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RESEARCH ARTICLE

ANTIBIOTIC EFFECTS OF SILVER NANOPARTICLES SYNTHESIZED FROM *STYCHONS NUX VOMICA* ETHYL ACETATE ROOT COLUMN FRACTION AGAINST CLINICAL RESISTANT *STAPHYLOCOCCUS* STRAINS

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Abstract

The present investigation was carried out to evaluate the antibacterial activity of leaf and root silver nanoparticles synthesized using different solvents. According to our studies the AgNP's of *Strychons nux vomica* synthesized from different solvent extract of root revealed to possess significant activity comparing to the antibacterial activity exhibited by AgNP's of leaf extract. Among the AgNPS tested, AgNP's synthesized using ethyl acetate extract showed highest inhibitory activity comparing to AgNP's synthesized using acetone and methanol extracts. The ethyl acetate extract based AgNP's showed highest zone of inhibition 29.2 mm noted against MRSA. Activity when compared with reference drugs used in the study, was found subtle low with the activity of Dicloxacillin (29.8mm) and high with Clindamycin (28.5mm). Leaf extracts exhibited antibacterial activity against clinically resistant S. aureus species. Among **AgNP's** tested, ethyl acetate extract, showed high growth inhibition activity against MMSA and MRSA with zone of inhibition 21.5 and 19.9 mm respectively. However, it is failed to inhibit the growth of VRSA. On the other hand, Ag NP's synthesized using acetone extract showed antibacterial activity against all three tested bacterial strains. The zone of inhibition 16.9, 17.8, 15.8mm was noted against MMSA, MRSA, VRSA respectively. Following to acetone extract Ag NP's, methanol extract Ag NP's are also exhibited antibacterial activity. The zone of inhibition 15.5, 12.1, 16.7 noted against MMSA, MRSA, VRSA respectively.

Introduction:-

Staphylococcus aureus (*S. aureus*) is a normal microflora of human and spread from one individual to another by direct contact of infected person, although colonized objects or surfaces also important considerations for the transmission of *S. aureus.* Approximately, 30-40% of people around the globe are the nosal carriers of this bacterium (CDC NNIS System, 2001). *S. aureus* is commonly susceptible to the antibiotics. By passing the decades *S. aureus* acquired resistance for the most important class of antibiotic Isoxazoyl penicillins such as, Flucoloxacillin, Methicillin and Oxacillin (Diekema *et al.*, 2001). The history of *S. aureus* resistance was begin in 1940's with amazing antibiotic Penicillin, thereafter a gradual development of resistance was observed to till the date to all most all contemporary licensed antibiotics. Since 1940's to till the date the new variants of *S. aureus* (MRSA-I, MRSA-II,

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MRSA-III, MRSA-IV, VISA, CA-MRSA, VRSA) are noted (Sakoulas et al., 2003; Wootton et al., 2006). With reference to the clinical failure of antibiotics, the current situation badly demands the development of new drugs with novel mechanism of action against Staphylococcal infections. The new drugs can be a natural (Microbial synthesis and plant derived) and artificial (chemical synthesis). Structural modification of existing antibiotic for effective combat against resistance mechanism is also important in the drug discovery. Nanoparticles (NP's) hold a great importance in drug delivery because they can easily diffuse or penetrate through cell membrane to target site. Moreover, the use of biodegradable polymers in the synthesis of NP's increases the efficiency of drug release at intracellular site. The physical and chemical methods of NP's Production include disadvantages like low solubility of NP's, production is high cost, the use of high toxic chemicals (sodium borohydride and hydrazine) in NP's Production, purification of NP's is difficult, time consuming processes, large scale production is not possible, high energy is required for NP's synthesis. In contrast, the green chemistry approach involves low energy, no utilization of toxic chemicals, eco-friendly NP's production methods and production of biohazard by-products during NP's production is not seen. Silver, Zinc, Copper and gold are commonly used to produce NP's from plants. Among these, the large surface area of these silver nanoparticles (AgNP's) is extensively applied in different medical applications especially, antibacterial applications.

In context to the multidrug resistance by bacteria and rapid discovery of new drugs from different sources, the present was designed to evaluate the antibacterial efficacy of Strychons nux vomica leaf a root extracts. The extracts which were shown significant result are further used for synthesis of AgNP's for evaluation of antibacterial activity.

Material and Methods:-

Collection of plant material

Using soil digging machine, approximately, two feet of the soil was removed at one side of the plant to collect the roots. The plant material was collected from Siddhapuram village, Warangal rural, Telangana. Before the process of root collection we have taken an oral permission from the village Sarpanch Sambaiah. The roots were bought to the laboratory and the associated soil was separated and subjected for gentle washing under running tap water for further cleaning the soil. The material was dried under shadow. Approximately after 45 days, the dried root material was made fine powder.

