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# ANTIMICROBIAL ACTIVITY OF COTTON FIBRES TREATED WITH PARTICLES EXTRACTED FROM CITRUS PLANTS: A REVIEW

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## ABSTRACT

Nanotechnology is an emerging technology in textile sector for the fabrication of functional textiles with different properties such as antibacterial, hydrophobicity, UV-protection, flame retardancy, anti-static and self-cleaning. In current COVID-19 crises, the development of antimicrobial textiles through the deposition of nanoparticles has emerged as a research subject of particular interest. Recently, the green-synthesis of nanoparticles from plant extracts has become an effective alternative to conventional physical and chemical synthesis methods due to being environmentally benign and nontoxic. In this review article, the significance of nanotechnology in antibacterial finishing of textiles, mechanism of antibacterial activity of nanoparticles, significance of green synthesis methods for nanoparticles have been discussed. The green-synthesis of different nanoparticles from the citrus plant extracts and their application on textiles for imparting antibacterial activity is reviewed in particular. The chemical composition of citrus plant extracts and their role as bio-reductants in the synthesis of nanoparticles is also highlighted. Moreover, different qualitative and quantitative standard testing protocols employed for the antimicrobial characterization of plant extracts and textiles have been discussed. The major challenges and limitations associated with the plant-based biosynthesis of nanoparticles have also been highlighted

## KEYWORDS

Nanotechnology; Nanoparticles; Green synthesis; Citrus plants; Antimicrobial Textiles.

## INTRODUCTION

The biosynthesis of nanoparticles remains executable through the usage of numerous natural resources involving microorganisms, fruits, plant tissues, plant extracts, and plants [1]. However, distinctive attention is being associated with the use of plant extracts. The popularity of the aforementioned pathway is primarily rooted in the features such as, simplicity of the process, facile synthesis and economic feasibility [2]. Further, the plant extracts are found to be of dual utility by paying the desirable character of acting as stabilizing agent as well as reducing agent. Moreover, the plant extracts with the addition of metallic nanoparticles are enumerated to reveal excellent antimicrobial attributes and thus have earned prime candidature in the fabrication of bioactive textiles [3]. The use of plant-oriented antimicrobial agents such as, Aloe vera, Eucalyptus, Turmeric, Neem, and Basil has already been delineated in stream of applications encapsulating health surveillance, e-textile and sport gears [4]. The demand for hygienic materials, such as biocidal coatings in textiles (sportswear, undergarments, and bedding linen), is escalating with the advancement of living standards. The antibacterial finishing treatments has become an essential aspect of medical, therapeutic, and healthcare practises due to various potentially infectious microorganisms in hospital environments that might cause cross-infection disorders [5]. Antimicrobial finishing is one of the most significant

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functional finishes applied to hospital textiles, as well as everyday garments. The negative consequences of microbial proliferation involve unpleasant smell, loss of tensile characteristics, staining, and discoloration of textile materials. Antimicrobial finishes are applied to textiles to prevent the growth of microorganisms. Numerous antimicrobial agents are applied, including quaternary ammonium compounds, triclosan, metal salts based on cobalt, copper, zinc and silver nanoparticles to prevent the deterioration of textile materials [6]. These synthetic chemicals are all hazardous and have a negative impact on the environment [7]. Due to this problem, synthetic antimicrobial agents are being replaced by natural antimicrobial agents that have strong antibacterial action. Neem, Aloe vera, Eucalyptus, Basil and Turmeric are a few examples of natural antibacterial agents that have been used for the finishing of textiles. Citrus fruit peel exhibits an effective anti-microbial agent, making it suitable for use in the development of healthcare textiles [8].

The antimicrobial activity of copper and silver nanoparticles coated textiles has been widely reported. However, the antimicrobial activity of Cu-coated textiles from citrus plants source has not been reported. In the current study, the green-synthesis of different nanoparticles from the citrus plant extracts and their application on textiles for imparting antibacterial activity is reviewed in particular. The chemical composition of citrus plant extracts and their role as bio-reductants in the synthesis of nanoparticles is also highlighted. The various qualitative and quantitative standard testing protocols utilized for the antimicrobial characterization of textiles have also discussed in this review. The developed Cu- coated textiles could be effectively applied in the field of hospital textiles for the preparation of antibacterial scrub suits, surgical gowns, panel covers, protective clothing, bedding textiles, coveralls, wound dressings, table covers, curtains, and chair covers etc.

## What are antimicrobials or antimicrobial agents (antimicrobial finishes)

The antimicrobial agents are usually projected as substances capable of killing or suppressing the microbial growth and thus provide protection against microbe-based adversities such as odor, discoloration, and degradation [9]. However, the performance of antimicrobial agents remains susceptible of multifaceted complexities such as, chemical structure, surrounding environment, in-plant handling and multiplicity of surface.

### *Natural antimicrobial agents*

The natural antimicrobial agents are plants-fetched or animal-derived substances popular for their bioactivity and ecological capabilities. A stream of applications associated with natural pathogens can be witnessed in textile-based functionalities, pharmaceutical manufacturing, and biomedical industries. One may notice the applicability of marine species, Aloe vera, neem, turmeric, tulsi, pomegranate, prickly chaff flower, clove, and other natural herbs in the role of antibacterial agent in textile finishing [9].

### *Necessity of antimicrobial agents*

Microbes are almost always present on the human body, with a mean density of 100–1000 microbes/cm$^2$, even with clean skin. Numerous organisms, including bacteria, yeast, and fungi, can invade the wearer at this level. Numerous unfavorable side effects of these microbial disorders include offensive odors and stains brought on by material discoloration. As a result, the person as well as the textile are both negatively impacted by the microbial activity on textiles. Textiles serve as an ideal medium for the adhesion, transfer, and proliferation of infection-causing microbial species due to their characteristics and proximity to the human body [10] [11]. Natural fibers are more susceptible to microbial invasion than synthetic fibers because of their permeability and hydrophilicity. The structure of natural fibers traps nutrients, moisture, and oxygen, providing the ideal supplemented culture for quick microbial growth. Direct contact with the human body also offers warmth, nutrients, and humidity, which fasters the growth of microorganisms. The infections are bacterial and fungal. Moreover, the algae can develop on them if they are kept damp for a long time. Fungi also produce an unpleasant odor and a slimy, sticky feeling, in addition to discoloring, damaging the fibers, and staining textiles. The chemistry and structure of the textile fibers may also promote the growth of microbes. The low molecular weight contaminated compounds in textile finishing may not facilitate microbial multiplication, but they may provide enough nourishment for damp microbe growth. This weakens the fabrics strength and gives out an unpleasant odor. It also stains the fabric. It is crucial to limit microbial growth on textiles throughout use and preservation because of all these reasons [12].

### *Interaction of microbes with textile fibres*

Natural and manmade fibers respond to microbial growth quite differently. Both may function as suitable substrates, however the underlying mechanisms vary significantly depending on the situation. Natural fibers are good targets for microbial breakdown because they retain water well and can easily have their polymer bonds hydrolyzed by microbial enzymes. Most protein and cellulose fibers are attacked by microbes. According to research, the fabrics most vulnerable to microbial attack are jute, cotton, flax, silk, and wool [13].

Microorganisms develop more slowly on synthetic fibers than on natural fibers because the polymeric framework of synthetic fibers does not hold as much water. On the other hand, these fibers increase the retention of state perspiration in the intercellular spaces, where bacteria grow faster. For instance, synthetic fiber socks have reportedly been found to be more likely than natural fiber socks to cause a foot infection. According to study, the proportion of polyester in a fabric affects how well bacteria attach to it. When synthetic fibers undergo finishing chemicals like polysiloxane and polyethylene emulsions, they are more vulnerable to microbial degradation [14]. These chemicals enable bacteria to break down the polymer into "chewable bites," initiating the hydrolysis cycle, by exploiting the acidic as well as basic metabolic by-products. In this way, even the hardest polyurethanes could well be decomposed. Polypropylene, polyester, and nylon synthetic fibers have all shown signs of microbial deterioration when exposed to conductive conditions [15].

