Assay of Antibacterial Activity of Zinc Oxide Nanoparticles (Capped with BSA), Leaf and Stem Extracts of *Andrographis paniculata* (Family: *Acanthaceae)* on the Bacterial Pathogens of Lives Stock

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ABSTRACT

Antibiotics and Chemotherapeutic agents were used for the cure of various human and animal diseases nowadays. Due to the increasing resistance exhibited by pathogens against these antibiotics and chemotherapeutics, there is an urgent need to develop new drugs or discover new components that might work effectively against the resistant pathogens. Medicinal plants do that job effectively in addition metal nanoparticles have also been tested by many workers for their antimicrobial activities. In the present study, the various concentrations (15 & 30µl) of chloroform extract of leaf and stem of *Andrographis paniculata* was used against four cattle pathogenic bacterial species such as, *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoniae* which are predominant disease-causing agents of cattles frequently occurring in the large population of cattles which were living in the dirty and unclean areas. The formation of zone of inhibition on the growth of was the clear evidence for the anti-microbial activity of the plant extracts. The clearance zone was found to vary according to the quantity of leaf and stem extracts. Among the experimental samples used in the study, the ZnO NPs (capped with BSA) mixed with leaf and stem extracts have higher antibacterial efficiency when compared to remaining samples used. Especially the *S.aureus* and *P.vulgaris* were seemed to be controlled effectively than the other bacterial species. In the present investigation, ZnO NPs (capped with BSA) and antibiotic tetracycline have maximum antibacterial activity against *E.coli* and *K.pneumoniae* than the other two cattle pathogens tested. The leaf and stem extracts of *A.paniculata,* which inhibited the growth of *K.pneumoniae.* Hence, the plant extract combined with ZnONPs (0.5%) (capped with BSA) used control these bacterial species in cattles.

KeyWord: *Andrographis paniculata*- ZnO Nanoparticles - Leaf and stem extracts - cattle Pathogens- Antibacterial activity

I. INTRODUCTION

Cattle farmers have problems of poor production, cattle diseases and the publica is vulnerable to zoonoses. Indigenous practices such as the use of herbal medicines and concoctions have been a form of therapy for cattle among resource-poor small holder farmers [1]. Indigenous practices are considered by scientists to be risky to both human and animal health. A few farmers use conventional veterinary drugs like antibiotics most times unnecessarily and this is making disease causing bacteria more resistant to the drugs and therefore becoming a threat to public health. Documentation and validation of indigenous medicine is therefore necessary because they are likely to be important in future especially given the trend of emerging diseases and the development of resistance of pathogens to drugs. To solve the problems facing the cattle industry, documentation of data about indigenous knowledge about the antibacterial activity of nanoparticles and herbal plants were carried out many workers [2]. India is primarily an agricultural country with the majority of the population residing in rural areas. Their main occupation is agriculture, in addition cattle are valuable assets of the rural poor and are critical in supporting their live hoods particularly during unfavorable times. Cattle are raised as livestock for meat, as dairy animals for milk and other dairy products, and as draft animals [3].

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [4]. Successful use of herbal remedies in modern health care systems is being achieved in China, India and the Soviet Union [5].The field of Nanotechnology is one of the most active areas of research in modern material science.

Nanotechnology is the application of Science and Technology to control matter at the molecular level. Nanoparticles are viewed as the fundamental building blocks of nanotechnology. They are the starting points for preparing many nano structured materials and devices [6], [7]. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications [8]. Three applications of nanotechnology are particularly suited to biomedicine: diagnostic techniques, drugs, and prostheses and implants. There are several previous reports on antibacterial activity of medicinal plants and nanoparticles against cattle pathogens. Have investigated that, the acetone and ethanol extracts of some medicinal plants and nanoparticles, have antibacterial activity against pathogenic bacteria [9]. There are several approaches are available for synthesize of Zinc Oxide nanoparticles for example, Zinc ions are reduced by radiation, chemical reduction, electrochemical, photochemical methods, Langmuir–Blodgett, sol-gel and biological techniques. It is well known that metal nanoparticles have good antimicrobial properties [10].

