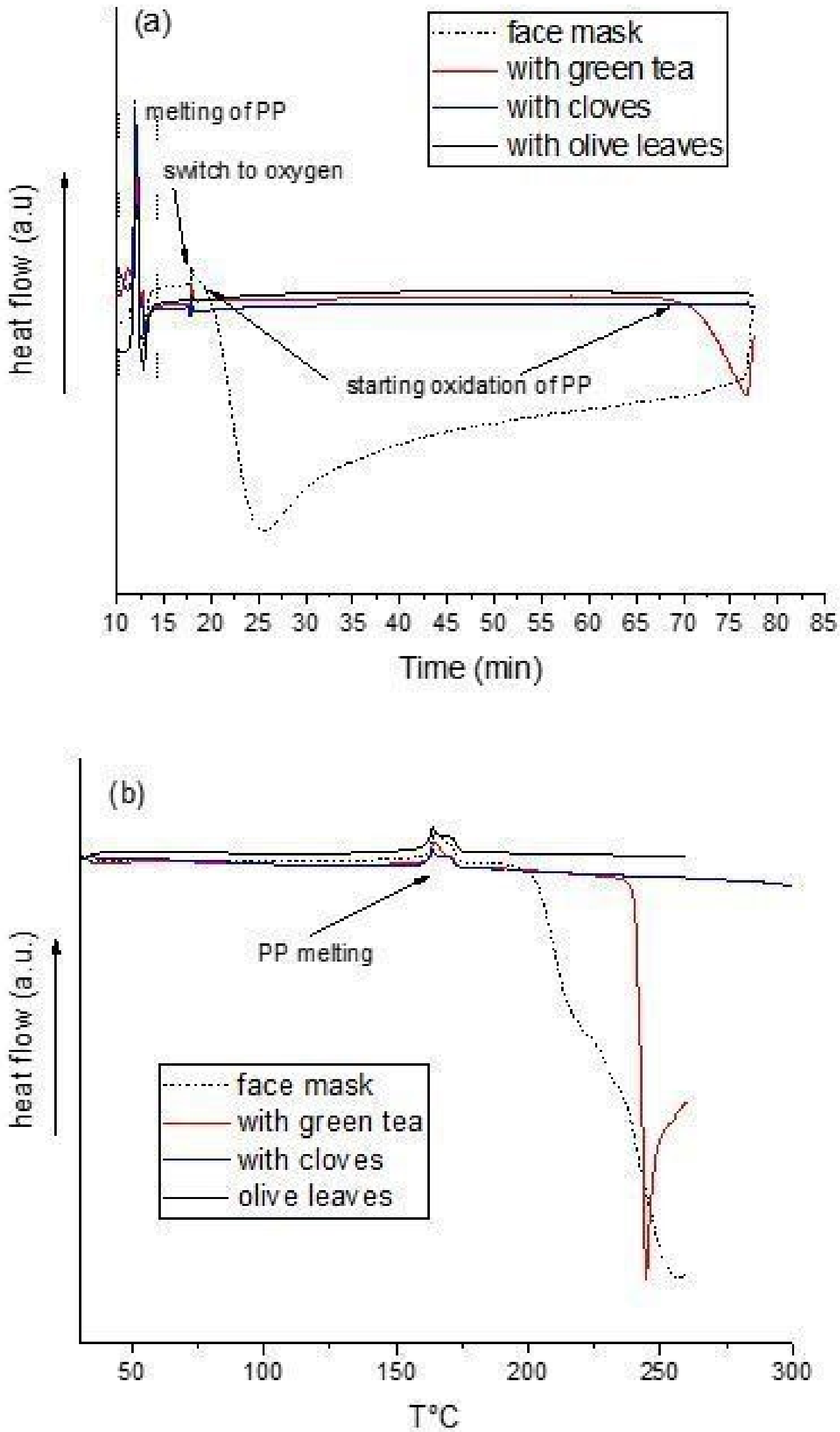

Figure 7. (a) OIT (carried out at 180 °C) and (b) OOT measurements of untreated face mask and face mask treated with green tea, cloves and olive leaves extracts.

Discussion

Following the recent H1 N1, H5 N1 and SARS-CoV-1 epidemics and the actual SARS-CoV-2 outbreak, many researchers focused on the development of efficient disinfection/sterilization methods to make PPEs reusable and to face their shortage.

During the first few months of the COVID-19 pandemic, the Center for Disease Control and Prevention recommended the decontamination and reuse of filtering facepiece respirators by

Table 3. Results of bactericidal test using clove bud and olive leaf extracts.

