Synthesis of antimicrobial extract from *wrightia tinctoria* leaves for medical applications

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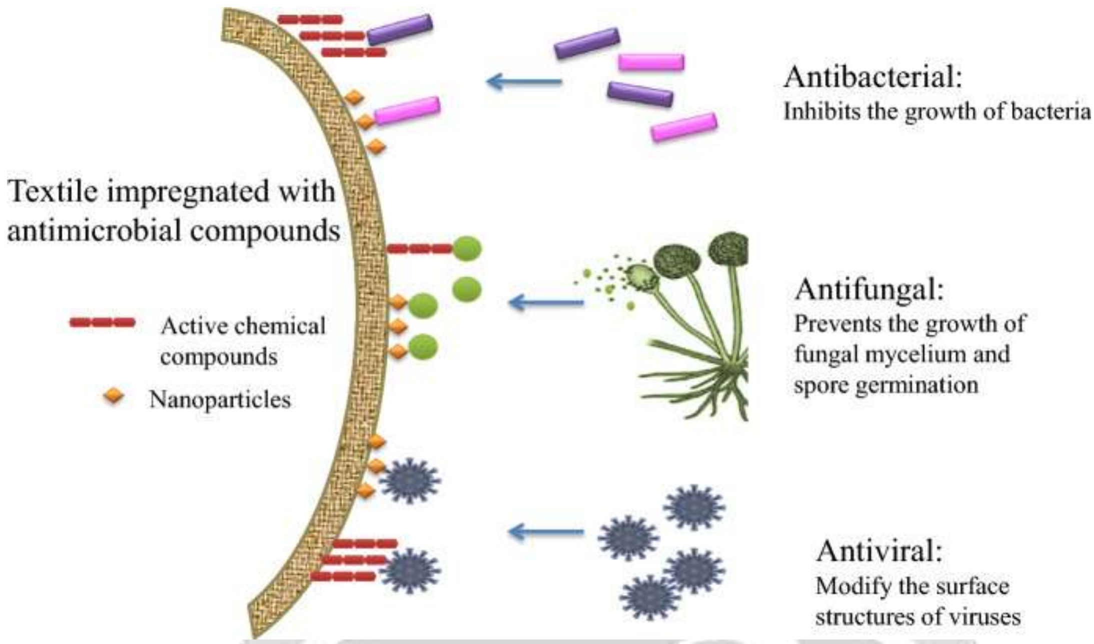
Abstract

Antimicrobial textiles are functionally active textiles, which may kill the microorganisms or inhibit their growth. The present article explores the applications of natural antimicrobial compounds used to prepare antimicrobial textiles. Different types of antimicrobial textiles including: antibacterial, antifungal, and antiviral have also been discussed. Different strategies and methods used for the detection of a textile's antimicrobial properties against bacterial and fungal pathogens as well as viral particles have also been highlighted. These antimicrobial textiles are used in a variety of applications ranging from households to commercial including air filters, food packaging, health care, hygiene, medical, sportswear, storage, ventilation, and water purification systems. Public awareness on antimicrobial textiles and growth in commercial opportunities has been observed during past few years. Not only antimicrobial properties, but its durability along with the color, prints and designing are also important for fashionable clothing; thus, many commercial brands are now focusing on such type of materials. Overall, this paper summarizes the scientific aspect dealing with different fabrics including natural antimicrobial agents along with their current functional perspective and future opportunities.

Keywords: Wrightia tinctoria, Antimicrobial, Antibacterial, Soxhlet.