II. MATERIAL AND METHODS

A) Preparation of leaf extracts:

The leaves of the *Andrographis paniculata* have been used in the present investigation were collected from agriculture field in around Sivagangai. The leaves of the plant were dried and then powdered, 15 grams of the air-dried powder of the plant was taken and added separately with 100 ml of chloroform in a conical flask plugged with cotton wool and then kept on a rotary shaker at 150 rpm for 48 hrs with intermittent shaking of the content. After 48 hours, the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume the help of water bath and stored at 4^0C in airtight bottles [11].

B) Collection of Test Organisms:

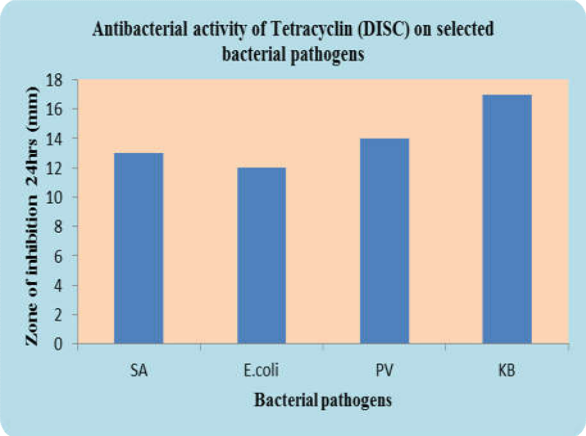
The bacterial pathogens of Cattles like *Staphylococcus aureus* (MTCC No: 96) and the three Gram-negative bacterial species such as, *Escherichia coli* (MTCC No: 1687), Institute of Microbial Technology (IMTECH), Chandigarh, India. The other strains of such as, *Proteus vulgaris* and *Klebsiella pneumonia* were obtained from King's Institute and Veterinary College, Chennai.

C) Assay of antimicrobial activity using disc diffusion method:

Liquid nutrient agar media and the Petriplates were sterilized by autoclaving at 120°C for 30 minutes. The sterilized petriplates were then labelled with the selected bacterial strains. Under aseptic conditions in the laminar flow hood, 20 ml of agar medium was dispersed into the petriplates labelled with bacterial strains. After solidification of the media, microbial strains (cattle pathogenic bacterial species) were swabbed on the surface of the agar plates separately. Using sterile forceps, the paper discs loaded with experimental samples (15 & 30µl) were placed on the surface of the agar plates. The plates were then incubated at 37°C for 24 hours. The measurement of zone of inhibition was recorded with 24 hours of interval for all the control and experimental samples.

IV. RESULTS AND DISCUSION

Antibacterial activity of Tetracycline (as a positive control) have higher inhibition on *K.pneumoniae* (17 mm) (Fig-1). ZnO NPs (capped with BSA) to have greater extent of anti-microbial activity on *E.coli* (10& 13 mm). Both the chloroform extract of leaf (12 & 13 mm) and stem extract (10 & 12 mm) of *Andrographis paniculata* have greater extent of anti-microbial activity on *K.pneumoniae*, the mixture of Zno Nps and the chloroform extract of leaf of *Andrographis paniculata* have greater extent on *S.aureus* (10 & 16mm) and the chloroform extract of stem of *Andrographis paniculata* have greater extent of antibacterial activity on *P.vulgaris* (10 & 16 mm). The results obtained with two different concentrations such as, 15 & 30 µl have indicated a notable variation in the inhibitory activity in the all the bacterial species (Table-1 and Figure-2).