However, the textiles might act as active agents in the proliferation of microbes as well as substrates for bacterial development, which is of more concern. Viruses can persist for up to 16 hours on materials including terry towels, washable wool suits, cotton shirts, polyester/cotton shirts, nylon jersey, and cotton/polyester shirts. Synthetic fibers are more suitable to viral survival and spread than cotton [16]. The virus could well be physically removed from the fabric during washing. However, it was found in the laundry wastewater indicating that it has not been completely inactivated. All of these bacterial growth requirements can be satisfied by textiles, which can have a number of negative side effects. The existence of microbes and their growth can cause odor, health problems, and eventually fabric deterioration [17]. Microbes can damage the additives used in textiles, causing staining and the loss of functional properties including tensile strength and elasticity. One of the most notable adverse effects is the formation of malodor. When microorganisms multiply, they break down nutrients found in the environment, such sweat and soil, to produce chemicals that give off odors. For instance, it is hypothesized that bacteria produces 3-methyl-2-hexenoic acid and is responsible for the distinctive body odor [18]. In addition to other factors, bacteria turn human perspiration into odorous substances like aldehydes, amines, and carboxylic acids that have an unpleasant odor [19].

## Significance of nanotechnology in textile finishing

Nanotechnology has evolved to be a major area with several applications for engineering science at the nano-scale to produce goods with distinctive features [20]. The science and engineering involved in developing, producing, and analysing nanostructures having outstanding functional qualities in comparison to their bulk counterparts can be summed up as nanotechnology. Additionally, nanotechnology is becoming increasingly important in interdisciplinary fields of science, including biology, chemistry, physics, and materials science [21]. As a result, synthesized nanomaterials are very useful in a variety of industries, such as energy electronics, biomedicine and pharmaceuticals, environmental remediation, and functional textiles. Among these applications, nanotechnology plays major role in the development of functional smart textiles with insect repellent, self-cleaning, flame-resistant, UV protection, hydrophobic, anti-static, and antimicrobial resistance properties [17]. The development of novel nanostructures and nanoparticles with promising bioactivity to be used in a variety of biomedical applications is mostly due to the acceleration of developments in the field of nanotechnology. Numerous studies have been conducted recently on the potential applications of nanoparticles in biomedicine, particularly as antimicrobial agents, and they have been recognized as a potential substitute for conventional antimicrobials to combat the multi drug resistance of microorganisms [22]. The overuse and exploitation of synthetic antibiotics, as well as the absence of new approaches for the production of antimicrobial compounds to address the issues, have all contributed to the rise in bacterial resistance to antibiotics, which has been a significant cause of concern. The development of innovative nanostructures for use in pharmaceutical and biomedical applications has benefited tremendously by bioactive features of nanoparticles, such as their antifungal, antibacterial, antiviral, antioxidant, and anti-inflammatory actions [23]. Nanoparticles' high surface-to-volume ratio improves their ability to interact with microorganisms and raises the level of their antibacterial activity. As shown in Fig. 4, different nanoparticle types, their chemistry, particle size, structure, and charge density all affect the antibacterial activity of nanosized particles such as nanocomposites, metal, carbon-based nanoparticles, and metal oxides [24]. Copper, platinum, gold, silver and other nanoscale metal particles are frequently used to exhibit antibacterial action, particularly to prevent bacterial development. Additionally, in recent years, these nanoparticles have been integrated into textile to prevent the growth and transmission of infectious bacterial strains through fabrics. Due to the increased demand for functional textiles in recent years, primary attention of researchers has thus shifted to developing these materials using nanoparticles [25].

Nanotechnology has added a new dimension to the finishing procedures used on textile materials. The fact that traditional or conventional finishing techniques frequently have short-term effects on textile materials (such as fabrics or clothing) is one of the key distinctions between them and nanotechnology finishing processes. These functional finishes lost some of their effectiveness after washing or wearing [26]. Fabric treated with nanotechnology, on the other hand, develops more durability. The increased durability of a certain functional effect is due to the larger surface area of nanoparticles relative to the same volume of material produced with larger particles [27].

## Mechanism of antibacterial activity of nanoparticles

The mode of antibacterial action for nanoparticles is currently unclear, and research is actively being done to determine the mechanism of antibacterial action. Recently, the proposed mechanism of antibacterial activity of nanoparticle include (i) bacterial cell wall and membrane destruction, (ii) invasion into bacteria, and (iii) oxidative stress [28]. Additionally, it has been noted that the presence of amino groups, carboxyl groups, and phosphate groups, gives negative charge on the surface of bacterial cell membrane. As a result, positively charged nanoparticles typically bind to the cell membranes by electrostatic interaction [29]. Eventually, the nanoparticles can directly enter the bacterial membrane and assimilate there, enabling them to interact with substances including protein, lipids, and DNA. According to Yun'an Qing et al. [29], the interactions of nanoparticles with cellular interactions cause cell damage and ultimately cell death. Additionally, in order to cause oxidative stress in the bacterial cells, nanoparticles release reactive oxygen species (ROS) including singlet oxygen (O2), superoxide radicals (O), hydrogen peroxide (H2O2), and hydroxyl radicals (OH-). The ions generated by the disintegration of nanoparticles cause ROS, which prevents bacterial growth or harms the organelles or cells of the bacteria. Additionally, the major factors that affect the nanoparticles' action mechanism towards bacteria are their size, topology, shape, morphologies, and surface charge [30]. Yan et al. [31] investigated the interaction of Ag nanoparticles interacted with *Pseudomonas aeruginosa* and performed detailed analysis to explain the antibacterial mechanism. The outcomes showed that the key mechanisms for their efficient antibacterial mechanism are the contact of the nanoparticle with the cellular membranes and the formation of ROS [31]. Similar to this, Concha Guerrero et al. [32] revealed the interaction of nanoparticles with bacteria by TEM analysis while examining the mechanism of antibacterial action for copper oxide nanoparticles with different 11 bacterial strains. The findings showed that the copper oxide nanoparticles have potent antibacterial action through membranes and bleb disintegration, hole and cavity production, and bacterial cellular disintegration and rupture [32]. It was proposed that the ionic interaction between nanoparticles and bacteria, as well as the formation of redox reactions and ROS in bacteria, are the causes of the damaging effects of nanoparticles [32]. Additionally, Azam et al. [33] demonstrated that copper oxide nanoparticles with sizes between 20 and 28 nm have size-dependent antibacterial efficacy against both gram-positive and gram-negative bacteria. According to the findings, the smaller-sized copper oxide nanoparticles had strong antibacterial action with very low minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) values. This study revealed that the mechanism of antimicrobial activities is also influenced by the size of nanoparticles other than the production of ROS and oxidative stress [33]. It should be emphasised that the nanoparticles deposited in textile fabrics will follow the same mechanism of antimicrobial activities as described above, and Figure 1 provides a review of the possible antibacterial processes that are hypothesised to be shown by nanoparticles.

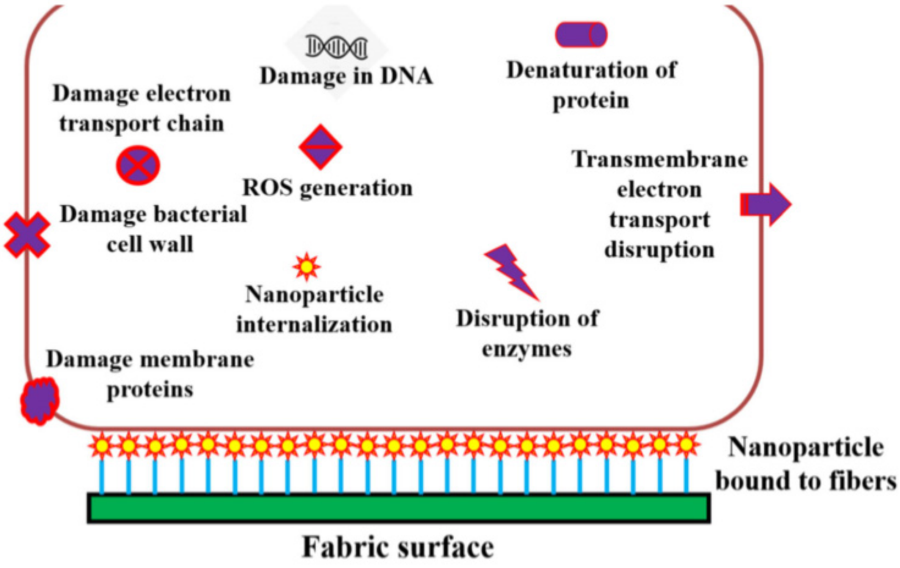

**Figure 1.** Possible antibacterial processes shown by nanoparticles. [34]

## Different synthesis approaches for nanoparticles and their limitation

The uniqueness of nanoparticles to be employed in intended applications is typically determined by synthesis methods. The synthesis techniques determine the surface charge, size, morphology, and topology of nanoparticles, which in turn affects their characteristics. The following are the three main synthesis methods for the production of nanoparticles:

