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IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES USAGE (*Curcuma Longa* L.) RHIZOMES AGAINST *HELICOBACTER PYLORI*

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Abstract

Nano-particles are of extensive scientific concern as they are effectively a bridge between bulk materials or molecular structures. The antibacterial activities of silver nanoparticles (Ag-NPs) using (*Curcuma Longa* L.) Rhizomes were studied with respect to *Helicobacter pylori (H.pylori).* Studies on curcumin, (*Curcuma Longa*) powder, have seen many biological effects such as antimicrobial activity. In this *in vitro* instruction, we trialed to assay the antibacterial activity of Ag-Nanoparticles using *Curcuma Longa* L. (Ag-NPs) the effect of the agent was examined on *H.pylori* is a micro-aerophilic bacterium. It has drawing as a major cause of gastrointestinal disorders such as gastritis and peptic ulcer. Extracellular biosynthesis of Ag- NPs was verified by utilizing medicinal extracts for the reduced a watery Ag ions at brief time. The silver nanoparticles changing the color of plant extract and then this changing in color confirmed by using "UV-Vis spectroscopy. Antibacterial action of Ag-NPs against*, H.pylori* studied by using disc diffusion method. The outgrowth of GN *H.pylori* was "inhibited by Ag-NPs. The antimicrobial activity was observed against" *H.pylori* (15.6 mm SD± 1.6), for Ag-NPs while for $Ag(NO_3)$ was (6.8 mm SD±0.6). Therefore, In Ag-NPs was reveal to be more antibacterial effect and could be advisable for further and more *in vivo* examinations.

Key words: *Curcuma Longa* L., Silver Nanoparticles, Antimicrobial activity

Introduction

Nano-science and nano-technology are overcoming and animated fields, which have many type of substances that are developed for different implementations (Rai *et al.*, 2009). Nanotechnology has premium effort in technology and most fields of sciences, on account of their volume and, or shape dependent intrinsic physiochemical, optoelectronic, catalytic & biological characteristic & larger face area (Hoyme, 1993). Nanotechnology preceding suitability to re-explore the biological features of which are known antimicrobial compounds by manipulating their size to improve the action.

Silver containing materials can be used to disregard microorganisms on textile fabrics (Crabtree *et al.*, 2009, and Ip, 2006). Nanoparticles attach to the surface of the cell. This interaction changes in bacterial structure, which effect the cell functions, such as permeability, causing pores, decreasing the activity of respiratory enzymes, and altimatly death of bacterial cell (Sharma, 2011 and Li, 2010).

Silver nanoparticles thought known to demonstrate killing bacteria because its toxic to cell to a large numbers of bacteria, and low toxicity to mammalian tissue (Pacios *et al.*, 2007). Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it (Chopra 2007 and Alliathy 2017) and examining for antibacterial actions. Many considerations have confirmed the "bactericidal effect of nanosilver against Gram negative bacteria (GNB) & gram Negative Bacteria (GPB), but the bactericidal mechanism of this compound has not been clearly elucidated (Calvo *et al.,* 2006, Pal *et al.,* 2007 and Lut 2008) defined the antibacterial activity of silver nanoparticles against *E. colli*, *Vibrio cholera, Pseudomonas aeruginosa* and *Salmonella typhi,* and suggested that Ag-NPs contact to the cell wall of bacteria and interacted with the action, and other biological systems penetrate bacteria, and release Ag ions (Upendra 2008,

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Saxena *et al.*, 2010 and Khandelwal *et al.,* 2010).

Helicobacter pylori is a micro-aerophilic bacterium with the ability to establish infections in human stomach and peptic ulcer (Thirumurgan *et al.*, 2010). The International Agency for Research on Cancer (INARC) classified *H. pylori* as a group I carcinogen by (Machado *et al.*, 2009). The destruction of bacteria. from patients remaining the good option for an effectual therapy of *H. pylori* linked disorders. Triple remedies, inclusion 2 antibiotics and a proton pump inhibitor, gives a high eradication rate (Toracchio *et al.*, 2000).

