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Concentration influence on antimicrobial activity of banana blossom extract-incorporated chitosan-polyethylene glycol (CS-PEG) blended film

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ABSTRACT

The development of antimicrobial agents derived from micro-organisms and chemotherapeutic agents from plants is a research area of the utmost importance. The present study was designed to evaluate the antimicrobial activity of banana (Musa sapientum) Blossom extract against Gram-positive and Gram-negative bacteria (Bacillus subtilis, Bacillus cereus and Escherichia coli) with extract-incorporated Chitosan (CS) - Polyethylene Glycol (PEG) blended film. The extraction was carried out in ethanol, chloroform and water. The antimicrobial activities of the extracts evaluated using disc methods and Minimum Inhibition Concentration (MIC).The compounds responsible for antimicrobial activity was conformed to confirmatory test. Ethanol extract showed an antibacterial activity against the tested microorganisms.

Keywords: Antimicrobial activity, Disc methods, Medicinal plants, Microorganism, Musa sapientum flower, Minimum Inhibition Concentration.

INTRODUCTION

The experience of human misery in the form of disease is perhaps as old as the inception of man on the earth. The history of medicine beyond record of human civilization is shrouded in the mistry of obscurity; it almost touches the boundaries of mythology, both East and West alike. Human or Animal sacrifices on altars of temples of god was a common practice even during the days when Indus, Nile, and Greek Civilizations were on their climax. Though these acts did not have any direct or otherwise bearing on the health of diseased or wounded, it had its own convincing or satisfying effects. In order to find remedy for illness and for providing relief to the wounded the man discovered its first resort in plants.

Knowledge of medicinal plants has been accumulated in course of many centuries. The “doctrine of signatures” also account for the use of several plants as medicinal agents. The

reason for extensive use of plants as drugs may be the fact that plants are available every where, wide range of medicinal plants and their distinct form and thus are procured without any trouble. Medicinal plants have a long history of use and their use is widespread in both developing and under developed countries. According to the report of The World Health Organisation, (WHO) 80% of the worlds population rely mainly on the traditional therapies which involve the use of plant extracts or their active substance [1]. Globally, more than 400,000 species. of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine [2]

Several pharmacological industries have evaluated new era for the search of effective antibiotics throughout the world but on the other hand resistance to these an antibiotic by microorganisms has increased [3]. It is known that Microorganisms have the genetic ability to transmit and acquire resistance towards drugs. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antimicrobial activity [3, 4]. They have a long evolution of resistance against microbial agents which has lead to alternative directions in drug development. Most of green plants represent a reservoir of effective chemo-therapeutants and can provide valuable sources of natural drugs, natural pesticides and biofertilizers. Therefore, extracts of plants and phytochemicals are getting more importance as they have the great potential sources for microbial and viral inhibitors during the recent decade. Plant parts used for this purposes are bulb, gel, leaves, roots, barks, peels etc. Different class of plant family and their respective parts has been used to treat threat throughout human culture. Among the most ancient recorded uses of medicinal plants are those found in China and India, where historic approach to the treatment of human diseases is still practiced [5].

Bananas are the fourth most important food crop in developing countries, after rice, wheat, and maize, with nearly 90% of the crops being grown for small-scale consumption and local trade [6] . Banana plants are cultivated in more than 100 countries throughout the tropical and subtropical regions, occupying around 10 million hectares, with an annual fruit production of approximately 88 million metric tons. It possesses many curative properties and prevents many kinds of illnesses and conditions. Different parts of plant are used very frequently in different worship ceremonies by the Indians among them banana have many beneficial nutritional properties. They are a good source of vitamins C, B6, A, potassium [7, 8] high content of carbohydrates and fibre, while they are low in protein [9]. Several references have been reported for hot and cold extraction method of banana plant [10-12]. In the present investigation, the anti- microbial activities of *Musa sapientum* flower against Gram-positive and Gram-negative bacteria *(Bacillus subtilis, Bacillus cereus and Escherichia coli)* by varying the assorted concentrations of extract in order to unravel its potential use in the treatment of microbial infection in polymeric CS –PEG blended film.

Incorporation is one of the innovative concepts that have been introduced as a response to the continuous changes in current microbial study and biomedical field for dressing. It can be defined as a mode of blending, in which polymer, the extract, interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the products. This definition of active polymer was chosen for the European FAIR-project CT 98-4170 [13]. In general, incorporation can provide several functions that do not exist in conventional microbial study. The direct incorporation of antimicrobial additives in polymer blending is a convenient means by which antimicrobial activity can be achieved. Several compounds have been proposed and tested for antimicrobial study using this method [14, 15].

EXPERIMENTAL SECTION

Plant material

Musa Sapientum (banana) flower used in this study were taken from local market. They were washed in 50μg/L hypochlorite solution, sliced and air-dried at 50°C in a hot air oven. Dried samples were ground to powder using a mechanical grinder, and kept separately until use.