1. Physical methods,
2. Chemical methods,
3. Biological methods.

Each of these methods for synthesising nanoparticles has a number of benefits and drawbacks that can affect how well they exhibit biological characteristics, particularly when it comes to using them to inhibit pathogenic bacterial species [35]. Furthermore, the synthesis method-mediated antibacterial activities play a significant role in defining the effectiveness of antibacterial action while depositing the nanoparticles on the surface of fabrics. Physical synthesis methods which include laser ablation [36], physical vapour deposition [37], and ball milling [38] are effective in order to produce monodispersed nanoparticles with the necessary morphology and size. Although these technologies produce nanoparticles with antibacterial property employing them for textile applications presents difficulties due to the high cost and difficult production method. The most widely used techniques for synthesizing nanoparticles include sol-gel, hydrothermal synthesis, and precipitation techniques. This approach produces stable nanoparticles with the appropriate form and size along with the potential to change their surface charge, which is useful in biological applications, particularly for their antibacterial property. However, the dispersion of nanoparticles and their cytotoxicity against human cells as a result of using toxic chemicals during synthesis are the challenging aspects which cannot be modified via chemical approach [39]. It also affects their efficiency in using them as an antibacterial agent for the pilot-scale production of bactericidal textiles. Green or biosynthetic methods have recently become increasingly important to synthesize nanoparticles that are safe for humans and the environment. Algae, plants, and bacteria, fungi, as well as their extracts, have been frequently used to synthesize biocompatible nanoparticles that were effective in medicinal applications [40, 41]. As a result, the green synthesis has become the most effective method in recent years for depositing nanoparticles on the surface of textiles as antibacterial finishes to suppress the growth of pathogenic microorganisms [34].

## Significance of green synthesis methods for nanoparticles

Due to the significance of ecologically sustainable production process, green synthesis of nanoparticle has attracted a lot of attention. The fabrication of nanoparticles using plant extracts is a suitable substitute for chemical reduction methods. The use of plant extracts as stabilizing or reducing agents for the synthesis of nanoparticles extracts has been found as alternative [42]. As a result, many researchers have adopted the green synthesis of NPs, which is seen as a viable alternative to chemical processing and used for a variety of applications. According to earlier research, phytochemicals stabilise metal nanomaterial for a longer period of time by reducing metal ions to metal nanoparticles. It facilitates this by capping the synthesised nanoparticles [43]. Additionally, green nanoparticles are said to have superior catalytic activity when compared to nanoparticles produced chemically.

Citrus plants from the *Rutaceae* family are among the many plants that have a lot of potential for use as bio-reductants in the environmentally friendly synthesis of nanoparticles. One of the most important and widely grown fruit crops is citrus tree [44]. Citrus plant extracts include numerous active phytochemicals that can be employed as a reducing and stabilising agent, making them ideal for use as a reducing agent in the synthesis of nanoparticles including zinc oxide and silver nanoparticle [45]. In addition, coumarins, cellulose, pectin, carotenoids, hemicellulose, lignin, essential volatile oils, phenolic components, etc. are found in the peel and juice of the majority of citrus fruits [46]. Citrus plant extracts have the potential to treat textiles for the improvement of functional properties of textiles which includes UV protection, antibacterial activity, and mosquito repellent properties [47]. Citrus plants are therefore thought to be effective bio-reductants for the formation of nanoparticles and active compounds for the antimicrobial finishing of textiles.

## Chemical composition of citrus plant extracts

Citrus peels are an excellent source of pectin as well as essential oils and contain a number of bioactive substances, particularly flavonoids [48]. Citrus peels have a variety of biological actions that have been attributed to their complex composition, including antioxidant properties, anti-inflammatory effects, and other biological advantages for human health, including the capacity to fend off cancer and cardiovascular disorders [49]. Citrus peels are not waste rather; they are a unique natural resource as they exhibit such important biological functions. Table 1 summarizes some of the important chemical components present in the extract of citrus fruits and plants.

**Table 1:** The names and amount of major functional groups present in Citrus plants extract.

| Sr. # | Chemical Component | Plant Name | | | | Ref. |
|---|---|---|---|---|---|---|
| | | **Orange** | **Lemon** | **Grapefruit** | **Mandarin** | |
| 1 | Hesperidin | 1.22–42.56 | 20.62 | 0.13–0.59 | 0.01–5.46 | [50] |
| 2 | Narirutin | 0.02–20.21 | | | 0.587 | [51] |
| 3 | Naringin | 0.002–0.03 | 0.81–5.37 | 0.09–1.60 | | [52] |
| 4 | Eriocitrin | | 0.01–0.21 | | | [53] |
| 5 | Nobiletin | 0.14–0.24 | 0.13–0.42 | 0.01–0.05 | 1.24–1.77 | [54] |
| 6 | Tangeretin | 0.02–27.5 | 0.07–0.21 | | 0.20–0.49 | [54] |
| 7 | 3,5,6,7,8,3',4'-hepatamethoxyflavones | 0.55–17.37 | | | | [50] |
| 8 | Sinensetin | 0.16–36.92 | | | 0.09–0.17 | [50] |
| 9 | 5-demethylnobiletin | | 0.02-0.36 | | | [53] |
| 10 | 5-demethyltangeretin | 0.35-0.12 | | | | [53] |
| 11 | 5-OH-3,6,7,8,3'4'-hexamethoxyflavone | | | 0.02-0.21 | | [53] |
| 12 | Sudachitin | 0.16-1.21 | | | 0.15 | [53] |
| 13 | Vicenin-2 | 0.17 | | | | [52] |
| 14 | Hesperetin | 0.002–0.007 | | | | [54] |
| 15 | Limonin | 0.03–0.14 | 0.26–0.32 | 0.02–0.20 | 0.05–0.20 | [54] |
| 16 | Nomilin | 0.01–0.12 | | | | [54] |
| 17 | Obacunon | | 0.03-0.12 | | | [54] |
| 18 | Limonexic acid | | | 0.02-0.62 | | [54] |
| 19 | Synephrine | 11.71–13.84 | | | | [55] |
| 20 | N-methyl tyramine | | | | | [55] |
| 21 | Limonene | 60.44–97.3 | 63.67–76.99 | 88.6–91.5 | 66.91–91.51 | [56] |
| 22 | β-Pinene | 1.45–1.82 | 9.31–14.74 | 0.8–1.2 | 0.73–1.90 | [57] |
| 23 | α-Pinene | 0.21–3.53 | 0.99–1.59 | 0.40–0.70 | 0.54–2.43 | [57] |
| 24 | Valencene | 0.14–0.40 | | | 0.03–0.74 | [56] |
| 25 | Linalool | 0.04–4.11 | | 0.12–1.10 | 0.10–3.23 | [57] |
| 26 | β-Elemene | 0.02-0.06 | 0.01-0.24 | | | [57] |
| 27 | α-Copaene | 0.04–0.24 | | 0.03–013. | 0.03–0.10 | [56] |
| 28 | γ-Terpinene | 0.02–0.32 | 5.51–10.38 | | 0.13–14.22 | [56] |
| 29 | α-Humulene | 0.02–0.04 | | 0.03–0.10 | | [56] |
| 30 | β-Myrcene | 0.03–0.05 | 0.38–0. 96 | 0.03–2.16 | 2.54–24.77 | [56] |
| 31 | Sabinene | 0.10–0.29 | | 0.16–0.63 | 0.30–0.60 | [56] |
| 32 | β-Sinensal | 0.04–0.14 | 0.05–2.21 | | | [56] |
| 33 | α-Sinensal | 0.02–0.04 | 0.20–0.60 | 0.34–2.13 | | [56] |