Many studies have detected that triple remedies is not constantly effective, and the obtaining by *H. pylori* of resistance to antibiotics, such as amoxicillin, metronidazole and clarithromycin, could represent a critical problems that may reduce treatment efficacy (Graham 1998). In view of the incomplete cure achieved with conventional therapy because of increasing resistant strains, harmful side effects (Myllyluoma *et al.*, 2005), the price of the antibiotics (Wong *et al.,* 2003), and a several other agents influencing to revise, there is pressing want to develop new treatment for *H. pylori* infection.

Because of the evolution of bacterial resistance to the accessible antibiotics and increasing currently of traditional remedy has lead researchers to search for the antimicrobial compounds in plants. *Curcuma Longa* is a medicative plant that botanically is related to Zingberiaceae family Turmeric (*Curcuma Longa* L.) is the underground rhizome used as a spice, herbacal medicine, coloring agent and cosmetics since Vedic age1(Kamada *et al.*, 2007). The dried rhizome is a rich source of protiable phenolic compounds known as the curcuminoids (Chattopadhyay *et al.*, 2004) Herbs and has been found to reduce inflammation, protect against infection, helps to cleanse the liver and the lungs and other organs, also protect the cell from damage which can leading to rheumatoid arthritis, osteoporosis, heart disease and others (Surh 2002).

There are many statements on induction of mono-carbonyl analogues of curcumin in order to increase antimicrobial and anticancer activity (Najah 2017).

This study is an attempt to test the anti-*H.pylori* activity of SNPs producted by using the *Curcuma Longa* L. abstract, which have been using in traditional medicine outside any attestation.

Materials and procedures

Helicobacter pylori culture:

Helicobacter pylori, isolated from Seventy-nine antral mucosal biopsy specimens of patients with gastritis or duodenal ulcers. *H. pylori* isolates were cultured on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI) then adding each of trimethoprim (5g/ml), vancomycin (8g/ml), and polymyxin B (10g/ml). The plates were incubated at 37°C in a microaerophilic atmosphere (10% CO_2) for 3 to 6 days. Stock cultivations were preserved at 70°C till use.

Determination of Minimum Inhibitory Concentrations (MICs):

The stock cultures were streaked on BHI agar with and incubated for 4-6 days. Growing *H. pylori* bacteria suspended insterile phosphate-buffered saline (PBS) and adjusted to an optical density of 0.1 at 600 nm.

Synthesis of silver nanoparticle and Plant material:

The dried Rhizomes of *Curcuma Longa* L were crunch to a powdery. 1 mM silver nitrate was added to the plant extract to reach a final solution of 200 ml and centrifuged at 19,000 rpm for 25 min. The supernatants were heated at 50 to 95ºC. The color changed within 10-15 mints., and these alterations indicate the formation of (SNPs). The decrease of pure sliver ions were measured by "UV-Vis spectrum of the allowance culture at 5 hours after diluting a small sample in distilled water by using systronic 118 UV-Vis Spectrophotometer.

Definition of antibacterial effectiveness by Disk-diffusion procedure:

Plates with Mueller Hinton agar (Difco) were attended to evaluating antibacterial activities. The colony forming (CFU) units of suspension of the tested isolates were determined and tested inoculums were adjusted to 1×10^5 cells/ml, matching with 0.5 McFarland. Inoculums (100µl) were separated on the agar dishes. 50 µl of Ag nanoparticles added to sterilized Whatman No. 1filter paper disks, others disks were impregnate with 50 µl silver nitrate (5µg/ml) and placed on the inoculated agar plates incubated at 37°C for overnight for bacterial culture, after incubation, plates were tested by limiting the zones of inhibition for control, SNPs and silver nitrate were measured. The tests were recurred 3 times and mean values of zone diameter were existent.

Statistical analysis:

The results of the study were enumerated as mean diameter of inhibition zone in mm ± standard deviation (mean ± SD).

Results

Ag-Nps" Has an effect on some organisms its effect on the bacteria under study generally obscure. The current investigation is the fore study about the effect of

Ag-Nps against this one of carcinogenic bacteria. The occurrence of yellowish brown color in the interaction means the figuration of silver nanoparticles (SNPs) (Fig. 1). UV-visible spectroscopy is a technique used for structural characterization of silver nanoparticles. The absorption spectrum of the pale yellow-brown silver colloids showed a surface Plasmon absorption band with a maximum of 418 nm indicating the presence of spherical Ag nanoparticles.

