Starch based antimicrobial food packaging film towards a sustainable environment

N Mallick¹, D Pal²ᵇ, A B Soni³ᶜ, D C Jhariya⁴ᵈ and D Singh⁵ᵉ.

¹Research Scholar, Department of Chemical Engineering, National Institute of Technology, Raipur-492010, CG, India
²Assistant Professor, Department of Chemical Engineering, National Institute of Technology, Raipur-492010, CG, India
³Professor, Department of Chemical Engineering, National Institute of Technology, Raipur-492010, CG, India
⁴Assistant Professor, Department of Applied Geology, National Institute of Technology, Raipur-492010, CG, India
⁵Associate Professor, Department of Chemical Engineering, Institute of Engineering & Technology Lucknow, U.P., India-226021

Email: ᵃnirlipta.nitu@gmail.com;ᵇdpsingh.che@nitrr.ac.in;ᶜabsoni.chem@nitrr.ac.in;ᵈdcjhariya.geo@nitrr.ac.in;ᵉdhananjay.singh@ietlucknow.ac.in

Abstract. Smart biodegradable packaging materials help restrict food-borne pathogens and reduce wastage of food items. Normally, food is packaged in plastic films, which are seldom reprocessed, which ultimately leads to an adverse impact on the environment. However, our ecosystem can be preserved by the use of biodegradable packaging materials. Deterioration of food caused by food-borne pathogens and microorganisms represent a severe problem. Therefore, a highly encouraging active packaging innovation is antimicrobial packaging. Various active compounds can be incorporated into the packaging film to extend the shelf-life of packaged foods. The starch-based film is considered as a cost-effective material for antimicrobial packaging. Moreover, essential oils can be incorporated to enhance the antimicrobial effect, i.e., slow down food-borne pathogens. This paper systematically examines the impact of three essential oils (clove, basil, and cinnamon) on biodegradable starch-based film's antimicrobial action. Three pathogenic microorganisms, namely: Staphylococcus aureus, Bacillus cereus, and Escherichia coli were are used for the assessment of antimicrobial properties. All the films containing different essential oils have shown a significant antimicrobial action against all the three microbes studied in this work.

Keywords: Antimicrobial activity, biodegradable starch films, clove oil, basil oil, cinnamon oil, smart packaging.

1. Introduction
Nowadays, biodegradable polymers are being utilized instead of conventional food packaging materials due to the unfavorable effect of synthetic material on the environment [1,2]. Therefore, a great effort has been made to develop biodegradable films from biopolymers to deliver an environmentally friendly packaging option in contrast to traditionally used plastic packaging films. The biodegradable films can be prepared using protein, polysaccharide, or lipid materials [3,4]. However, to maintain the food's
quality and freshness, it is necessary to select the correct packaging materials and technologies. During the storage of food, some food-borne pathogenic microorganisms cause the deterioration of food. A couple of examinations exhibited that packaging films incorporated with antimicrobial agents could reduce food-borne microorganisms' impact. To inhibit bacterial growth, the food industry has found a proper way to restrain the bacterial growth, an extension of the shelf life of the product, and, ultimately, ensure food safety. Therefore research on packaging is getting considerable attention due to the evolution of biodegradable packaging materials containing natural antimicrobial agents [5,6]. This kind of packaging can improve food safety by restraining pathogenic bacteria while conveying the effect of diminishing food and packaging waste [1]. This research is on studying the inhibitory action of starch-based films (loaded with various essential oils) towards food-borne pathogenic microorganisms.

1.1. Clove essential oil
Clove essential oil possessed antioxidant [7,8], antifungal [9], anticancer, and antibacterial properties. It restrains Gram-negative and Gram-positive bacteria as well as yeast. Some investigations revealed that one of its principal parts, which function as an antifungal agent, is eugenol (4-allyl-2-methoxy phenol), [10,11]. According to the United States Food and Drug Administration (FDA), Eugenol is considered a safe substance [12].

1.2. Basil essential oil
_Ocimumbasilicum_ L. (Basil oil) is often used as a culinary herb and belongs to the family _Lamiaceae_ (Labiateae) [13]. Basil leaves contain 0.2-1% essential oil, with fundamental components such as linalool and estragole. Basil oil has been utilized for a long time for nourishment products, especially in bakery items and the meat industry. It is also used in different dental and oral medicines due to its antiseptic effects [14]. Basil essential oils show antimicrobial activity against both Gram-negative and Gram-positive bacteria.

1.3. Cinnamon essential oil
The cinnamon essential oil has an effectual antimicrobial activity and is recognized as “safe-GRAS” by the Food and Drug Administration. Cinnamaldehyde is a principle part of cinnamon oil and a sweet-smelling natural compound that provides explicit taste and smell [15-17]. It is less poisonous, fat dissolvable, permeable through living cell membranes, effectively degradable, and acknowledged as biocompatible [18]. Cinnamaldehyde demonstrates the capacity to hinder _Escherichia coli_. A few examinations revealed that cinnamaldehyde damages the membrane's integrity and the penetrability of _Staphylococcus aureus_ and _E. coli_ [19].

2. Materials and Methods

2.1. Materials
The three essential oils, namely; clove, cinnamon, basil were obtained from a nearby market of Pune, India. Glycerol (MW; 92.09) was bought from Estelle Chemicals, Maharashtra, whereas Polyvinyl Alcohol (PVA, MW; 20,000) was purchased from Loba Chemie Pvt. Ltd. India. Rice husk was procured from a local rice production unit (Maharashtra, India) for reinforcement.