Introduction:

Textiles are omnipresent and play an essential part in human society. Cloths may contain certain types of microbes, which has been recently discussed as clothing microbiology and the effect and interaction of cloths with human skin microflora. Coatings of natural antimicrobial agents on the textiles or fabrics date back to ancient times, when the Egyptians used spices and herbal coatings on cloths to prepare the mummy wrap. Traditionally, the Chinese used bamboo fibres, which contained an antimicrobial compound called 'Bamboo-kun' for housing structure. Bamboo fibers have also been explored for their natural antibacterial and antifungal properties, which are mediated by 2–6-dimethoxy-p-benzoquinone and dendrocin. Application of antibiotics developed during the Second World War; at the same time, the use of antimicrobials to prevent textiles from rotting was also in demand. To protect the fabric from microbial colonization and increase their durability, several military fabrics were treated with antimony salts, copper, and a mixture of chlorinated waxes, which not only stiffened the fabrics but also gave them a distinct Odor. During initial times, the side effects of these antimicrobials were not considered, however, more attention was paid toward the adverse effects of these chemicals on the environment and health. The concept of safer antimicrobial compounds and textiles came into existence following the publication of Rachel Carson's book *Silent Spring* in 1962. Different sectors including ecologists, scientists, industrialists, and chemists worked collectively to produce eco-friendly antibiotics.



In the present era, antimicrobial textiles are very helpful in hospitals, environment and places that are prone to microbes, which are baleful. The clothes are worn by the patients, healthcare workers and doctors may have a lot of microbes present on them, which can be transmitted easily from one person to another. Commercial opportunities abound for antimicrobial fabrics whenever it is about controlling the spread of infectious microorganisms.

Antimicrobial textiles can be termed based on their specificity against microbes, i.e., antibacterial, antifungal, or/and antiviral. Several antimicrobial textiles may also act against bacteria, fungi, and viruses simultaneously. Some chemicals may be used to target a broad range of microbes and are generally termed as antimicrobial. In common public area including hotels, restaurants, or trains such type of fabric is highly demanded, e.g., the towel which is used to mop up fluids, curtain and carpet could be a source of infection. There are also noticeable unfulfilled requirements for Odor control, which is another expanding research area in this field. The textile may contain several microorganisms that are anathema and may transfer from an infected person to others. The only possible and effective way for reducing the microbial load from textile is by continuous laundering of clothes but this case is not possible in hospitals where there are continuous shifts. On the other hand, another way to reduce the chances of microbial infection from one person to others through the textile is by developing antimicrobial textiles. These antimicrobial textiles may also be useful for the people involved in sanitary-related work and those who are working in sewage treatment, where there is a high risk of getting infected. Surface modification of the textile including electrospinning, nanotechnologies, plasma treatment, polymerization, microencapsulation, and sol–gel techniques has been done to impart some novel functional properties to textile, e.g., water-repellent, flame-retardant, and antibacterial activity.

Antimicrobial winter wear is gaining importance as these clothes are not washed frequently and rarely exposed to sunlight. These clothes are generally stored for a longer time, which may enable the growth of microbes and thus antimicrobial type fabric may be an appropriate option. Similarly, the antimicrobial textiles may also be useful in those places where non-plastic bags are used. Food packaging which generally involves degradable material is safer for the environment as well as does not affect the food properties, however, the concept of antimicrobial coating in such wrappers is important to reduce the growth of pathogenic and food spoilage microbes. Primarily, the antimicrobial textile is required by the following sectors along with the appealing combination of colour, print and design:

- Apparel: caps, jackets, sanitary pads, sportswear, undergarment, winter wear
- Commercial: carpets, covering for seats, window, vehicle, etc.; dusting cloths, military fabric, tent, uniform
- Health care: bandage, earbuds, scrub, mask, lab coats, protective kits
- Households: bedding, carpet, cover, curtains, mop, pillows, towel

SELECTION OF NATURAL ANTI MICROBIAL AGENTS

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and “leads” which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. This review attempts to summarize the status of botanical screening efforts, as well as in vivo studies of their effectiveness and toxicity. The structure and antimicrobial properties of phytochemicals are also addressed. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations.