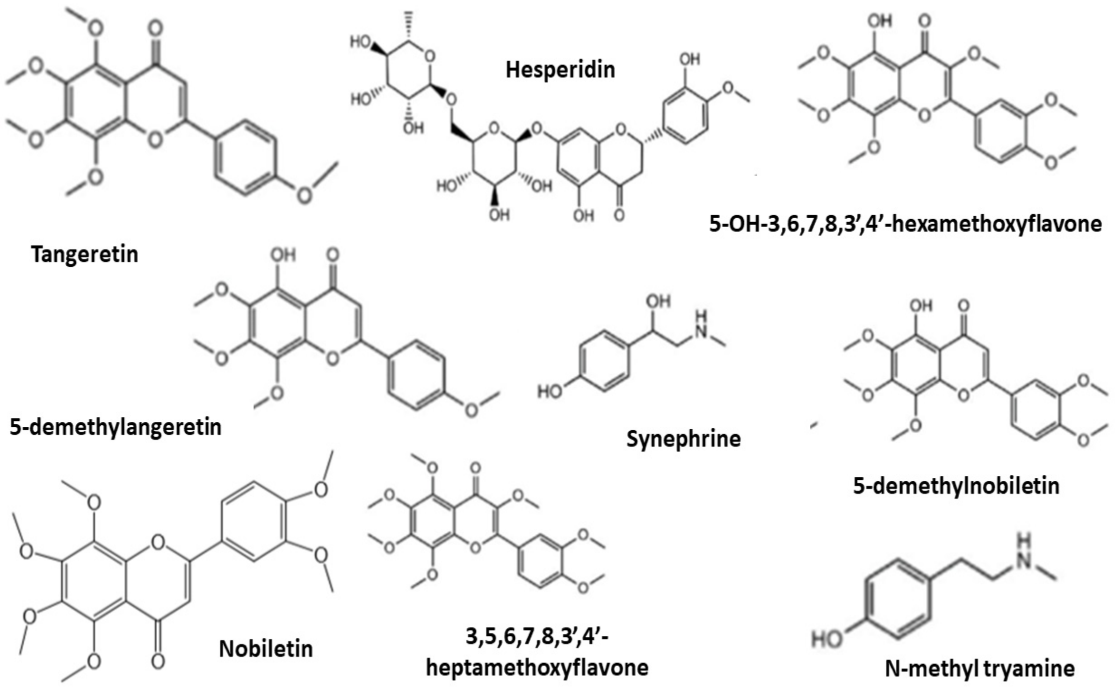

**Figure 2.** Molecular structure of flavonoids and alkaloids present in citrus plant extract [54].

**Table 2.** The type and name of phenolic compounds detected by DART-MS analysis in Citrus plants extract.

| Sr #. | Compound | Type | m/z |
|---|---|---|---|
| 1. | Nobiletin | Flavonoids | 403 |
| 2. | Catechin | Flavonoids | 290 |
| 3. | Sinensetin | Flavonoids | 373 |
| 4. | Ferulic acid | Hydroxycinnamic acid | 194 |
| 5. | Vanillic acid | Hydroxycinnamic acid | 168 |
| 6. | Gallic acid | Hydroxycinnamic acid | 172 |
| 7. | Caffeic acid | Hydroxycinnamic acid | 180 |
| 8. | Coumaric acid | Hydroxycinnamic acid | 164 |

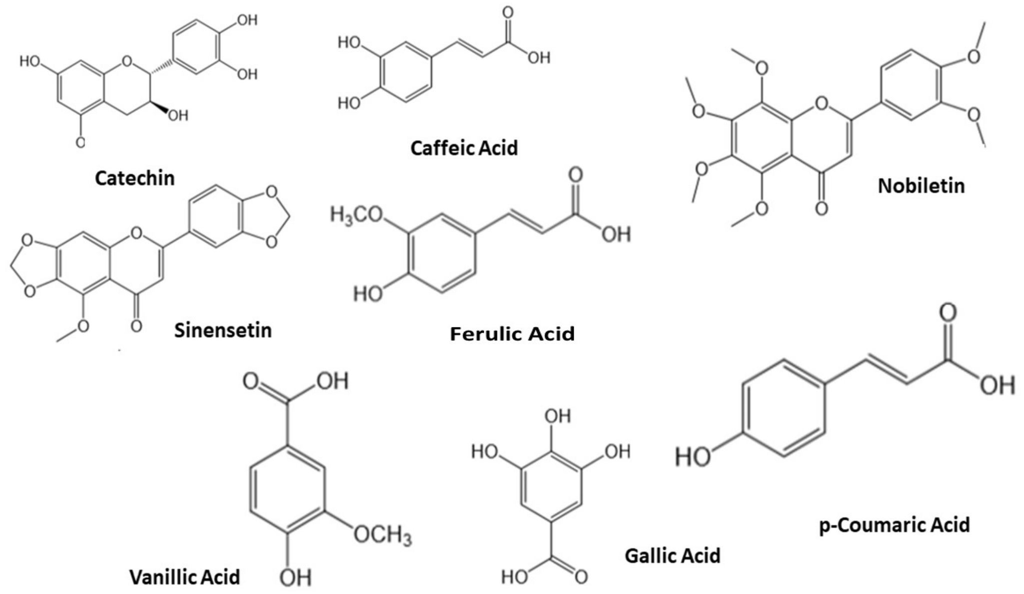

**Figure 3.** Molecular structure of phenolic components detected in citrus plat extracts [59].

Loizzo et al. [58] used HPLC to investigate extracts in order to determine and measure the most prevalent flavonoids in the polar extracts considering that flavonoids are primarily responsible for citrus's health benefits. Citrus is a major source of many kinds of flavonoids, including flavonols, flavones, flavanones, and polymethoxylated flavones, according to a review of the literature available on citrus species. They chose to assess the quantitative and qualitative features of ten flavonoids, including rutin, naringenin,naringin, quercetin, hesperidin,tangeretin, hesperetin, diosmin,apigenin, and nobiletin. It was also noted that environmental and genetic factors play a significant role in the contents and dispersion of flavonoids in several Citrus species [58]. Figure 2 shows the molecular structure of alkaloids and flavonoids present in the citrus fruits and plant extract.

Rehan et al. [59] effectively used DART-MS for the identification of phenolic components in the aqueous extract of orange peel (OP) (Figure 3). The determination of OP components was made by examining the extracted-ion analysis results of the ion flow at m/z levels comparable to [M - H]- and [M + H]+ ions of the examined compounds. Orange peel extracts were subjected to a DART-MS analysis, which revealed the presence of eight phenolic chemicals, including three flavonoids and five phenolic acids (Table 2) [59].

In addition to being one of the richest sources of vitamin C, citrus plant extracts also include significant levels of essential oils, carotenoids, flavonoids, and several minerals. Due to the presence of these compounds, citrus fruit peel exhibits an effective anti-microbial activity which makes it ideal for use in the production of medical fabrics [60].

## Methods for extraction of essential oils from citrus plants

Citrus essential oils have been employed as flavoring agents and for aromatherapy in a variety of goods, including meals, drinks, cosmetics, and medications. They are also employed for their anticancer, antioxidant, and bactericidal effects. Monoterpene hydrocarbons, which have a high amount of unsaturation and are typically unstable due to several causes, including light, heat, oxidation, and hydration, dominate citrus essential oils. Few studies have been carried out so far regarding the application of novel isolation techniques for citrus fruit essential oils. According to ISO specifications, essential oils are products obtained from unprocessed plant sources that can only be physically extracted. The physical procedures include dry distillation of organic substances, expression (often referred as "cold pressing" for citrus peel oils), and distillation (water, steam, and steam/water) [61]. The essential oils are physically isolated from the aqueous phase after distillation or expression. The citrus peels are often cold pressed in order to isolate volatile components to be utilized as essential oils. The citrus fruits peel and cuticles contain oil glands or sacs at various depths that contain citrus essential oil. Cold pressing physically removes peels and cuticle oils, and as cold pressing produces an aqueous emulsion, this emulsion is subsequently centrifuged to isolate the essential oil [61].

In certain countries, distillation is also utilized to economically collect essential oils from citrus by-products. Citrus peels subjected to steam or hot water during distillation liberate their essential oils via evaporation process. By distilling two immiscible liquids, particularly essential oils and water, the extraction of the essential oils is assisted on the assumption that, at boiling temperature, the total vapor pressure of the liquid matches the ambient pressure. As a result, the components of essential oils having boiling points typically ranging from 200 to 300 °C, evaporate at a temperature similar to that of water. The essential oil-filled steam passes and reaches a small tube that is being cooled from the outside. Steam as well as the essential oil droplets are recovered and separated after condensation in a container known as a "Florentine flask,". Essential oil floats on top being less dense than water, while water sinks to the bottom and could be readily separated [62].