Fig-1: The color of curcumin extracts changed following adding silver nitrate, (A) curcumin extracts. (B) Silver nanoparticles.

Table (1) indicate the diameter of inhibition zones(mm) round each disc with AgNPs againest *H. pylori* (15.6mm SD± 1.6).

Table 1: Inhibition Zone (mm) of nanoparticles against *H.pylori*

Organism	Inhibition Zone (mm) ±SD		
	Control	$Ag(NO_3)_2$	SNPs
H.pylori	15.6±1.6	6.8± 0.6	0

The antibacterial activity of AgNPs against *H. pylori* is represented in Fig. 2.

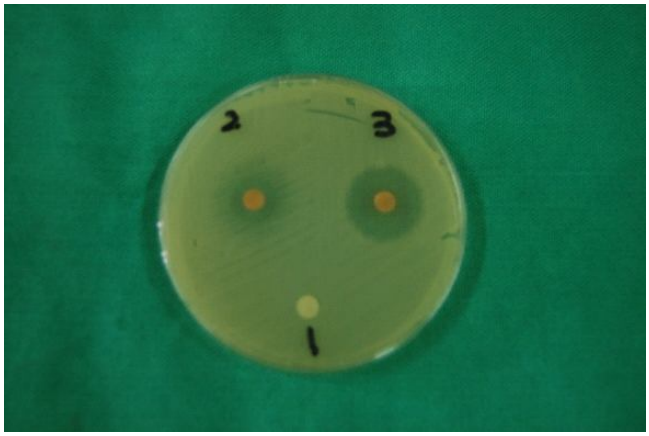

Fig. 2: Antibacterial activity of silver nanoparticles using *Curcuma Longa* L. extract. (1=Control. 2=Sliver nitrate. 3= Nanoparticals).

Discussion

Due to spreading of the infectious diseases and increased resistance of the pathogens to antibiotic have made to search for new antimicrobials unavoidable. In the prevalent situation, currently one of the most original and expected treatment is the nanoparticals. Even if the extended knowledge of microbial agents and implementations of new curative, the mortality morbidity related with the microbial contagion remaining height (Kolar *et al.*, 2001). Therefore, there is insistent claimed to find new strategies & correspond new antimicrobial appliances from plants and inorganic materials to evolve the following generation of medicine to control microbial infections.

It is recognized that silver nanoparticles demonstration yellowish - brown color in watery solution due to simulative of surface plasmon hesitation in Ag-NPs (Phull 2010). Silver has been used for its well known antimicrobial properties since roman time however the advances in generating Ag-NPs have made possible advancement of the use of silver as a strong bactericidal (18). Many prospector (Khandelwal *et al.*, 2010) used *Escherichia coli* as a model for G -ve bacteria and confirm that Ag-NPs may be used as an antimicrobial agent". Other workers (Morones *et al.,* 2005) also opined that the Ag-NPs have an antibacterial effect on *S. aureus and E. coli*.

Synthesis of mineral NPs biologically is considered a traditional method and the use of plant extraction has knowledge to control the diseases, besides being safe and no phytotoxic effects (Savithramma *et al.*, 2011). "Silver nitrate is utilized as reducing agent as silver has distinctive properties such as good connectivity, catalytic and chemical stability. The watery Ag ions when bared to medicinal extractions were reduced in solution, by leading to the formation of Ag hydrosol. The antimicrobial mechanism of silver nanoparticales is by casing pore and damage in cell membrane as a result to formation of free radicals. These free radicals may be derived from the surface of silver nanoparticals and responsible for the antimicrobial activity (Petica *et al.*, 2008). Silver nanoparticles lead to the formalization of pores bacterial cell wall, and these nanoparticles could enter into protoplasm through the pores and damage the bacterial cell membrane, then enter into the protoplasm, which not only thiching deoxyribonucleic acid, but also coagulated & interact with the cytoplasm of damaged bacteria. Finally, particles resulted in the drain of cytoplasmic component (Feng *et al.*, 2003 and Yamanaka *et al.*, 2005). Moreover, the silver nanoparticles could increase the disintegrate of (DNA). Silver has an significant

antimicrobial effect which is depended on superficial contact in these silver can restraint enzymes of the respiratory chain and DNA synthesis.

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