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

SELECTION OF WRIGHTIA TINCTORIA

Wrightia tinctoria (Family: Apocynaceae) commonly called “Indrajau” is distributed throughout the world and occurs abundantly in India. It is a deciduous tree with white fragrant flowers. The seeds and bark of this plant are used in Indian traditional medicine as anti-diarrheal and anti-dysenteric. Sweet Indrajao is a small, deciduous tree with a light gray, scaly smooth bark. Native to India and Burma, Wrightia is named after a Scottish physician and botanist William Wright (1740-1827). From a distance, the white flowers may appear like snowflakes on a tree. The fruits pendulous, long-paired follicles joined at their tips. The hairy seeds are released as the fruit dehisces. The leaves of this tree yield a blue dye called Pala Indigo. Sweet Indrajao is called dhudi (Hindi) because of its preservative nature. Supposedly, a few drops of its sap in milk prevent curdling and enhance its shelf life, without the need to refrigerate. The wood of Sweet Indrajao is extensively used for all classes of turnery. It is made into cups, plates, combs, pen holders, pencils, and bed stead legs. It is commonly used for making Chennapatna toys.

***Medicinal uses*:** Ethnomedically, the bark of this plant is used as a galactagogue to treat abdominal pain, skin diseases and wounds, as an anti-pyretic, anti-dysenteric, anti-diarrheal- and anti-hemorrhagic agents, and as an antidote for snake poison. Seeds of this plant are also used as an aphrodisiac. In view of the reported severe health hazards of estrogen, such as increased risk of endometrial hyperplasia and carcinoma, breast cancer, and thromboembolic diseases. A large number of natural products showing promising anti-fertility activity in preliminary studies could not be pursued due to their associated estrogen-agonistic activity.

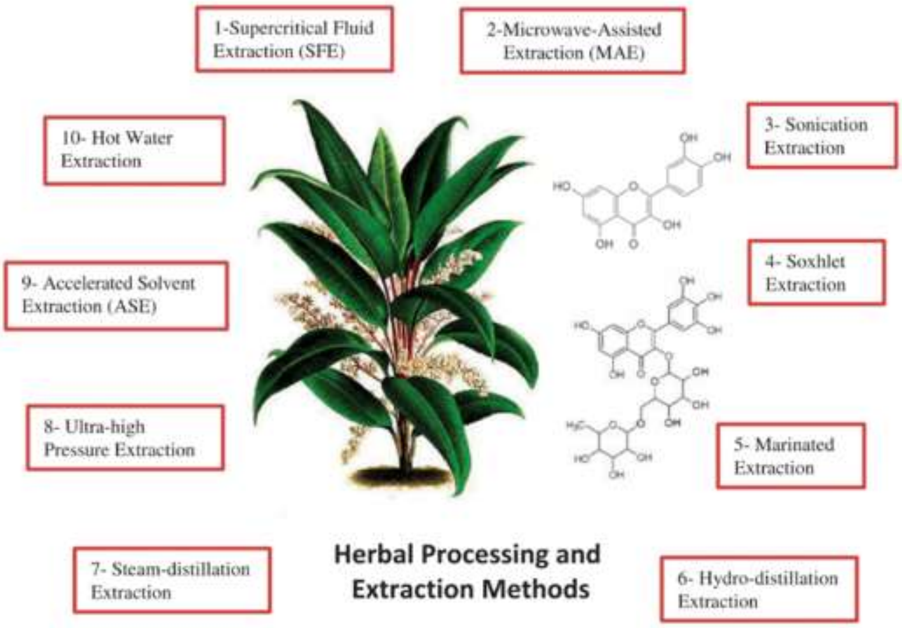
The leaves are applied as a poultice for mumps and herpes. Sometimes, they are also munched to relieve toothache. In folk medicine, the dried and powdered roots of *Wrightia* along with *Phyllanthus amarus* (keezhanelli) and *Vitex negundo* (nochi) are mixed with milk and orally administered to women for improving fertility. The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-inflammatory and anti-dandruff properties and hence is used in hair oil preparations.