There are some drawbacks to using traditional methods to extract citrus essential oils. The high temperatures and extended extraction times during hydro-distillation and steam distillation can change the chemical composition of the oil components and frequently lead to the loss of the most volatile compounds. Citrus essential oils are vigorously stirred with water during cold pressing, and the concentration of terpene alcohols and citral gradually decreases. Additionally, air is whipped into the liquid during agitation, which promotes resinification, hydrolysis, and oxidation. Due to these drawbacks, the use of novel essential oil and aroma extraction methods has been taken into consideration [63]. These methods include ultrasound extraction [64], a controlled pressure drop process [65], supercritical fluid extraction [66], subcritical water extraction [67], and microwave extraction [68]. These methods typically increase the quantity of essential oils, preserve their quality, and consume less energy.

A novel patented process called fast microwave-accelerated distillation, also referred to as microwave "dry" distillation or "MAD," isolates essential oils from natural materials by fusing microwave radiation with traditional distillation [69]. In this technique, fresh vegetable materials are added to a microwave reactor. The in-situ liquid (mostly water) within the plant material expands as a result of heat produced, causing the glands and oleiferous vesicles to rupture. Thus, the essential oils that were trapped in the in-situ water of the plant matter via azeotropic distillation is released through this method. After passing via a condenser outside of the microwave cavity, the vapors are then condensed. In a bottomed flask, the distillate is continually collected. Cohobation restores the moisture of the plant material, refluxes surplus water and recycles it to the extraction vessel. The essential oil is immediately extracted and dried w ithout performing an additional solvent extraction step. Essential oils have been extracted using MAD from a variety of source materials. The modified microwave-assisted extraction (MAE) approach, which employs organic substances, and the modified hydro-distillation (HD) technique, which needs a lot of water, are not comparable to the MAD method. Essential oils from aromatic spices and herbs such as thyme, basil, mint, star anise, ajowan, and cumin have been extracted using MAD [69].

## Citrus plants as bio-reductants in synthesis of nanoparticles

Nanotechnology is becoming a significant research area due to its various potential applications in all domains of science, technology, healthcare, pharmacology, and other related fields. It includes materials as well as their applications with a single dimension between 1 and 100 nm. Nanoparticles (NPs) are often synthesized using a variety of processes, including milling, laser ablation, sputtering, chemical reduction, etc. These traditional methods, including the chemical reduction method, which uses a variety of harmful chemicals to synthesize nanoparticles, later pose several health problems due to their toxicity and raise severe environmental concerns, while other methods are expensive and require a considerable amount of energy. However, for biomedical application where purity is a priority, the biogenic synthesis process to synthesize nanoparticles is ecologically responsible and free of chemical pollutants. The extracts of natural plants, proteins or enzymes, are employed as reducing or stabilizing agents in the biological process of nanoparticles synthesis. Considering this, Ain Samat et al. employed *Citrus aurantifolia* extract in the biosynthesis of zinc oxide (ZnO) nanoparticles. They used zinc acetate as the precursor of Zn at various concentrations, and they reported that the synthesized ZnO nanoparticles were between 50 and 200 nm in size using FESEM [5]

Manal et al. [70] attempted to synthesize Ag-nanoparticles using biological waste material from citrus lime peels, and to characterize the produced green Ag-nanoparticles for their antibacterial properties and cytotoxic effects. According to a UV-visible spectrophotometer analysis of the synthesized Ag-nanoparticles, spherical and irregularly agglomerated Ag-nanoparticles development was reported. According to DLS measurements, the synthesized Ag NPs had an average size of 59.74 nm. The majority of the examined human pathogenic bacteria exhibited significant activity against the produced Ag-nanoparticles in varied degrees in antimicrobial experiments. At last, two types of cell cultures human breast cancer cell line (MCF-7) and colon carcinoma cell line (HCT-116) were used to assess the cytotoxic effect of the greenly produced Ag-nanoparticles. The findings showed that cell viability and concentration are directly related [70]. Amanulla et al. [71] used extract of orange peel to synthesize TiO2 nanoparticles. An agar well diffusion was used to measure their antibacterial action against *S. aureus, E. coli, and P. aeruginosa. P. aeruginosa* exhibited more activity when exposed to the nanoparticles [71].

In another study, orange peel waste can be used to produce silver nanoparticles in a natural, sustainable, and environmentally beneficial way. Orange peel-extracted Ag nanoparticles have strong photocatalytic properties against dyes and can also be widely used to treat dye effluent and treat wastewater [60].

Employing a biosynthetic process that uses plant extracts can greatly increase anti-microbial efficacy of zinc oxide nanoparticles. The presence of components (phytochemicals) that are intrinsically resistant to microorganisms may be the primary reason for the enhanced anti-microbial action of ZnO nanoparticles using plant extracts. This suggests that biologically synthesized zinc oxide nanoparticles could be an efficient substitute of conventional agents because of their benefits associated, such as their affordability and eco-friendliness. According to the literature, *Citrus aurantifolia, Citrus limon, and Limonia acidissima* are the three citrus species that were most frequently used in the green synthesis of ZnO nanoparticles [72]. N. Ain Samat and R. Md Nor [73] used *Citrus aurantifolia* fruits to prepare the extract. The fruit-skin was removed from these fruits after they were peeled, and the pulp was chopped and homogenized with deionized water. Following filtration, the filtrate was employed in further experiments. They used the zinc acetate ($Zn(OAc)_2.2H_2O$) as zinc precursor. Various amounts of zinc acetate were added in the Citrus aurantifolia extract. The mixture was then agitated at 90°C for three hours. This produced a white precipitate, which was then washed and dried at 100°C for 6 hours. The ZnO nanoparticles were successfully synthesized from this method [72]. In contrast to other citrus fruit (lime, grapefruit, lemon, tangelo, and orange) peel extracts, orange peel extract produced Ag nanoparticles (7.36 8.06 nm) in 15 minutes when employed in the microwave-assisted production of Ag-nanoparticles. These extracts revealed improved inhibitory activity against *E. coli, P. aeruginosa, S. aureus, and B. subtilis* [74].

In another study, plant powders most commonly are obtained from, *Citrus sinensis, Centella asiatica, Solanum tricobatum, and Syzygium cumini* were used to synthesize silver nanoparticles from AgNO3 solution as silver precursor. Agar well diffusion approach was used to test the antibacterial action of green-synthesized silver nanoparticles. The Ag-NPs synthesized from *C. sinensis* and *C. asiatica* exhibited highest antibacterial action (16 mm) towards *Pseudomonas aeruginosa*. It was observed that the Ag-nanoparticles produced by this technique have effective antibacterial activity towards harmful microorganisms [75]. Naikoo et al. [76] reported the synthesis of Au nanoparticles using citrus fruits with lemons and reticulate. Lemons were first smashed to extract the juice, which was then placed over polyamide mesh with fine pores. The extract was centrifuged at 10,000 rpm for ten minutes. In order to facilitate vigorous homogeneous mixing, 1 mM of a 50 mL composition of hydrogen tetrachloroaurate trihydrate was eventually heated to a boil. The extracted juice was diluted with the host solution using a range of 1- 6 ml. There was a noticeable transition in colour from colorless to dark violet to ruby red. The granular solution was vigorously agitated again after 20 minutes, and it was then cooled to room temperature. After that, it was moved to another container where it was stored. Peak of the surface plasmon resonance was seen in the 530–550 nm range. In-depth TEM analysis was done to elucidate the structural characteristics of Au nanoparticles [76].

Sudhir et al. reported a non-toxic, low-cost, and environmentally friendly technique for the production of copper nanoparticles by employing citron juice (Citrus medica). The biologically active copper nanoparticles were identified using a UV-Vis spectrophotometer, which exhibited a typical resonance (SPR) for Cu-nanoparticles at about 631 nm. The FCC composition of nanoparticles with a mean size of 20 nm was determined using X-ray diffraction. The antibacterial activity of copper nanoparticles was assessed by using the Kirby-Bauer disc diffusion method on specific chosen bacterial species and plant pathogenic fungi. *E. coli* strains were found to be the first pathogen that the produced Cu-nanoparticles significantly inhibited, following *K. pneumoniae, P. aeruginosa, Cutibacterium acnes*, and *Salmonella typhi*. *Fusarium graminearum, Fusarium culmorum*, and *Fusarium oxysporum*, were shown to be the most vulnerable plant pathogenic fungus [77].