2.2. Film preparation
The biodegradable starch-based films in this work were prepared as per the procedure mentioned here. Initially, 3 g of starch powder was dissolved in 70 ml of distilled water and then stirred for 10 minutes with a magnetic stirrer's help. After that 1 gm of polyvinyl alcohol, 2 g of rice husk powder, and 0.75 g of glycerol was added to the starch solution. The solution was heated at 90 °C for 60 minutes in a water bath. Subsequently, cinnamon, cloves, and basil essential oils (0.40 g, 0.60g, and 0.80 g) were added to the mixture [12, 14, 15, 23]. The produced films were dried at room temperature for 24 hours. The starch film without essential oils was utilized as a control. The solution was homogenized by using a
homogenizer for 5 minutes and then degassed to release the dissolved air. The solution was poured into a Teflon plate and was dried for 5 days at room temperature. The dried films were then stored in a plastic bag at ambient conditions (~25°C) after peeling off from the plates for further characterization.

2.3. Antimicrobial activity - disk diffusion method

The disc diffusion method was employed to assess the antimicrobial activity of cloves, basil, and cinnamon essential oil against the entire three microorganisms, namely; Bacillus cereus, Staphylococcus aureus, and Escherichia coli. The antimicrobial activity of essential oil incorporated starch-based films was determined by cutting the samples into circular shapes with a diameter of 1.5 cm and placed on a medium surface. Incubation of the Petri dishes was carried out for 24 hours at 37 °C. The antimicrobial agents' effectiveness was assessed by the formation of an inhibitory zone around the disc samples, which was characterized by the surrounding clear areas.

3. Results & Discussions

3.1. Antimicrobial activity assessment

In this study, Figure 1-9 shows inhibition areas (determined by the disk diffusion method) produced by essential oils (cloves, cinnamon, and basil oils) modified starch-based biocomposite film against Gram-positive and Gram-negative bacteria. The antibacterial activity of Starch (St)/Polyvinyl Alcohol (PVA)/Rice Husk (RH) biocomposite film depends on the concentration of essential oils. High concentration levels of essential oils showed greater inhibitory activity, suppressing microbial growth. The starch film's degree of inhibition incorporated with different concentrations of cloves, cinnamon, basil essential oils was found to vary considerably. There was certainly no anti-microbial effect for the film without essential oils (control). The starch-based films showed remarkable antimicrobial property against all the studied bacterial strains, namely; Bacillus cereus, Escherichia coli, and Staphylococcus aureus. The inhibitory action against Gram-positive and Gram-negative bacteria increases proportionally with respect to the content of clove oil. A 20% of clove oil incorporated film showed an inhibition zone of 4-20 mm in diameter. The various constituents present in clove oil viz. eugenol, β-caryophyllene, and eugenyl acetate are primarily responsible for demonstrating the inhibitory activity. The structure of proteins and phospholipids in cell membranes are changed due to the presence of these components. It is believed that the damage of cell structure, ion exchange, inhibition of breathing, and finally death of cell occurs due to the interactions of essential oils (owing to hydrophilic properties) with the lipid structure, such as the cell membrane of Gram-negative bacteria, mitochondria [20, 21, 22]. Staphylococcus aureus has minimal affectability to clove oil components in film, particularly eugenol, which primarily inhibit fungal activity due to smooth spores and micelles. The starch film based biocomposites antibacterial properties on cinnamon essential oil represent an inhibition zone ranging from 5 to 19 mm in diameter.
Figure (1-9) Inhibition zone against *Escherichia Coli*, *Bacillus circus*, *Staphylococcus aureus* of Starch (St)/Polyvinyl Alcohol (PVA)/ Rice Husk (RH) films incorporated with different concentration of clove, basil, and cinnamon essential oils.
When cinnamon essential oil was used, a better inhibition was observed at a higher concentration. The presence of cinnamaldehyde is mainly accounted for the inhibitory activity of cinnamon essential oil. Cinnamon oil has the least sensitivity against \( E. \) coli. The starch biocomposite film based on basil essential oil showed the lowest inhibition zone of 3-14 mm in diameter and has the least sensitivity towards \( E. \) coli.

4. Conclusion
Over the last few years, essential oils have got much attention due to their safe and natural healing properties. Its antiseptic and anti-inflammatory properties are widely known, but its antifungal capabilities are often overlooked. Growing concerns about risks to mankind and environmental contagion, sustainable conventional processing, and the development of resistant strains of pathogenic fungi are the principal issues identified with the utilization of conventional synthetic fungicides. The current tendency proposes new formulations of antibacterial packaging based on natural antibacterial agents to protect fresh food, improve nutritional values, and extend the shelf life. Bio-based films play a key role in food packaging and protecting it from environmental contaminations. Present work revealed that cinnamon and basil essential oil showed higher inhibitory action against \( Staphylococcus aureus \) as compared to \( Bacillus cereus \) and \( Escherichia coli \). Clove essential oil showed the highest inhibitory action against \( Escherichia coli \) as compared to \( Bacillus cereus \) and \( Staphylococcus aureus \). It was assumed that the essential oil interacts with the cell wall and then the cytoplasmic membrane, thereby inhibiting the normal synthesis of DNA, which ultimately resulted in bacterial death. This study concludes that clove essential oil has more inhibitory efficiency than cinnamon and basil essential oil, with a maximum inhibition zone of 20 mm in diameter. However, much research is still needed to study antimicrobial mechanisms and toxicity. Determination of optimal concentrations of clove, basil, cinnamon essential oils is necessary to observe their effect on foods' deterioration.

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