ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 4, Issue 11, pp 478-481, November 2015

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

PREPARATION OF COMPOSITES AND EVALUATION OF ANTIBACTERIAL EFFICACY

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A R T I C L E I N F O

Article History:

Received 19th, September, 2015
Received in revised form 28th, September, 2015
Accepted 13th, November, 2015
Published online 28th, November, 2015

Key words:
Lantana camara, Copper Oxide nanoparticles, Microencapsulation, Gauze/Fabric finishing, Antibacterial Activity.

A B S T R A C T

Lantana camara is an evergreen shrub is spread throughout the world in a ubiquitous manner. Although it was introduced as an ornamental species and soon took over as a weed, its medicinal properties did not go unnoticed and as such its various biological activities have been used for a long time. Nanotechnology is an ever rising field of science that has helped the medical world to step up in drug production and site targeted drug release and such therapies. In this study, we have tried to establish that composites of herbal particles and nanoparticles as a measure against serious infections is always a promising field of research. 80% ethanolic extracts of *Lantana camara* leaves and Copper Oxide nanoparticles were mixed in different ratio to produce composites and then their antibacterial activity was assessed, and as expected the composites yielded a better result than the herbal particles or nanoparticles alone.

INTRODUCTION

Lantana camara is an ornamental plant, native to the American tropics that are spread throughout the world as a weed and renowned for its medicinal properties (Floridata, 2007; Kalita and Sanjeeb, 2012). Out of the many medicinal properties it exhibits, antibacterial activity has been studied the most. It has been found to be more effective against Gram positive bacterial strains like *Bacillus cereus, Staphylococcus aureus*etc And less effective against Gram negative strains like *Klebsilla pneumioniae, Pseudomonas aeruginosa* etc (Sardhara Rahul and Sathiya Gopal, 2013). Ethyl acetate extracts of the plant has been found to be very effective against most bacteria. Flower extracts have also been found to be more effective than leaf extracts but *Eschericia coli* were found to be most susceptible (Ganjewala, 2009) But in case of methanol extracts, leaf extracts have been proved more effective than flowers or root (Badakhshan, 2009). Resistance to antimicrobial agents is a major global public health problem (Floridata, 2007). Infectious diseases account for approximately one-half of all death in tropics. Despite the progress made in the understanding of microorganisms and their control in industrialized nations, incidents due to drug resistant microorganisms and the emergence of unknown disease causing microbes, posed enormous public concern disease causing microbes, posed enormous public health concern (Kalita and Sanjeeb, 2012).

Copper is an outstanding material for biocide applications. Copper ions (Cu2+), either alone or in copper complexes, have been used for centuries to disinfect liquids, solids and human tissues. Today, copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide, antibacterial and antifouling agent (Borkow and Gavia, 2005). Moreover, copper is one of the relatively small groups of metallic elements that are essential to human health, and today, it is considered as a safe material to humans. This knowledge is based on empirical evidence that has been rationalized during the last decades. For example, by testing a set of metal surfaces, it was shown that those containing copper are the most effective in reducing bacterial viability (Yoon *et al*., 2007). With the development of nanotechnology, a new generation of copper-based nano particles (CNP) can be produced, which also show biocidal behaviour, although just a few studies have been reported. By comparing silver nanoparticles (3 nm) with CNP (9 nm), showed that both particles are biocidal, although their bactericidal sensitivity varies depending on the microbial species (Saikia Anil Kumar and Ranjan Kumar Sahoo,2011).CNP (100 nm) have better performance than silver nanoparticles (40 nm) against *E. coli* and *B. subtilis* (Yoon *et al*., 2007).