In another study, researchers used *Citrus sinensis* peel extract as a reducing and capping agent in a novel, efficient green chemistry process to produce silver nanoparticles [53]. The particles were 10 and 35 nm in size at 60 and 25 ° C., respectively according to TEM measurements. Generally spherical particles with a diameter range from 15 to 50 nm were produced during the development of stable Ag nanoparticles at various concentrations of AgNO3. The reductive potency of orange peel (*Citrus sinensis*), a typical waste of the food processing industry, has been documented to be used to generate biodegradable polymer templated "green" silver nanoparticles in an attempt to make nanoscale research more environmentally sustainable. TEM analysis revealed spherical particles of 3–12 nm in size. The fact that the majority of the particles had a diameter of 6 nm was particularly noteworthy. The synthesized Ag nanoparticles revealed excellent antibacterial activity [78]. Ali et al. [79] reported the use of green synthesized (GS) ZnO nanoparticles to make nanostructured ZnO thin films by employing a spray pyrolysis technique. Investigations were conducted into how the deposition rate affected the optical, electrical, and structural characteristics of the ZnO thin films. In order to prepare the ZnO nanoparticles, a Zn (Ac)2.2H2O solution was dissolved in an aqueous *Citrus reticulata* peel extract. FE-SEM was used to describe the morphological properties of the GS samples, and XRD analysis was used to determine the crystal structures [79].

## Citrus plant extracts as antimicrobial finishing for cotton fabric

The textile dyeing and finishing sector has established the significance of green finishing compositions. K. A. Hammer et al. [80] investigated the antibacterial activities of 52 plant oils and extracts against different Gram positive, Gram negative bacteria, and yeast such as *Acinetobacter baumanni, Aeromonas veronii* by using agar and broth dilution procedures. At concentrations under 2.0% (v/v), it was revealed that bay, lemon grass, and oregano prevented the growth of microorganisms [80]. Thangamani, K., and Periasamy, R. [81] conducted a research study on the herbal finishing of natural fabrics, such as soya bean, cotton, and bamboo fabrics to assess their antimicrobial property. They selected *Plectranthus amboinicus, Terminalia chebula, Ocimum tenuiflorum (tulsi), Aloe barbadensis, Cymbopogon flexuosus* and *Asteraceae* as natural antimicrobials. These herbal sources antibacterial methanol extracts were applied directly to fabrics using the pad-dry-cure procedure. They observed that textiles made up of cotton and bamboo treated with asteraceae exhibited no antibacterial action. However, soya bean fabric had good microbial activity. Lemon grass oil applied to cotton fabric was found to have the strongest antibacterial effects on both Gram-negative and Gram-positive bacteria [81]. $FeCl_2.4H_2O$ and FeCl3.6H2O were added in 100 ml distilled water in a 250 ml conical flask for the bio-synthesis of magnetite nanoparticles. The mixture was then heated at 80 °C with gentle stirring using a magnetic stirrer. The aqueous solution of lemongrass leaves extract was added to the above mixture after 10 minutes, and it was heated for 5 minutes while being constantly stirred. For homogenous magnetite precipitations, 20 ml of 0.1N NaOH aqueous solution were added to the stirring solution at a rate of 3 ml/min after 5 minutes. The solution was then cooled down to the room temperature. The aqueous solution was diluted using sterile distilled water and centrifuged at a speed of 15,000 rpm for 15 minutes after being decanted. After centrifugation, the magnetite particles were separated and dried for 48 hours at 80°C in a hot air oven. The obtained dried form was mashed with a pestle and mortar. The ultrasonication process and dipping were used to coat the particles onto the textiles. Magnetite nanoparticles were successfully coated onto cotton textile samples using the ultrasonication process. The antibacterial action of the produced magnetite nanoparticles towards *B. subtilis* and *E. coli* was evaluated using the agar well diffusion method and the disc-diffusion method. In the disc diffusion method for *E. coli*, the first disc was the sterilized untreated fabric with as control sample for ampicillin while the second disc was treated cotton sample, which was also sterilized. There was a significant inhibition of microbial growth from it in the disc diffusion method (Figure 4) [82].

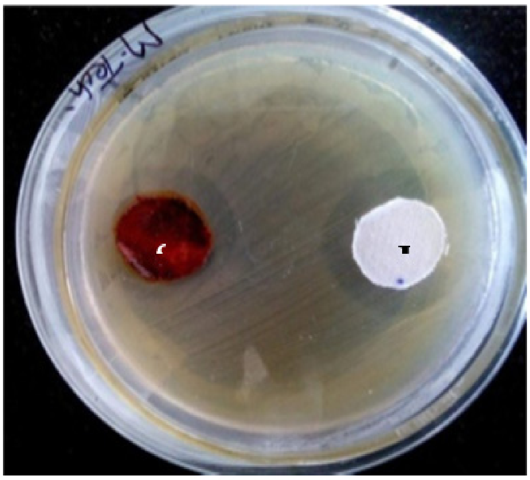

**Figure 4.** Antibacterial action of fabric treated with synthesized NPs and control fabric (ampicillin) against *E. coli.*

Biological synthesis of nanoparticles has always been significant because there are no hazardous synthetic substances involved in the process. This study also reported the use of citrus plant extract in the synthesis of nanoparticles. *Citrus sinensis* fruit fresh peel juice was used to make Ag nanoparticles. Field emission scanning electron microscopy (FESEM), X-ray diffraction, and UV-visible spectroscopy were used to analyze the synthesized nanoparticles. The produced Ag nanoparticles were immobilized on the cotton fabric after being characterized. The antifungal and antibacterial properties of synthesized Ag nanoparticles were also evaluated. Ag nanoparticles exhibited significant growth reduction against methicillin-resistant *S.aureus, Candida albicans*, and *Candida tropicalis* in antimicrobial investigations [83]. Vankar and Shukla [84] used *Citrus limon* leaf aqueous extract to produce silver nanoparticles. The synthesized Ag nanoparticles were then tested for antifungal action on silk and cotton fabrics. Agar diffusion was used to measure the antifungal activity

**Table 3.** The values of zone of inhibitions (ZOI) of citrus lemon peel extract against *S. aureus* and *E. coli*.

| **Sr #.** | **Zone of Inhibition (mm)** | | |
|---|---|---|---|
| | **Sample** | ***S. aureus*** | ***E. coli*** |
| 1. | Orange lemon | 20-24 | 17-21 |
| 2. | Green lemon | 24-30 | 22-26 |
| 3. | Black lemon | 18-26 | 18-25 |

towards *Fusarium oxysporum* and *Alternaria brassicicola*. The antifungal activity of the cotton and silk materials was strong and long-lasting [84].

This research work was aimed to investigate, identify, and assess the bioactive substances that can be obtained from orange peels (OP) using an ultrasonic extraction technique as a potential eco-friendly additive for multifunctional cellulosic textiles and fibres. The novel approach employed two methods. An environment friendly in-situ synthesis of Ag, ZnO, and ZnO/Ag nanoparticles was successfully established for the fabrication of multifunctional viscose fibres using phenolic chemicals derived from OP. The treated viscose fibres provide exceptional antioxidant, antibacterial, UV protection, photo catalytic and self-cleaning activities [59].

Similarly, another research work was conducted to investigate, assess, and compare the antibacterial properties of cotton coated with essential oils isolatedfrom black, green, and orange (a combination of orange and green) lemon peel (Citrus limon). The of orange and green) lemon peel (Citrus limon). The citrus limon peel is highly nutritious, including essential oils and flavonoids, which have antibacterial properties. The finishing agent i.e., lemon peel extract, was obtained by steam distillation methods after being treated with methanol. By measuring the zone of inhibition, the antibacterial activity was assessed against the gram-negative *E. coli* bacteria and the gram-positive *S. aureus* bacteria. In comparison to orange and black lemon, cotton treated with green lemon peel extract exhibited strong antibacterial activity against *S. aureus* (26-30mm) and *E. coli* (20-25mm) test microbes. In comparison to orange lemon peel, black lemon peel extract had stronger antibacterial activity against S. aureus (18-26mm) and E. coli (18-25mm) test microbes. Furthermore, the durability of the biological finishing agent on cotton fabric was assessed both before and after washing, and the results were the same. The results from this study indicated that the antibacterial action of cotton fabric treated with the biological finishing agent was same before and after laundering. This study showed that citrus lemons had more robust, long-lasting antibacterial capability, with extract of green lemon peels having the most effective effect [8].

