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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF *Bougainvillea glabra* FLOWER EXTRACT DOPED MgS NANOPARTICLES ON *Escherichia coli*, *Salmonella typhi* AND *Pseudomonas aeruginosa*

*¹Shoba, V., ²Krishnapriya, K., ³Boopathy Raja, A., ¹Elanchezhyan, C., and ¹Selvisabhanayakam

¹Department of Zoology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu

²Department of Microbiology, Faculty of Agriculture, Annamalai University

³Department of Zoology, Nehru Memorial College, Puthanampatti, Trichy

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ABSTRACT

Nanotechnology has the potential to impact many aspects food and agricultural systems. Food security disease treatment delivery methods new tools for molecular and cellular biology new material for pathogen detection and protection of the environment are examples of the important links of nano technology to the science and engineering of agriculture's and food systems. In the present study, the red and white color paper flower ethanolic extracts doped with MgS (Magnesium Sulphate) nanoparticles were used to determine their antibacterial activity. Both paper flower ethanolic extracts doped with MgS nanoparticles are having potential antibacterial activity when compared with standard antibiotic erythromycin. The white colored flower extracts doped MgS nanoparticles are having more antibacterial activity than the red colored flower extracts doped MgS nanoparticles. The white flowers may contain active antibacterial component. The combination of *Bougainvillea glabra* flower extracts and MgS nanoparticle may be suggested as a antibiotic agent because of their active potential to inhibit the bacterial growth.

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INTRODUCTION

Human being is very commonly infected by micro organism in the living environment, which sometimes results in the illness and other health hazards. Microorganism harmful to human being is termed as pathogens. In the recent past, due to the emergence and increase of such pathogenic strains resistant to multiple antibiotics (Desselberger *et al.*, 2000). Nanotechnology is the area of science which deals with developing and producing extremely small tools and machines by controlling the arrangement of individual atoms thorough, inexpensive control of the structure of matter based on molecule-by-molecule control of products and by products; the products and processes of molecular manufacturing, including molecular machinery (Liam *et al.*, 2010). Nanoparticles is a special group of materials with unique features and extensive application in diverse field (Matei, 2008) of material which were considered to be safe develop toxicity at nano size ranges which is mainly related to the increased specific surface area and high reactivity of nano size materials (Nagarajan and Rajagopalan, 2008). There are reports that metal nanoparticles have high specific surface area and high fraction of surface atoms and have been studied extensively owing to their unique antibacterial activity. Much research has also been done to study to the antibacterial activity of metal oxide powders and nanoparticles. In this regard, metal nanoparticles have received increasing attention over the years. The antibacterial activity of ZnO powder and nanoparticles has been effectively studied against some of the multi resistance pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Okouchi *et al.*, 1995 & Selahattin *et al.*, 1998). Metallic nanoparticles are most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Gong *et al.*, 2007).

The antimicrobial properties of silver ions were known since ancient times and silver ions are widely used as bactericide. Several reports are available that silver and copper nano particles are having potential antibacterial activities. The considerable antimicrobial activities of inorganic metaloxide nanoparticles such as ZnO, MgO, TiO₂, SiO₂, and their selective toxicity to biological systems suggest their potential application as therapeutic, diagnostic, surgical devices and nanomedicine based antimicrobial agents (Mohsen and Zahra, 2008; Sawai and Yoshikava, 2003 and Reddy *et al.*, 2007). The advantages of using these inorganic oxide nanoparticles as antimicrobial agents are their greater effectiveness on resistant strains of microbial pathogens less toxicity and heat resistant strains. Among metal oxide nanoparticles, ZnO nanoparticles have many significant features such as chemical and pluvial stability, high catalysis activity effective antibacterial activity as well as intensive ultraviolet and infrared adsorption. Outbreaks of food borne pathogens such as *Escherichia coli* and *salmonella spp* continue to draw public attention to food safety. There is a need to develop new antimicrobial to ensure food safety and extend self life. The use of antimicrobial agents directly added to foods or through antimicrobial packaging is one effective approach.

Nanotechnology has the potential to impact many aspects food and agricultural systems. Food security disease treatment delivery methods new tools for molecular and cellular biology new material for pathogen detection and protection of the environment are examples of the important links of nano technology to the science and engineering of agriculture's and food systems (Weiss *et al.*, 2006). Nanoparticles have been reported for the application in nano sensor and nanotracer (Moraw *et al.*, 2003). Natural products perform various functions, and many of them have interesting and useful biological activities Galal (1991). In India, nearly 9,500 registered herbal industries and a multitude of unregistered cottage-level herbal units depend upon the continuous supply of medicinal plants for