Wrightia tinctoria is a small deciduous tree widely found throughout various parts of India. The tree is used for enormous medicinal purposes. The leaves are used in Ayurvedic medicines for treating toothache and hypertension; the bark and seeds are used to treat various indigestion and skin problems. The leaves are especially useful in treating skin diseases. They are used in Siddha medicine for the treatment of psoriasis. The major constituents such as indigotin, indirubin, tryptanthrin, isatin, anthranilate and rutin are isolated from W. tinctoria leaves. They also contain β-amyrin, lupeol, β-sitosterol and ursolic acid. The leaves are found to be acrid whereas the bark and seed are found to be bitter. The leaves have thermogenic and hypotensive properties. The bark and seeds have thermogenic, carminative, anthelmintic, depurative, and aphrodisiac properties.

EXTRACTION METHODS

Natural medicines were the only option for the prevention and treatment of human diseases for thousands of years. Natural products are important sources for drug development. The amounts of bioactive natural products in natural medicines are always low. Today, it is very crucial to develop effective and selective methods for the extraction and isolation of those bioactive natural products. This paper intends to provide a comprehensive view of a variety of methods used in the extraction and isolation of natural products. This paper also presents the advantage, disadvantage and practical examples of conventional and modern techniques involved in natural products research. The lab-intensive and time-consuming extraction and isolation process has been the bottle neck of the application of natural products in drug development. There is an urgent need to develop effective and selective methods for the extraction and isolation of bioactive natural products. This review intends to provide a comprehensive view of a variety of methods used in the extraction and isolation of natural products. Extraction is the first step to separate the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages: (1) the solvent penetrates the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature, and the extraction duration will affect the extraction efficiency.

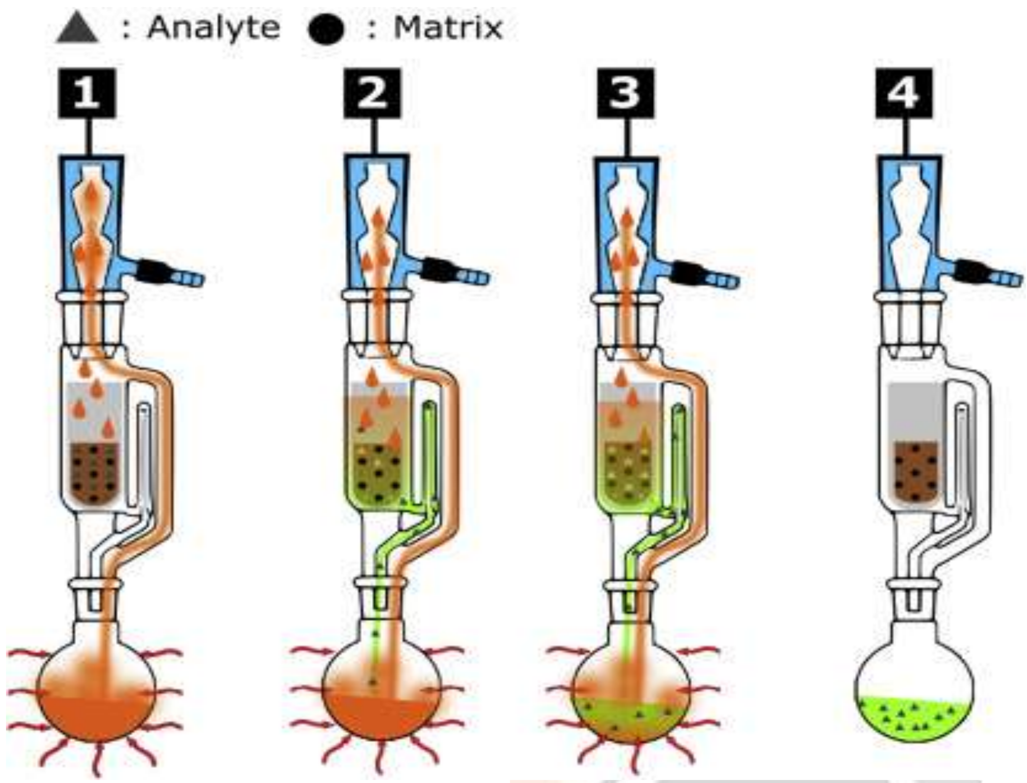
The selection of the solvent is crucial for solvent extraction. Selectivity, solubility, cost, and safety should be considered in selection of solvents. Based on the law of similarity and intermiscibility (like dissolves like), solvents with a polarity value near to the polarity of the solute are likely to perform better and vice versa. Alcohols (EtOH and MeOH) are universal solvents in solvent extraction for phytochemical investigation. Generally, the finer the particle size is, the better result the extraction achieves. The extraction efficiency will be enhanced by the small particle size due to the enhanced penetration of solvents and diffusion of solutes. Too fine particle size, however, will cost the excessive absorption of solute in solid and difficulty in subsequent filtration. High temperatures increase the solubility and diffusion. Temperatures that too high, however, may cause solvents to be lost, leading to extracts of undesirable impurities and the decomposition of thermolabile components. The extraction efficiency increases with the increase in extraction duration in a certain time range. Increasing time will not affect the extraction after the equilibrium of the solute is reached inside and outside the solid material. The greater the solvent-to-solid ratio is, the higher the extraction yield is; however, a solvent-to-solid ratio that is too high will cause excessive extraction solvent and requires a long time for concentration. The conventional extraction methods, including maceration, percolation and reflux extraction, usually use organic solvents and require a large volume of solvents and long extraction time. Some modern or greener extraction methods such as super critical fluid extraction (SFC), pressurized liquid extraction (PLE) and microwave assisted extraction (MAE), have also been applied in natural products extraction, and they offer some advantages such as lower organic solvent consumption, shorter extraction time and higher selectivity.