Orange peels (OP) are one of the fruit wastes produced mostly by the juicing companies that contribute to environmental issues because of their high production volume and physicochemical features, which include water and soil contamination. Orange peel has antibacterial capabilities as it is flavonoid-rich. According to a study, orange peel and papaya skin supplemented with silver nanoparticles enhance the anti-microbial properties of the treated textiles [60]. Citrus waste extracts from orange and lemon peels were tested to evaluate the washing durability of biological antibacterial coatings. The antibacterial activity of the fabric samples against various bacterial strains was evaluated quantitatively by AATCC testing method 100-2004 following various washing conditions. The findings revealed that the cotton treated with biological finishes exhibits resistance to microbes [85]. The antimicrobial properties for textile material (56% cotton/44% polyester) was evaluated using the essential oils isolated from rosemary *(Rosmarinus officinalis*) and orange (*Citrus sinensis*) at concentrations of 1%, 3%, and 5% for each oil. The antimicrobial action was evaluated against each strain. The obtained results supported the use of textiles functionalized with orange and rosemary essential oils as effective active antimicrobial inhibitors, with a maximum reduction of 92.48% for orange extracts and 56.99% for rosemary extract [86]. With the objective of encouraging bio-based substances in textile dyeing and finishing, Shahid et al. [87] presented the use of chitosan combined with the extract of *Citrus sinensis* peel biomolecules as a method to increase the natural dyeing efficiency of cotton fabric at optimal conditions. Chitosan polymer was first soaked in acetic acid and then, using the pad-dry process, coated on cotton. Cotton samples that had been coated with chitosan were then dyed using a *Citrus sinensis* peel extract to enable the employment of chitosan biological mordant to enhance colorimetric results. This work offers great potential for use in natural dyeing technique by revealing essential information about the synergistic effects between two natural chemicals in imparting semi-durable antibacterial and antioxidant action against S. aureus and E. coli bacteria [87]. Cotton fabric was dyed with the citrus extract under various conditions, such as dye concentration, temperature, pH, and, duration and evaluated for fastness and antimicrobial effectiveness in order to investigate the possible future use of *Citrus grandis* in biological applications for strengthening human skin health. The optimised dyeing of the cotton fabric was achieved at a pH of 3, a dyeing temperature of 60°C, a dyeing time of 60 min, and a dye concentration of 80% owf. Cotton dyed with the maximum concentration of antimicrobial agent exhibited outstanding bacterial growth reduction rates against S. aureus for up to five launderings, indicating the need to increase washing durability [88]. In another investigation, two techniques were used to apply lemongrass oil to fabric: exhaust method and oil microcapsules applied through padding. Complex coacervation techniques were used to develop the lemongrass oil microcapsules, which were then applied to cloth using the pad dry cure method. The AATCC-100 was used

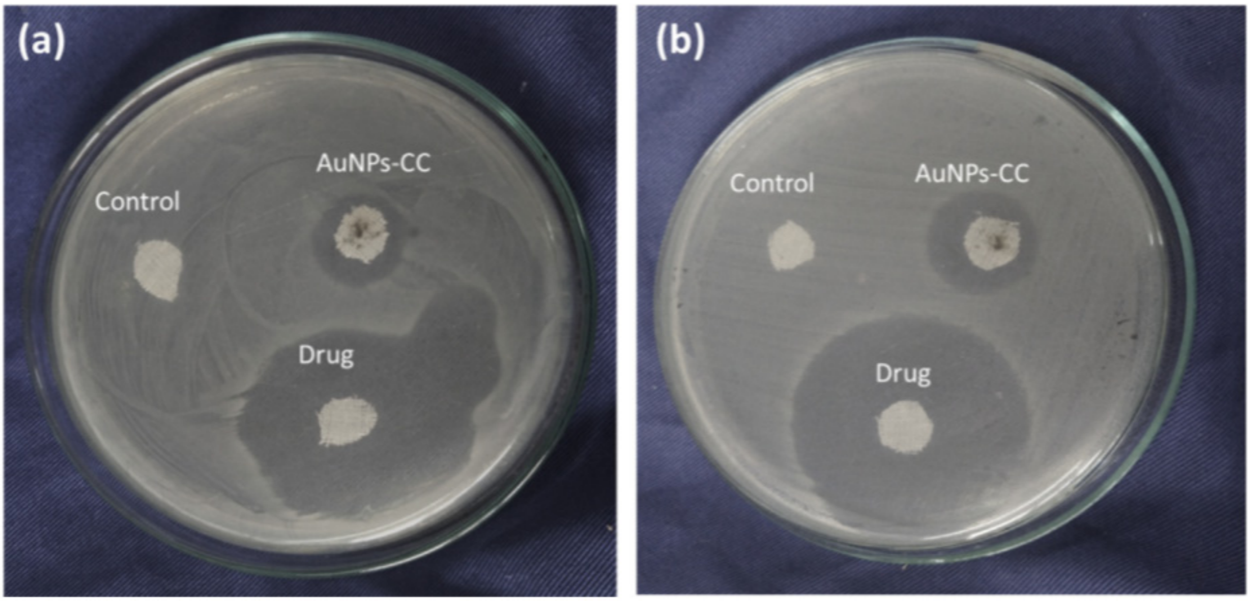

**Figure 5.** Antibacterial action of cotton fabric treated with synthesized nanoparticles, negative control (CC) positive control (drug) towards E. coli (a) and S. aureus (b) [90].

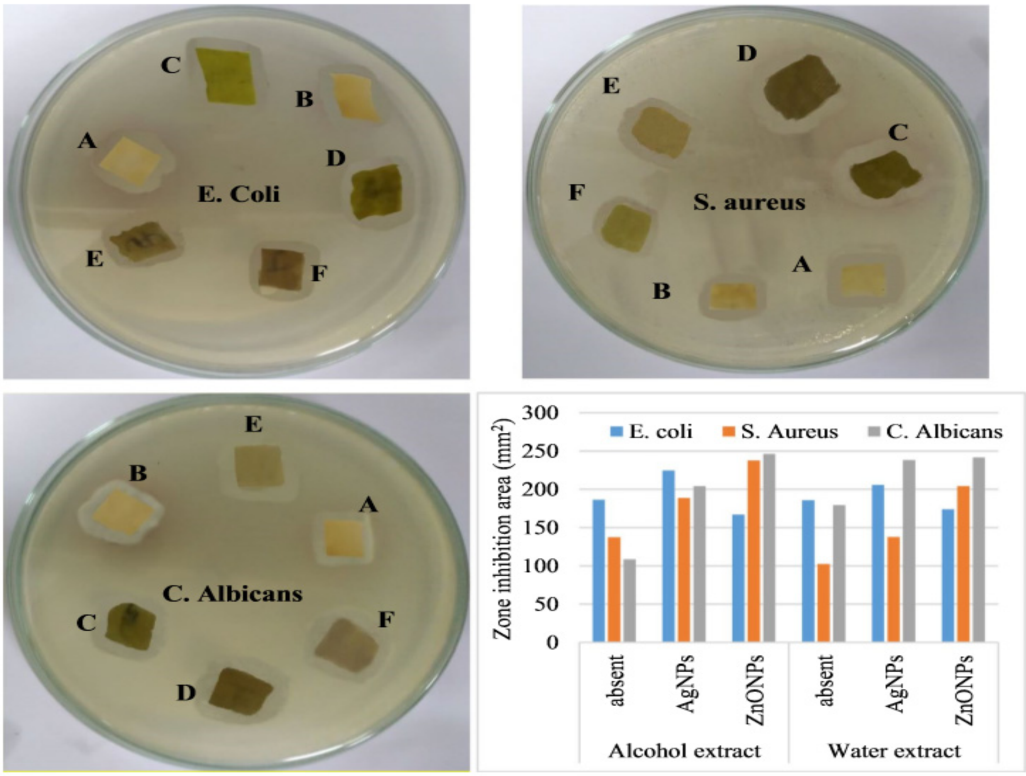

**Figure 6.** Antibacterial activity of cotton fabric treated with alcoholic and water extract of citrus plants [47].

to evaluate the antibacterial properties of untreated and treated cotton fabrics. The fabric treated with lemongrass oil microcapsules demonstrated an 80% bacterial growth inhibition [89].

In another study, a facile and environmentally sustainable method for producing biologically active gold nanoparticles on cotton fabric (AuNPs-CC) was established. It was established that the -OH groups in the cellulose polymers, which are abundant in cotton, ultimately reduced the Au ions into gold nanoparticles. A citrus limon juice extract was used to expedite the kinetic process of AuNP production. Energy-dispersive spectroscopy (EDS), field emission scanning electron microscopy (FESEM), and other spectroscopic techniques were used to evaluate the developed samples. The FESEM results clearly showed he 22 nm size Au nanoparticles adhered to the cotton fabric. The development of bioactive Au nanoparticles over the CC surface was verified by the XPS and XRD. The bactericidal abilities of the various strains of the pristine-CC and AuNPs-CC were investigated. The Citrus limon assisted synthesized AuNP-CC revealed excellent antibacterial properties against bacterial pathogens [90].