*Corresponding author: Shobav09@gmail.com

manufacture of herbal medical formulations based on Indian Systems of Medicine. It is estimated that more than 6,000 higher plant species forming about 40% of the higher plant diversity of the country are used in its codified and folk healthcare traditions. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002). In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Clark and Hufford, 1993). The abuse of antimicrobial drugs, pesticides, and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria and questions of safety Esiobu (2002). Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (De Boer *et al.*, 2005). Herbal drugs have become increasingly popular and their use is widespread. Clear-cut proof of their efficacy in microorganisms inducing pathogenesis is yet to be explored. Over 50% of all modern clinical drugs are of natural product origin Stiffness and Douros (1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995), Samy and Ignacimuthu (2000), Srinivasan *et al.* (2001) are a few examples among many works dealing with antibacterial activities. The therapies with pure nanoparticle leads to some toxic effects to the patients are reported earlier by many scientists. Hence, the present study is aimed to evaluate the antibacterial activity of *Bougainvillea glabra* extract (red and white color flower) doped MgS.

MATERIALS AND METHODS

Collection of Plant Materials

The *Bougainvillea glabra* were collected from in and around Annamalainagar, Cuddalore district, Tamil Nadu, India. The plant materials were deposited at Zoology Department, Annamalai University, India.

Preparation of Ethanolic Extract

The ethanolic extract of *Bougainvillea glabra* flower was prepared according to the method of Hossain *et al.* (1992). 500gms of fresh *Bougainvillea glabra* dried flower was powdered and then soaked in 1500 ml of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 hours and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapor at 40^o - 50^oC under reduced pressure. A 1.87% semi solid dark brown material obtained was stored at 0 - 4^oC until used.

Synthesis of nanoparticle

The *Bougainvillea glabra* extract doped MgS nanoparticle was synthesized in chemical method (Murugadoss *et al.*, 2010).

Selection of bacterial strain

Escherichia coli, *Salmonella typhi* and *Pseudomonas aeruginosa*

Culturing the test organisms

Mueller-Hinton Agar Medium

Of the many media available, Mueller-Hinton agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
- It gives satisfactory growth of most non fastidious pathogens.
- A large body of data and experience has been collected concerning susceptibility tests performed with this medium

Anti-bacterial Activity

The antimicrobial assay of ethanolic extract was performed by agar disc diffusion method (Bauer *et al.*, 1966 & Parekh and Chanda, 2007).

RESUT AND DISCUSSION

The ethanolic flower extracts of *Bougainvillea glabra* doped with MgS nanoparticle found to be very active against *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. To screen the antibacterial activity against tested organisms, erythromycin was used as a standard. The result of the antibacterial activity against *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* by ethanolic extract of *Bougainvillea glabra* (both red and white flower) doped with MgS nanoparticle were shown in table 1 & Plate.

Escherichia coli

The 40 mg of ethanolic red flower extract doped MgS nanoparticle showed higher inhibition zone about 13 mm against *Escherichia coli*. The 40 mg of ethanolic white flower extract doped MgS nanoparticle showed inhibition zone about 15mm against *Escherichia coli*. The 40 µg of Erythromycin showed the inhibition zone of 10 mm against *Escherichia coli*.

Salmonella typhi

The 40 mg of ethanolic red flower extract doped MgS nanoparticle showed inhibition zone about 15 mm against *Salmonella typhi*. The 40 mg of ethanolic white flower extract doped MgS nanoparticle showed higher inhibition zone about 20mm against *Salmonella typhi*. The 40 µg of Erythromycin showed the inhibition zone of 11 mm against *Salmonella typhi*.

Pseudomonas aeruginosa

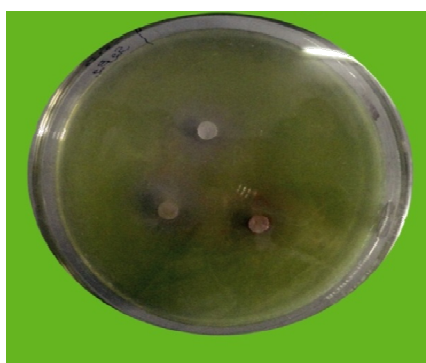
The 40 mg of ethanolic red flower extract doped MgS nanoparticle showed higher inhibition zone about 15 mm against *Pseudomonas aeruginosa*. The 40 mg of ethanolic white flower extract doped MgS nanoparticle showed inhibition zone about 17mm against *Pseudomonas aeruginosa*. The 40 µg of Erythromycin showed the inhibition zone of 8 mm against *Pseudomonas aeruginosa*. In the present study, the red and white color paper flower ethanolic extracts doped with MgS nanoparticles were used to determine their antibacterial activity. Both paper flower ethanolic extracts doped with MgS nanoparticles are having potential antibacterial activity when compared with standard antibiotic erythromycin. The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. However, there has been a parallel and alarming increase in bacterial resistance to existing chemotherapeutic agents as a result of their injudicious use (Service, R. F. 1995). In many countries such as India and China, thousands of tribal communities still use folklore medicinal plants to cure sicknesses. The great interest in the use and importance of Indian medicinal plants by the World Health Organization in many developing countries has led to intensified efforts on the documentation of ethno medicinal data of medicinal plants (Waller, 1993 & Perumal-Samy and Patricraja, 1996). Biologically active compounds from natural sources have always been of great interest to working on infectious diseases. A number of studies have been reported, dealing with antimicrobial