Extraction procedures:

The powders of dried flowers (100g) were subjected to extraction with ethyl alcohol (200ml), chloroform (200ml) and water (200ml) by cold maceration in sterile conical flask and rotated with constant stirring overnight [16].The extracts obtained were separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 1). The ethanolic extracts were centrifuged at 8000 rpm for 10 minutes.

Test Microorganisms

The screening of the anti microbial activity of crude extracted *Musa sapientum* flower were carried out individually on active cultures of, *Bacillus subtilis*, *Bacillus cereus and Escherichia coli.*

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Agar slants (peptone 1g, meat extract 0.5 g, sodium chloride 0.25 g and agar 1 g 100 mL^{-1} H_2O) at +4ºC . The bacterial culture medium was prepared as following, nutrient broth (peptone 0.5 g, meat extracts 0.25 g, sodium and chloride 0.25 g 50 mL^{-1} H_2O) and soft agar medium (peptone 0.5 g, meat extracts 0.25 g, sodium chloride 0.125 g and agar 0.2 g 50 mL^{-1} H_2O) adjusted to pH 6.6 and autoclaved at 121°C for 20min. Cell suspensions were prepared by inoculation of each bacteria into 10 ml of Nutrient Broth (NB Hi media). Incubation was performed at 37ºC for 24 h. On the next day Nutrient Agar Plate was prepared and cooled to 45ºC.

Antimicrobial activity assay

For antimicrobial activity the microorganisms were grown / cultured in Nutrient Broth at 37°C overnight. First we prepared sterile nutrient agar plates, for contaminations of other microorganism in plates; it should be incubate in incubator. After 24 hrs we were select the nutrient agar plates which was not contaminated. With the help of spread plate technique we were maintain the desired bacterial environment in each plate. In this process we used 100 micro liter inoculums (Bacteria) in solid nutrient agar plates and via spreader we spread all the inoculums in nutrient agar plates until the agar surface in the plates absorb all the inoculums. After the spreading filter paper disc which were sterilized and impregnated with different concentration and allow to dry completely 10-15 min and then placed it on surface of previously spresded nutrient agar plate. The Petri dishes were incubated at 36 C for 24 hrs.

Antimicrobial Activity of Medicinal Plant Ethanolic Extract-Incorporated Chitosan-Polyethylene Glycol (CS-PEG)) blended Film:

Bacterial growth occurs mainly at the surface; attempts have been made to solve this by using antibacterial sprays or dips [17, 18]. However, direct surface application of antibacterial substances has limited benefits, because the active substances are neutralized on contact or diffused rapidly [19, 20] Potential properties and applications of blended films and coating on materials have been extensively reviewed [21-25] The method is different from direct application, as the incorporation of antimicrobial agents into blended film or coating the functional effect at the surface. The antimicrobial agents are slowly released to the surface,

and therefore, they remain at high concentrations for extended periods of time [26] Blend CS (chitosan) and PEG (Polyethylene glycol) film was used as the base material for extract encapsulation [27-29]. The determined zone of inhibition was 8.4mm, 8.2 mm in respect of control against *Bacillus subtalis*, *Bacillus cereus* shown in Fig1(A,B,C). At the lower concentration than 10.0 and 20.0 % (v/v), the zone of inhibition could not be detected.