Copper oxide nanoparticles with sizes ranging from 20 to 95 nm also showed activity against several bacterial pathogens, including *Staphylococcus aureus* and *E. coli* (Ruparelia, 2008). Although some papers show the potential toxicity of these nanoparticles to human, theyn conclude that the particles themselves, and not the ions, are responsible for this (Yoon *et al*., 2007; Karlsson *et al*., 2008). Copper Oxide nanoparticles generated by thermal plasma technology have been found to show effective antibacterial activity against MRSA and *E. coli* (Ruparelia, 2008). Copper Oxide nanoparticles in polypropylene matrix have also been able to show strong antimicrobial behavior against *E. coli* (Delgado, 2011). Both Gram positive and Gram negative organisms have been found to be almost equally susceptible to Copper Oxide nanoparticles (Pattnaik *et al*., 2010; Abboud *et al*., 2014). Wound dressings with Copper Oxide coatings have been shown to reduce infections and also stimulate wound repair (Chen *et al*., 2006). The present study aims to investigate the phytochemicals, Copper Oxide nanoparticles have been combined and studied by application on gauze materials and antibacterial activity assessment of *Lantana camara* plant leaf extract.

MATERIALS AND METHODS

The chemicals used in the experiment were purchased from HiMedia, Mumbai, India. Nutrient Agar was used as the culture medium for the growth of the bacteria. Cotton Gauze - Mill scoured and bleached 100% cotton gauze fabric (55 g/m^2) purchased from Swamy Cotton Mill, Tirupur.

Bacterial Strains used

The test organisms used in the study were *S. aureus* (ATCC 11226) and *E. coli* (ATCC 6529).

Extraction process

Plant leaf was dried, powdered and 20g was extracted using 250ml of 80% alcohol in a Soxhlet apparatus for 7 hours. The extract was dried and stored for further use.

Phytochemical analysis

Phytochemical screening of the L. camara was performed to detect the presence of different classes of constituents, such as Alkaloids, Carbohydrates, Saponins, Flavonoids, Tannins, Sterols (Hamid and Aiyelaagbe, 2011; Kumar *et al*., 2011).

Preparation of Copper Oxide Nanoparticles

Copper sulphate (3.45g) was added to 50ml of distilled water. To another 50ml of distilled water, 17.3g of Sodium Potassium tartarate and 6g of Sodium Hydroxide was added. Both solution were shaken well and mixed well. 5g of Glucose was added and mixed well for 10 minutes. It was then kept in a water bath at 60°C for 10 minutes, then cooled and centrifuged at 2000 rpm. The supernatant was discarded and the pellet was washed with distilled water and ethanol twice and dried. The weight of the dried pellet was noted (Kooti and Matouri, 2011).

Microencapsulation of Plant Extracts

Sodium alginate 1% was prepared by dissolving 0.5g in 50ml of distilled water. 1g of extract was dissolved in another 50ml of distilled water. Both were mixed together and stirred in a shaker for 5 minutes at 1000 rpm and it was allowed to settle. Calcium chloride solution of 25mM concentration was prepared by dissolving 0.55495g in 200ml of distilled water. The plant extract-sodium alginate mixture is sprayed in to Calcium chloride solution which was in constant stirring at 3000rpm at room temperature. The whole solution was kept in the stirrer for another hour and then allowed to settle. The supernatant was discarded and the pellet was used for antibacterial activity assay (Wilks *et al*., 2005).

FINISHING OF TEXTILE MATERIAL

The prepared composite was finished on fabric was done by Exhaustion method, where citric acid is used as a binder. Six number of 13X13 cm gauze cloth pieces were cut and soaked in a solution (ML ratio 1:20) of the following preparations

Test 1- Control (Distilled water)
Test 2 - Copper oxide nanoparticle (CONP) of 10 mg/ml concentration
Test 3 - Microencapsulated plant extracts (MEPE)
Test 4 - MEPE: CONP (1:1)
Test 5 - MEPE: CONP (1:2)
Test 6 - MEPE: CONP (1:3)

Citric acid (8%) was added to all the above preparations and shaken well before soaking the fabric. After finishing, they were removed, squeezed and dried at 80°C in the oven for 5 minutes and then cured at 120°C for 2minutes (Wilks *et al*., 2005).