	Initial bacterium titer	Titer after test (Mean ± SD)	Log10 Reduction	Percentage Reduction
Clove buds extract				
NDM-K.pneumoniae	$1.8\times10^{9}\pm5.6\times10^{9}$	$3.2\times10^{8}\pm7.21\times10^{8}$	0.74	68.71 %
Olive leaves extract				
NDM-K.pneumoniae	$1.8\times10^{9}\pm5.6\times10^{9}$	$3.3\times10^{8}\pm1.65\times10^{8}$	0.73	67.79 %

ultraviolet germicidal irradiation, vaporous hydrogen peroxide, and moist heat. These methods showed to retain filtration performance, fit characteristics achieved prior to decontamination, and safety for the wearer by inactivating SARS-CoV-2.

An alternative approach, exploited in this work, was to extend the lifetime of the PPEs by treating them externally with substances having antiviral properties. Since PPEs are in close contact with skin, the use of compounds harmless with respect to the human beings is advisable. Besides the well documented antioxidant and anti-inflammatory properties of polyphenols, evidences highlighted the antiviral potential exerted by this class of bioactive compounds. Moreover, when obtained as natural products from the extraction of agri-food resources, they could be better perceived and accepted by the public respect to chemical disinfectants. The use of ethanol, a solvent that is relatively cheap, reusable and nontoxic, could lend an environmentally friendly aspect to the low-cost preparation of potentially bioactive extracts from agri-food solid wastes in a sustainable framework. Moreover, the use of a mixture of water and ethanol, compared to pure alcohol, improves the extraction yield by increasing the swelling of plant materials and the contact area between the vegetable matrix and the solvent.

By relying on these premises, we investigated the potentiality of hydroalcoholic extracts from olive leaves, clove buds and green tea leaves as "natural antiviral barrier". NWF_PP face masks were impregnated with each hydroalcoholic extract, and the composite material characterized firstly by chemical-physical techniques and then by antibacterial/antiviral tests against NDM-producing *Klebsiella pneumoniae* and respiratory viruses (Human Adenovirus type 5 and Human Coronavirus 229E).

Despite from TGA the non-homogeneous covering of the sample was evident, we observed that the extracts adsorbed on NWF_PP degraded at higher temperature with respect to the crude extracts, probably benefiting from the physical entrapment in NWF_PP, despite its easy treatment by impregnation and drying at RT.

This study suggests also that polyphenols-covered NWF_PP exhibits improvements in oxidation resistance, as measured by OIT, in comparison with the original material. Natural polyphenols as free radical scavengers have been widely used in food and pharmaceutics as potent antioxidants, and it is reported that the resistance of crosslinked ultra-high-molecular-weight polyethylene blended with gallic acid and dodecyl gallate towards oxidation was proved.[52]

Further research is needed to deepen the effect on microorganisms. In particular, even if a virucidal effect was demonstrated confirming the well-known action of polyphenols,[3,53,54] the titer reduction, especially for the enveloped virus chosen (*Human Coronavirus*), was very low with a maximum of 1 log, not reaching to the results of a similar study.[36] The optimization of the polyphenol extract concentration adsorbed on the surfaces is a key point to be addressed in order to improve the virucidal activity.[36,55–58]

Most plant extracts such as hydroxytyrosol, tyrosol and luteolin show indeed good activity against gram-positive bacteria, while activity against gram-negative bacteria is a critical measure of success.[56,59] However, the different activities against gram-negative and gram-positive bacteria can be rationalized by considering the differences in the composition of the cell wall. Polyphenols photo irradiated with blue light were found to be capable of causing oxidative damage of bacterial DNA acting against Gram-negative bacteria through their incorporation into bacterial wall.[60] Gram-negative bacteria have a lipopolysaccharide component in their outer membrane which makes them more resistant to antibacterial compounds. This would explain the low percentage of reduction obtained in this study in comparison of *K. pneumoniae.*

Conclusions

Hydroalcoholic extracts of clove buds, olive leaves and green tea were found to have a valuable content of polyphenols and they were employed to modify non-woven tissue. The NWF_PP masks soaked in polyphenol extracts were characterized by FTIR, TGA, OIT and OOT and their virucidal and antimicrobic properties were tested. In particular, HAdV5 shows similar sensitivity to olive leaves and dry clove buds, with a viral abatement of 91.5 % (1.07 log reduction) and 95.6 % (1.36 log reduction), respectively. Instead, HCoV229E was more sensitive to olive leave extracts, with an abatement of 89.1 % (0.96 log reduction), than to dry clove buds (32.2 %, 0.16 log reduction). Comparing the two viruses, our experimental data showed that coronavirus was more resistant to the tested polyphenolic compounds than adenovirus.

This work confirmed the role of natural compounds as antiviral molecules that could be used for improving the barrier effect of masks. The choice of these vegetable raw materials (clove buds, olive leaves and green tea), easily available in common markets or from pruning waste material from agriculture as well as the easy preparation of the extracts compatible with the use of kitchen tools, aimed to address their sustainable application both at individual and industrial level.

Supporting Information Summary

In the Supporting Information the experimental procedures are reported (details of plants treatment and extracts characterization, face-mask preparation and characterization, biological tests) as well as additional figures.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Antiviral agents · agri-food waste · biomass · personal protective equipment · polyphenols

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