Table 1. Antimicrobial activity of *Bougainvillea glabra* flower extract doped MgS nanoparticles on different bacterial strains

| S. No. | Strains | Name of the treatment | Concentration (mg) | Zone of inhibition (mm) |
|--------|---------------------|--------------------------------|--------------------|-------------------------|
| 1. | <i>E.coli</i> | White flower extract doped MgS | 40 | 15 |
| | | Red flower extract doped MgS | 40 | 13 |
| | | Erythromycin | 40 | 10 |
| 2. | <i>S. typhi</i> | White flower extract doped MgS | 40 | 20 |
| | | Red flower extract doped MgS | 40 | 15 |
| | | Erythromycin | 40 | 11 |
| 3. | <i>P.aeruginosa</i> | White flower extract doped MgS | 40 | 17 |
| | | Red flower extract doped MgS | 40 | 15 |
| | | Erythromycin | 40 | 8 |

PLATE

Antimicrobial Activity

Escherichia coli*Salmonella typhi**Pseudomonas aeruginosa*

screening of extracts of medicinal plants (Brantner, and Grein, 1994 & Perumal-Samy and Ignacimuthu, 1997). There is an ever-increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices; they also have no side effects. It is evident that the metal based nanoparticles constitute an effective anti microbial agent against common pathogenic microorganisms. Therefore, some of the nanoparticles such as Silver, Titanium di Oxide and ZnO are receiving considerable attention as antimicrobial and additives consumer health related and industrial products (Dibro *et al.*, 2002). As Silver nanoparticles have a broad spectrum antimicrobial activity against several pathogens. They are increasingly incorporated into various materials to extend their utility in materials and biomedical applications (Sankar *et al.*, 2007). They are used as additives in health related product such as bandages, catheters and other materials to prevent infection, particularly during the healing of wounds and burns. They are currently being added to many common household products such as bedding, washer, water purification systems, tooth paste, shampoo, fabrics deodorants, filters paints, kitchen utensils, toys and humidifiers to impact antimicrobial properties (Baker *et al.*, 2005). Nanoparticles of Titanium dioxide are used in cosmetics, filters that exhibit strong germicidal properties and odors and in conjunction with Silver as anti microbial agents. Moreover due to the photo catalytic activity, it has been used in waste water treatment. It is considered nontoxic and has been approved by the American Food

and Drug Administration (FDA) for use in human food, drugs, cosmetics and food contact materials (Wist *et al.*, 2004). Nowadays Titanium dioxide nanoparticles are finding wide application as self cleaning and self disinfecting materials for surface coating in many applications and in food industries for disinfecting equipments (Yoshimuram *et al.*, 1994). ZnO nanoparticles are used in the wallpapers in hospitals as antimicrobials. ZnO powder is an active ingredient for dermatological application in creams, lotions, and ointments on account of its antibacterial properties. The high thermal sensitivity of egg components prevents the application of more intense heat treatment. To use new antimicrobial agents are others nanothermal processing techniques to ensure the safety of liquid egg products while their desired qualities are not altered (Leasor and Foegeding, 1989). Disc diffusion methods are extensively used to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined. Differences in MIC values of bacteria may be related to differential susceptibility of bacterial cell wall, which is the functional barrier to minor differences present in the outer membrane in the cell wall composition (Zhao *et al.*, 2001). The gram-positive and gram-negative microorganisms differ in several aspects other than with respect to the structure of their cellular walls, mainly with regard to the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria that form a barrier to hydrophobic compounds (Zhao *et al.*, 2001; Mazutti *et al.*, 2008). In the present study also the Minimum inhibitory

concentration (MIC) values are differ to species to species. Thus, it shows that *Bougainvillea glabra* flower extracts doped MgS nanoparticles can be used as potentially effective antimicrobial agent. The white colored flower extracts doped MgS nanoparticles are having more antibacterial activity than the red colored flower extracts doped MgS nanoparticles. The white flowers may contain active antibacterial component. The combination of *Bougainvillea glabra* flower extracts and MgS nanoparticle may be suggested as a antibiotic agent because of their active potential to inhibit the bacterial growth.

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