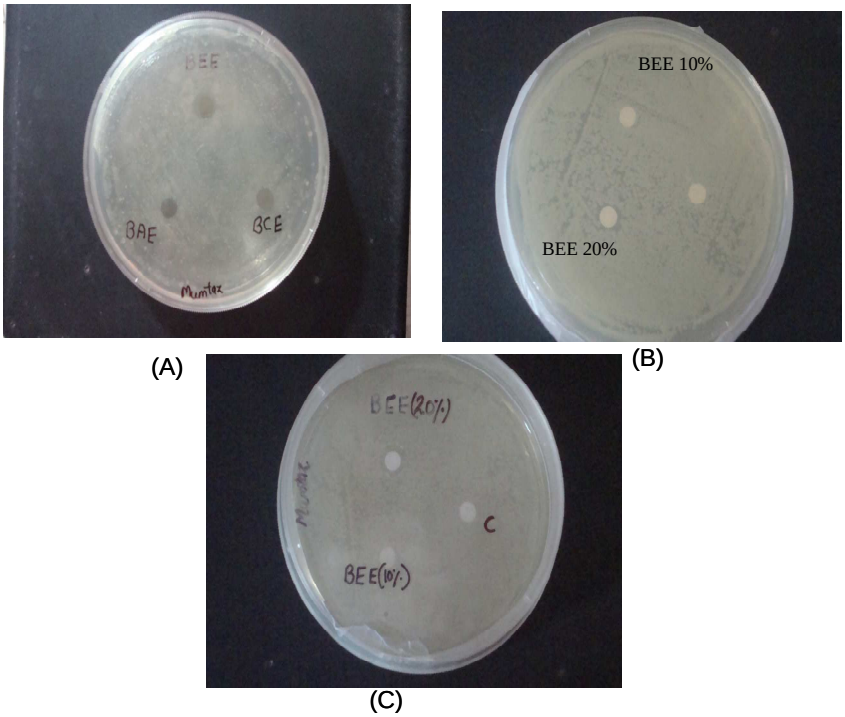

Figure 1 (A): Antimicrobial Activity of Banana Flower Ethanolic Extract (BEE), Banana Flower Chloroform extract (BCE), Banana Flower Aqueous extract (BAE).

Figure 1 (B, C): Effect of Concentration of Banana Flower Ethanolic Extract (BEE) -Incorporated Chitosan-Polyethylene Glycol (CS-PEG)) blended Film on antibacterial study against *Bacillus cereus, Bacillus subtilis.*

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) were defined as the lowest concentration (ppm) of the extract in agar plates showing no visible bacterial growth. The soft nutrient agar was then added onto a Petri dish containing 15 mL hard agar as mentioned above. The samples were dissolved in chloroform ethanol. The solutions were then individually added at different concentrations from (0 (control) to 20 mL^{-1}) to soft agar and mixed well before being poured into sterile Petri dishes containing 15 mL hard agar. The cultures (5 μL) were taken from nutrient broth and added to three places on the medium surface and incubated at 37°C.

RESULTS AND DISCUSSION

The result obtained in the antibacterial activity and MIC indicated that the chloroform and water extracts of *Musa Sapientum* flower had negligible bacterial effect but the ethanolic extract showed (Table 1) antibacterial activity against bacteria Bacillus (Table 2). β-sistosterol, 12- hydroxystrearic acid, palmitic acid and d-malic acid were bioactive compounds isolated from *Musa Sapientum* and conformed by confirmatory test [30]. The bioactive compounds were tested against, *Bacillus subtilis*, *Bacillus cereus*, and *Escherichia coli*. β- sitosterol and malic acid are active against responsible for antibacterial effect, while palmitic acid was had less effective. This study indicated that malic acid exhibited a stronger

antibacterial activity compared to β-sistosterol and palmitic acid, while, 12-hydroxystrearic acid recorded weak antimicrobial activity when measured by disk method. According to this investigation, it could be indicated that antimicrobial activity of the ethanolic extract of *Musa sapientum* L. is due to the present of those bioactive compounds.

Table 1: Antibacterial study of *Musa sapientum* flower extract using disc diffusion method

Bacteria	Flower extract/mean length of inhibition zones(mm)±S.D		
	Chloroform	Ethanol	Water extract
Bacillus subtalis	_	+	_
Bacillus cereus	_	+	_
Escherichia coli	_	_	_

Zone values are means ±S.D (Total Zone = Disc + Zone area)
Used disc was sterile paper disc (6 mm diameter)
+: Represent an inhibitory effect
_: Represent no inhibitory effect

The results showed that the CS-PEG blended films encapsulated the ethanolic extracts of *Musa sapientum* were effective against the test organisms. With increasing concentrations of the ethanolic extract, the zone of inhibition also increased (Table 2). Ethanolic extract film at a concentration of 20.0 % (v/v) showed the best inhibitory effect.

Table 2: Antimicrobial Activity of *Musa sapientum* flower Ethanolic Extract-Incorporated CS-PEG blended Film

Bacteria	Ethanolic extract concentrations (% v/v)										
	C	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0
Bacillus subtalis	_	_	_	_	_	_	_	_	_	+	+
Bacillus cereus	_	_	_	_	_	_	_	_	_	+	+
Escherichia coli	_	_	_	_	_	_	_	_	_	_	_

+: Represent an inhibitory effect
_: Represent no inhibitory effect
C= Control (pure blended film)
Used disc was sterile extract incorporated CS-PEG Blended Film (6 mm diameter)

CONCLUSION

The determination of the antimicrobial activity of medicinal plant *Musa sapientum* extracts. The appropriate extraction process with an outstanding antimicrobial activity of the extract was the alcoholic extraction with 50% ethanol for 24 hours. Among all the solvent for *Musa* used in this study e*thanol* showed an antibacterial activity against the tested microorganisms. The study show that the natural antimicrobial compounds of *Musa sapientum* can be successfully incorporated into CS-PEG blended film and retain their inhibitory effect against microbial growth in model media based on the inhibitory zone, inhibitory underneath the films, and suspension test. The excellent antimicrobial activity was obtained from CS-PEG blended film incorporated with ethanolic extract. In future this work can be extended for wound dressing and drug delivery system.

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