SOXHELT EXTRACTION PROCESS

Soxhlet extraction is one of the most popular techniques for extraction of analytes from solid materials. Since its discovery in 1879, the standard Soxhlet technique has been routinely applied in almost every analytical laboratory. Up to this day, Soxhlet extraction technique remains a standard technique to which the performance of modern extraction techniques is compared. Over the years, intensive research on different modifications has been carried out to overcome the main disadvantages of conventional Soxhlet technique. New approaches of this technique have been implemented to reduce extraction times and extractant volumes. SOX has been a standard analytical practice for over a century and is certainly the most widely used and accepted method for the extraction of SVOCs from solid samples. Although considered a more traditional technique, SOX is the accepted standard for comparison with other extraction approaches and therefore of central importance to the US EPA methods, as discussed in EPA Method .

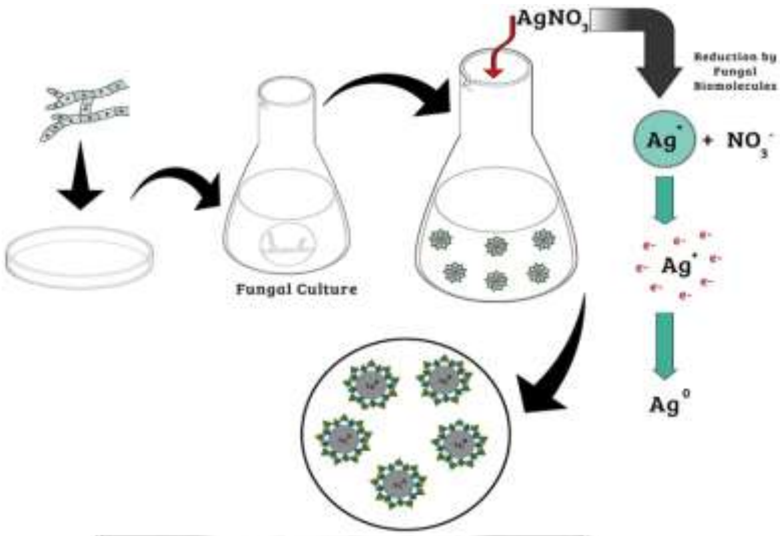
A schematic illustration of the SOX workflow. In the first step, the sample is placed in a disposable thimble that is positioned in the Soxhlet apparatus. Continuous extraction takes place by refluxing the solvent. Once the extraction chamber is filled, the extract is transferred to the boiling flask. This process is repeated until complete extraction is anticipated. Typical extraction times range between 12 and 24 h, making SOX a time-consuming technique. Additionally, the most common extractors use large amounts of purified solvent (several hundred millilitres).