*Citrus Sinensis* peel (orange peel) extract was utilized in an environmentally friendly process to endow cotton fabric with multifunctional qualities. The extract has been made using both water and ethanol as solvents. Both extractions have been employed as stabilizing and reducing agents in the synthesis of zinc oxide and silver nanoparticles. Different methods, including Fourier transform infrared (FTIR), total phenolic contents, antioxidant properties, particle size analyzer, transmission electronic microscopy (TEM), and X-ray diffraction, have been used to characterize the prepared extracts and synthesized nanoparticles. Silver nanoparticles, chitosan-loaded zinc oxide nanoparticles, and orange-peel extraction were applied on cotton fabric. Scanning electron microscopy, UPF rating, antibacterial activity, mosquito repellent effects, and self-cleaning features were evaluated to examine the treated cotton samples [47].

## Evaluation methods of antibacterial fabrics

Multiple testing methods have been devised to assess the potency of antibacterial textile materials. These testing standards can be divided into two main categories: quantitative analysis methods and qualitative analysis methods. The procedures to carry out these tests are briefly discussed below.

### *Qualitative testing protocol*

Some of the methods for qualitative antimicrobial tests usually referred to as agar diffusion or disc diffusion tests include SN 195920-1992 (Swiss Norm), JIS L1902-2002 (Japanese Industrial Standards), and AATCC 147-2004 (American Association of Textile Chemists and Colorists) [91]. These assessments are only qualitative, but they are simple to do and work best when screening lots of samples for antimicrobial effect. To conduct this experiment, microbial cells are loaded onto nutrient medium plates. The untreated and treated fabric samples are then placed on the injected agar plates. The plates loaded with samples are checked for bacterial growth from around test samples (zone of inhibition) and underneath the fabrics after being incubated at 37°C for 18 to 24 hours. The lack of bacterial activity right beneath the textile swatch indicates antimicrobial efficacy. If the antimicrobial agent is strongly covalently attached to the textiles, it is unable to penetrate into the agar media, which results in the absence of a zone of inhibition. If the antibacterial chemical diffuses in the agar media, a zone of inhibition (ZOI) is developed. Its diameter gives an approximate estimate of the potential for antibacterial activities. The diameter of the ZOI is determined using equations. Although each approach employs a different calculation method, all of the abovementioned techniques follow the same methodology. The AATCC-147 method is advised because it offers information that is generally accurate in terms of the diameter of the inhibition zone.

Another qualitative test method exists and is recognised as an international standard by the European Union (EN ISO 20645) [92]. This method of testing evaluates the impact of antibacterial treatments on plain woven, knit, and other textile cloths. The hygiene surfaces of hydrophilic and permeable materials, or antibacterial additives applied to the fibre, are subject to this standard testing. The smallest antibacterial treatment diffusion is needed for this agar test technique. Other materials should also adhere to the ISO 20645 standard for it to be effective. Testing textile items treated by antibacterial methods which react with agar is not appropriate for such a testing method [93].

### *Quantitative testing protocol*

The primary quantitative assessment methods used for the evaluations of antibacterial are AATCC 100-2004, JIS L1902-2002, SN 195924-1992, and ISO-20743 [91,94,95]. These procedures are substantially more time-consuming than qualitative antibacterial screening, but they offer precise quantitative evaluation of the antibacterial materials. According to the appropriate norms, approximately 1ml of inoculum is completely absorbed onto test fabric swatches of different sizes. This step ensures that the analysed samples and the microorganisms have direct contact. According to standard protocol, the bacterial suspension-loaded textile sample is incubated for the required amount of time. The precise quantity of injected and recovered bacteria is counted by employing the serial dilution procedure followed by plating of dilutions on agar media plates. By comparing the quantity of injected and recovered bacteria at 0 h and after a given incubation period, antibacterial activity is assessed in terms of percentage reduction and log reduction. In order to ensure that the obtained reduction in microbial population is substantially due to the antibacterial finishing, suitable control samples, such as fabric samples that have undergone the identical processing steps without the antibacterial finishing, must be tested in each experiment. The choice of an appropriate calculation equation could be crucial. Quantitative evaluation can be done using any method, but the ISO-20743 method is believed to be the most useful for evaluating the microbe resistance of textiles. This is because no other method can accurately simulate hospital textiles as hospital textiles practically prohibit the complete absorption of an inoculum dose (typically 1 ml) onto the fabric. In this method, textile swatches are placed on infected culture plate for a certain amount of time (60 seconds), after which they are removed for further analysis.

### *Minimum inhibitory concentration assays*

Broth tube dilution tests [96] and Liofilchem strip test procedures are used to determine the minimum inhibitory concentration (MIC) of antimicrobials [97]. The MIC value represents the lowest concentration at which the test microorganisms cannot grow. In order to assess MIC in fresh growth media, microorganism sub-cultures are subjected to various doses of the antimicrobial agent. The MIC value of that active agent is defined as the concentration of antimicrobial at which no growth is seen. The Liofilchem strip test are not economically sound due to the high cost of the strips required for this test. As a result, broth dilution tests are preferable because they do not require any additional materials like strip test methods do.

## Challenges associated with green synthesis of nanoparticles

Research on nanoparticles and promising applications has advanced significantly in recent years. The green synthesis of metallic nanoparticles using a variety of biological sources, including plants, bacteria, fungi, and yeast, has been documented in numerous investigations. However, there are still a number of issues that prevent its widespread manufacture and ensuing uses. The following is a summary of some of the challenging issues encountered during the synthesis:

- Extensive optimization studies on raw materials (plant extract) and processing parameters (temperature, rotational speed, pH, etc.) are required to regulate the size and form of the nanoparticles.
- Investigations are also needed to focus on optimizing different physicochemical properties of nanoparticles for certain applications.
- It is important to thoroughly examine the effect of each metabolite found in plant extracts and cellular elements of microorganisms on the synthesis of nanoparticles.
- Prioritization must be given to expanding the synthesis of nanoparticles for industrial applications by employing green synthesis techniques.
- Optimization of different reaction parameters for the improvement of nanoparticles stability and yield with reduced synthesis time is required.

Biosynthesized nanoparticles have limited their applications in textiles due to their instability and the weak bond that forms between the textile material and nanoparticles. Another major issue that must be investigated is the extraction and purification of nanoparticles from the reaction medium.

By overcoming these challenges, it might be possible to produce nanoparticles on a large scale more inexpensively and efficiently using green synthesis techniques than with traditional techniques.

## Problems associated with green synthesised nanoparticles

Long-term stability is constrained by current techniques of nanoparticle functionalization [94]. As nanoparticles are removed from fibre material, the nanoparticle dopants are vulnerable to leaching. This would be especially important for textiles and garments that go through multiple cycles of washing and drying as well as mechanical stress. Depending upon the product, metal nanoparticle emission from textiles might reach 80% within the first wash. Additionally, copper as well as other nanoparticles like silver that are used in textiles have shown to be harmful in ecological systems. The biological treatment procedures utilised in both municipal and commercial water treatment facilities can be interfered with by these antimicrobial nanoparticles [98].

## CONCLUSION

Metallic nanoparticles have found potential application in both the engineering and biological applications. In recent years, huge increase in their demand has been observed, and this development is not expected to halt. This review was aimed at summarising the significance of nanotechnology and nanoparticles (NPs) in the development of bioactive textiles, biosynthesis of NPs facilitated by the use of plant extracts while focusing on the utilization of citrus fruits and plant extracts. The chemical composition of citrus plants and fruits extract as well as the methods for the extraction of these biologically active components from citrus plants have been mentioned. This review also included the application of bio-synthesized biogenic nanoparticles on the cotton fabric for antibacterial properties. account of limitations, and challenges faced during the green synthesis of nanoparticles. The possible mechanisms exhibited by the nanoparticles to show antibacterial effect is also explained. This paper also summarizes different qualitative and quantitative standard testing protocols employed for the antimicrobial characterization of plant extracts and treated textiles. Moreover, the major challenges and limitations faced during the plant-based biosynthesis of nanoparticles have also been highlighted.

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