Antibacterial Activity Assessment EN ISO 20645

The antibacterial activity of the finished fabric was tested according to EN ISO 20645 against *S.aureus* and *E.coli.* Nutrient agar plates were prepared and allowed to solidify for 5 minutes. Inoculum (0.01%) was swabbed uniformly and allowed to dry for 5 minutes. The finished fabric with a diameter of 2.0±0.1 cm was placed on the surface of medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, the zone of inhibition formed around the fabric was measured in millimeters and recorded.

RESULTS AND DISCUSSION

The phytochemical analysis indicated the presence of alkaloids, tannins, flavonoids, saponins, alkaloids, carbohydrates and sterols and this result is in accordance with all the reports on phytochemical study of *Lantana camara* (Saxena Mamta and Saxena Jyoti, 2012; Sumithra M and VasugiRaaja, 2012).

In the present study it was inferred that *S.aureus* to be more susceptible to the herbal particles and also towards Copper Oxide nanoparticles in a consistent manner. Gram negative bacteria, on the other hand, possess tough outer membrane

Table 1 Phytochemical Tests

S.No.	Phytochemical test	Observation	Results
1	Alkaloids	Development of turbidity	+
2	Sterols	Development of bluish green colour	+
3	Flavonoids	Development of yellow fluorescence	+
4	Saponins	Development of layer of foam	+
5	Tannins	Development of green black colour for catecholic tannins	+
6	Carbohydrates	Development of red precipitate	+

formed by lipopolysaccharide layer along with proteins and phospholipids. This structure hinders the access of most compounds to the peptidoglycan layer of the cell wall (Ruparelia, 2008). Composites blended with CuO-NPs were found have quite higher zone of inhibition than those noted for the herb particle or Copper Oxide nanoparticle individually. At the 2:1 ratio of CONP: MEPE the composite was found to be highly effective against *S. aureus*, while it failed to bring the same range of result in *E. coli*. Similarly at 3:1 ratio the CONP: MEPE. Composites affected the organisms in almost the same range.

Antibacterial Activity

The maximum antibacterial activity of *E. coli* and *S. aureus* were found to be around 33mm and 30mm of zone of incubation in CONP:MEPE (3:1) and MEPE, respectively. The minimum antibacterial activity of *E. coli* and *S. aureus* were found to be around 20mm of zone of incubation in CONP: MEPE (1:1) . No zone formation observed in control sample (Table 2).

Table 2 Antibacterial Activity Assessment

S.No.	Samples	Diameter of Zone of Inhibition (in mm)	
		E. coli	*S. aureus*
1	Control	0	0
2	CONP	20	25
3	MEPE	25	30
4	CONP:MEPE (1:1)	20	20
5	CONP:MEPE (2:1)	20	28
6	CONP:MEPE (3:1)	33	30

Bacterial inhibition zones can only be created by diffusion of antimicrobial material and only the Cu control composites Demonstrated inhibition zones due to the solubility of Cu^{+}. The insolubility of the CuO-NPs prevented diffusion of a sufficient amount of Cu^{2+} to the surrounding environment to impart an observable antibiotic effect, rendering the NPs ineffective for non-contact bio film inhibition. Therefore it was inferred that the prepared composites have better antibacterial activity than the individual components that make up the composite itself. Further extensive study is very much essential to elucidate the aspect of mechanism of toxicity and remedial measures be adopted before application in the medical field.

CONCLUSION

Lantana camara is an evergreen ubiquitous shrub that was introduced as an ornamental species and later turned a weed. Its medicinal properties did not go unnoticed and as such its various biological activities have been used for a long time. Nanotechnology is an ever rising field of science that has helped the medical world to step up in pharmaceutical production and site targeted drug release. In this study, we have tried to establish that composites of herbal particles and nanoparticles as a measure against serious infections is always a promising field of research. Copper Oxide nanoparticles and 80% ethanolic extracts of *Lantana camara* leaves were mixed in ratios to produce composites that were coated on gauze materials. Then their antibacterial activity against *E.coli* and *S.aureus* was assessed and as expected the composites yielded a better result. The present study could pave way to suitable alternatives for the synthetic antibacterial finishes. Further testing and analysis on toxicity of Copper should be done before application in the medical field.

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