The advantages of the Soxhlet extractor are: simple and clear design, production process continuity, ease of visual monitoring of the process, a low flow of solvent and the possibility of its reuse after stripping and distillation. Typically, Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.

SYNTHESIS OF NANOPARTICLES

Biosynthesized nanoparticles are gaining attention because of biologically active plant secondary metabolites that help in green synthesis and due to their unique biological applications. This study reports a facile, ecofriendly, reliable, and cost-effective synthesis of silver nanoparticles using the aqueous leaf extract of *wrightia tinctoria* and their antibacterial and antiproliferative activity. Silver nanoparticles were biosynthesized using the aqueous leaf extract of *wrightia tinctoria*, which acted as a reducing and capping agent. The biosynthesized *wrightia tinctoria* silver nanoparticles (Wt-AgNPs) were characterized using different techniques, such as UV–visible spectroscopy, dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDAX). Phytochemical analysis was performed to determine the phytochemicals responsible for the reduction and capping of the biosynthesized Wt-AgNPs. The antioxidant activity of the biosynthesized nanoparticles was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays.



Their antibacterial activity was checked against Staphylococcus aureus (Gram-positive) and Salmonella typhi (Gram-negative) bacteria. The biosynthesized nanoparticles showed dosage-dependent inhibition activity with a significant zone of inhibition and were more effective toward S. typhi as compared to S. aureus. This work revealed that the biosynthesized silver nanoparticles using *wrightia tinctoria* leaf extract were associated with good antibacterial activity and antiproliferative potential against selected cancer cell lines. The biosynthesized *wrightia tinctoria* AgNPs can be further exploited as a potential candidate for antioxidant, antibacterial, and anticancer agents.

COATING METHOD FOR ANTI-MICROBIAL PRODUCT

The formulation of a textile coating is complicated, and it can contain a wide range of chemicals depending on the nature of the polymer, the additives for the specific end use and the type of coating machinery used for its application.

Spread Coating

Using precision ‘knife-over-air’ and ‘knife-over-roller’ techniques we can apply solvent-based polymer coatings directly onto the fabric at weights from 8gsm up to 300gsm on one or two sides of the material. The solvent medium is then evaporated leaving a deposited layer of polymer on the fabric which is subsequently cured at high temperatures in specially designed ovens to vulcanise the coating and fix its final characteristics. Alternatively, fabrics can be supplied unvulcanised (semi-cured) if required for further processing by our customers.

Dip Coating

Dip-coating is used for the application of a wide variety of water-based solutions and fabric treatments. Chemical formulations can be tailor-made for a variety of end uses from basic mechanical strength promoters, sealing yarns, waterproofing, improving dimensional stability, through to using the desired chemical functional groups to improve adhesion and compatibility to further industrial processes such as PU priming and RFL (Resorcinol Formaldehyde Latex) dipping to prepare fabric for rubber reinforcement purposes. We can dip or impregnate one or both sides with processes that are designed to add low chemical treatments to fabric.