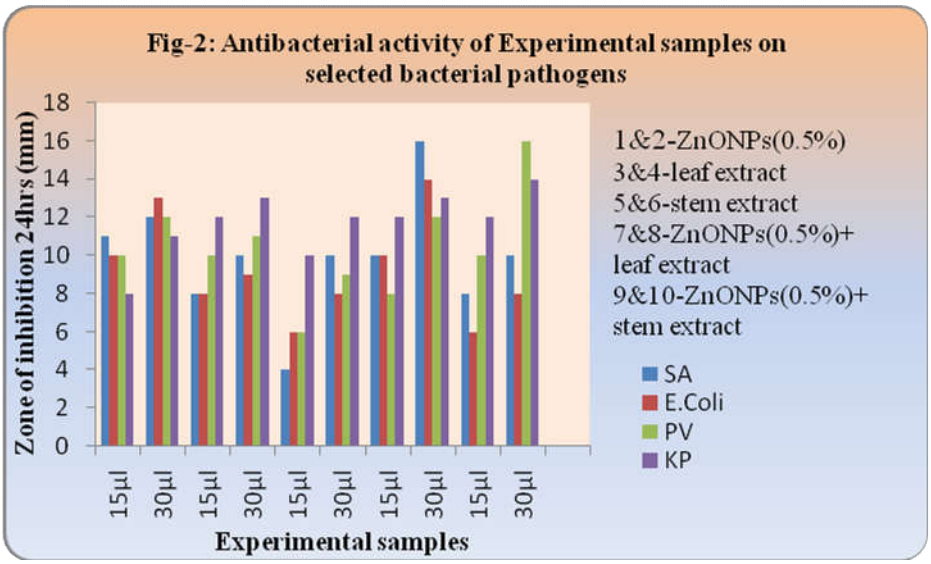


Table-1: Antibacterial activity of experimental samples

S.No	Experimental samples	Concentration used	Zone of inhibition (mm) 24hrs			
			S.aureus	*E.coli*	*P.vulgaris*	*K.pneumoniae*
1	Zno NPs	15µl	11	10	10	8
		30 µl	12	13	12	11
2	Leaf Extract	15µl	8	8	10	12
		30µl	10	9	11	13
3	Stem Extract	15µl	4	6	6	10
		30µl	10	8	9	12
4	Leaf Extract + Zno NPs	15µl	10	10	8	12
		30µl	16	14	12	13
5	Stem Extract + ZnoNPs	15µl	8	6	10	12
		30µl	10	8	16	14
6	Tetracyclin		13	12	14	17

Many studies have shown that some NPs made of metal oxides, such as ZnO NPs (capped with BSA), have selective toxicity to bacteria and only exhibit minimal effect on human cells, which recommend their prospective uses in agricultural and food industries. The ZnO nanoparticles (capped with BSA) have a significant effect on their antibacterial activity. The nanoparticles treated at a higher temperature leads to a lower activity [12].The mechanisms of the antibacterial activity of ZnO particles (capped with BSA) are not well understood although [13] have proposed that the generation of hydrogen peroxide be a main

factor of the antibacterial activity, while have indicated that the binding of the particles on the bacterial surface due to the electrostatic forces could be a mechanism. The phytochemical present in the plant extract have also significant impact on the pathogenic bacteria. The reports of [14] pertaining to antimicrobial activity of ZnO NPs were in close agreement with the present study reports.

In the present study, Over all analysis of result indicated that the mixture of plant extract and ZnONPs have greater antibacterial activity when compared to the experimental samples used in this study. The formation of zone of inhibition was the clear evidence for the anti-microbial activity of the medicinal plants. The clearance zone was found to vary according to the leaf extract of medicinal plant species. The presence of antimicrobial activity has been positively tested in many species of higher plants [15].

In the present investigation, the mixture of Zno Nps and chloroform extracts of leaf and stem of *Andrographis paniculata* have maximum antibacterial activity against *S.aureus* and *K.pneumoniae* than the rest of the bacterial cultures used in the study. Thus the experimental samples have showed different antibacterial activity on the 4 cattle pathogens used in the study.

V. CONCLUSION

Medicinal plants have numerous bioactive components such as antioxidant and antimicrobial compounds. The antimicrobial assay in the study are more significant and thus the study suggests that the active components are responsible for antibacterial active and the results are found to be interesting. Thus exploration of such biological agents might be a probable resource of an array of biologically active compounds and the present results will ensure a starting point for exploiting natural bioactive substances present in the extracts of plants. Hence the through investigations with the objectives of isolation and identification of the antimicrobial components in Andrographis paniculata must be carried out for clinical application. It is hoped that this study would lead to the establishment of some compounds that used to formulate new and more potent antimicrobial drugs of natural origin.

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