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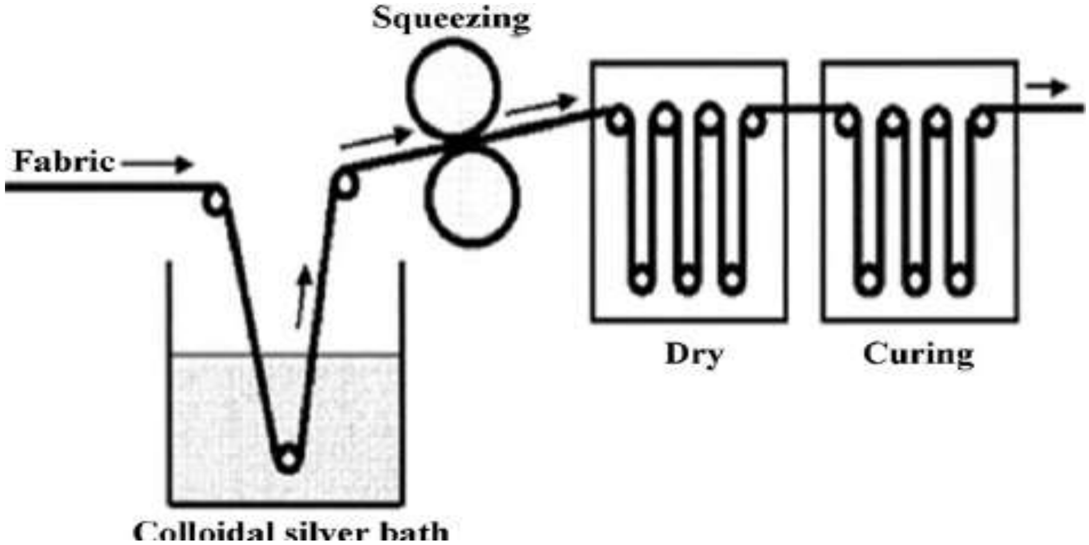
Fabric Lamination

The development of lamination process allows us to take individual fabric substrates and combine them into unique multi-layer bonded textile structures. These hybrid fabrics are combined through heat and pressure, resulting in the capability to produce combinations such as tri-laminates with specific performance characteristics.

PAD-DRY-CURE METHOD

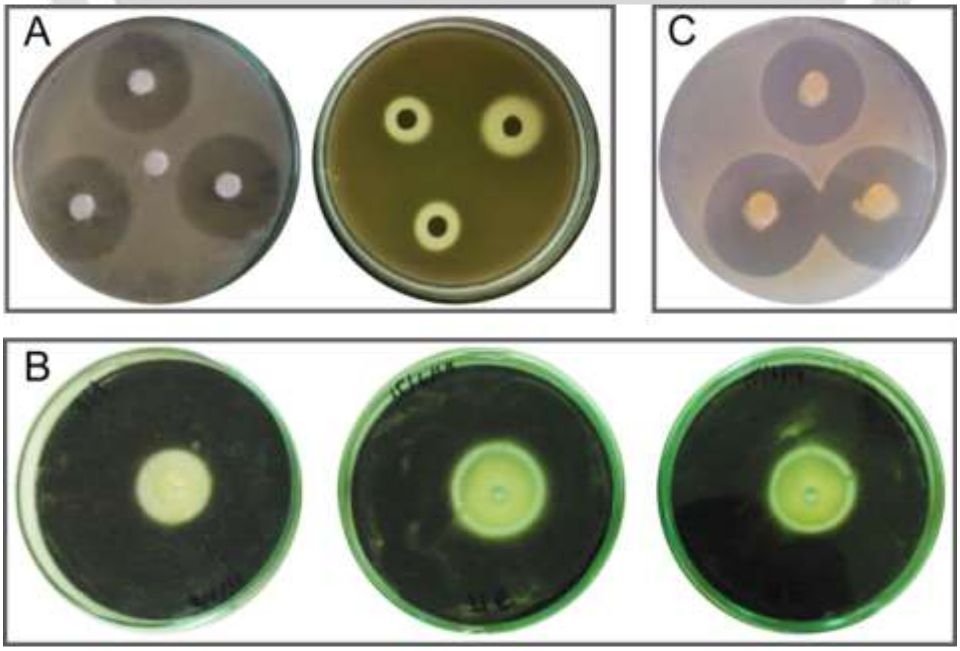
Each step of pad-dry- cure method for coating requires a separate machine. Depending upon the range of temperature require for drying and curing, the machine can same or different. Generally, dryer need to perform at 50- 60 degree C, and curing machine is required to work at around 160 degree C for various coating. Coating may be done using padding machine (called padder), however, specialized coating machines are available. A good coating process should enable even distribution of coating on the surface of textile, and even penetration of coating in the textile structure.



TESTING

Agar disk-diffusion method

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing. Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones. In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.



Antimicrobial gradient method (Etest)

The antimicrobial gradient method combines the principle of dilution methods with that of diffusion methods to determine the MIC value. It is based on the possibility of creating a concentration gradient of the antimicrobial agent tested in the agar medium. The Etest® (BioMérieux) is a commercial version of this technique. In the procedure, a strip impregnated with an increasing concentration gradient of the antimicrobial agent from one end to the other is deposited on the agar surface, previously inoculated with the microorganism tested. This method is used for the MIC determination of antibiotics, antifungals and antimycobacterial. MIC value is determined at the intersection of the strip and the growth inhibition ellipse. It is simple to implement; thus, it is routinely used to meet the demands of clinicians. However, Etest® strips cost about $2–3 each. Therefore, this approach becomes costly if numerous drugs are tested. Several previous studies have shown a good correlation between the MIC values determined by Etest and those obtained by broth dilution or agar dilution method. This technique can also be performed to investigate the antimicrobial interaction between two drugs. To study the combined effect of two antibiotics, an Etest strip, impregnated with a first antibiotic, is placed on a pre-inoculated agar plate surface. After one hour, the strip is removed and replaced by another one impregnated with a second antibiotic. The synergy is detected by a decrease of the MIC of the combination by at least two dilutions compared to that of the most active antibiotic tested alone. Also, for the same purpose, the Etest strips can be deposited on the agar medium in a cross formation with a 90° angle at the intersection between the scales at the respective MICs for the microorganism tested.

Direct bioautography

Direct bioautography is the most applied method among these three methods. The developed TLC plate is dipped into or sprayed with a microbial suspension. Then, bio-autogram is incubated at 25 °C for 48 h under humid condition. For visualization of the microbial growth, tetrazolium salts are frequently used. These salts undergo a conversion to corresponding intensely coloured formazan by the dehydrogenases of living cells. p-Iodonitrotetrazolium violet is the most suitable detection reagent. These salts are sprayed onto the bio-autogram, which is re-incubated at 25 °C for 24 h or at 37 °C for 3–4 h. The Mueller Hinton Broth supplemented with agar has been recommended to give a medium sufficient fluid to allow a best adherence to the TLC plate and maintain appropriate humidity for bacterial growth.

Conclusions:

The development of new antimicrobial products is a critical priority to address the growing threat of antimicrobial resistance (AMR). AMR occurs when microbes (e.g., bacteria, viruses, fungi, and parasites) develop the ability to defeat the drugs designed to kill them. This can happen due to overuse or misuse of antibiotics, or through natural selection. The development of new antimicrobial products is a critical priority to address the growing threat of antimicrobial resistance (AMR). AMR occurs when microbes (e.g., bacteria, viruses, fungi, and parasites) develop the ability to defeat the drugs designed to kill them. This can happen due to overuse or misuse of antibiotics, or through natural selection.

One promising approach is the development of antimicrobial products using plant extracts. Plant extracts have been used for centuries to treat infections, and they have a proven track record of safety and efficacy. In recent years, there has been a growing interest in developing plant-based antimicrobial products for a variety of applications, including: Human medicine, Veterinary medicine, Agriculture, Food preservation.

The development of antimicrobial products using plant extracts is a promising approach to address the growing problem of antimicrobial resistance. Plant extracts contain a wide range of phytochemicals with antimicrobial properties, and many of these compounds have different mechanisms of action than conventional antibiotics. This makes them less likely to contribute to the development of resistance.

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