Extraction

We have used Ethyl acetate, Acetone, Methanol solvents to extract 250g of *Strychons nux vomica* leaf and root powder using Soxhlet continuous hot extraction method. The solvent was evaporated using rota evaporator for the collection of crude extract.

Synthesis of Silver nanoparticles

The synthesis of silver nanoparticles (AgNP's) of different extracts of leaf and root was followed according to the method described by (Song and Kim, 2009). Separately, to 40 mL of 1 mM $AgNO_3$, 10 mL of 10% (w/v) active fractions was transferred slowly with continuous agitation. The mixture was maintained at room temperature and observed for the golden colour development which indicates the synthesis of AgNP's. This solution was incubated for overnight in a closed dark chamber. The upper layer (supernatant) was discarded and the sediment was centrifuged at 10,000 RPM for about 15-20 min. Discard the supernatant and add double distilled water to the sediment particles and centrifuged at 10,000 RPM for about 5min. Discard the supernatant and add ethanol and centrifuge at 10, 000 RPM for about 5 min. The supernatant was discarded and the AgNP's were oven-dried for 24 h at 50-55^{0}C (Song and Kim, 2009).

Anti-Bacterial activity

The antibacterial efficacy of *Strychons nux vomica* leaf and root extracts with and without AgNP's was investigated against Methicillin-susceptible **S. aureus** (MSSA), Methicillin- resistant **S. aureus** (MRSA) and Vancomycin Resistant **S. aureus *(VRSA)*** by agar well diffusion method (Chung *et al*., 1990). The media plates containing, test organisms were created 6mm wells and filled with 100 µl of synthesized nanoparticle solution and incubated at 27 ± 2^0 C. The zone of inhibitions was recorded in mm.

Statistical analysis

The results are expressed in Mean ± SD. The significance between the groups is carried out using student "*t*" test.

Result:-

Antibacterial activity of silver nanoparticles of leaf extracts

The AgNP's of Strychons nux vomica leaf extracts exhibited antibacterial activity against clinically resistant **S**. aureus species. Among **AgNP's** tested, ethyl acetate extract, showed high growth inhibition activity against MMSA and MRSA with zone of inhibition 21.5 and 19.9 mm respectively. However, it is failed to inhibit the growth of VRSA. On the other hand, Ag NP's synthesized using acetone extract showed antibacterial activity against all three tested bacterial strains. The zone of inhibition 16.9, 17.8, 15.8mm was noted against MMSA, MRSA, VRSA respectively. Following to acetone extract Ag NP's, methanol extract Ag NP's are also exhibited antibacterial activity. The zone of inhibition 15.5, 12.1, 16.7 noted against MMSA, MRSA, VRSA respectively. The results are shown in table 1.

Table 1:- Antibacterial activity of AgNP's synthesized from *Strychons nux vomica* leaf extracts.

Test strains	Ethyl acetate (Ag NP's)			Acetone (Ag NP's)			Methanol (Ag NP's)			CLD[#]	DCL[#]
	25	50	75	25	50	75	25	50	75	10	10
MMSA	13.5 ± 0.1	17.3 ± 1.1	21.5[*] ± 0.5	9.2 ± 1.1	13.8 ± 1.0	16.9 ± 1.5	7.0 ± 1.1	11.7 ± 0.1	15.5 ± 1.2	27.3 ± 0.5	30.9 ± 1.1
MRSA	12.2 ± 1.1	14.8 ± 0.5	19.9[*] ± 1.1	8.5 ± 1.6	14.1 ± 0.5	17.8 ± 1.1	6.2 ± 1.5	9.4 ± 0.3	12.1 ± 1.0	28.5 ± 0.2	29.8 ± 0.5
VRSA	--	--	--	9.5 ± 1.1	12.2 ± 0.2	15.8 ± 0.8	8.5 ± 1.2	13.9 ± 0.3	16.7 ± 1.1	28.4 ± 0.1	28.7 ± 0.5
CLD- Clindamycin, DCL- Dicloxacillin, [#]Concentration of antibiotics (µg/mL), [#]Concentration of Ag NP's (mg/mL), [*]P<0.05, n=4											

Antibacterial activity of AgNP's of Root extracts

The antibacterial activity of AgNP's synthesized from different solvent extract of root revealed to possess significant activity comparing to the antibacterial activity exhibited by AgNP's of leaf extract. Among the AgNPS tested, AgNP's synthesized using ethyl acetate extract showed highest inhibitory activity comparing to AgNP's synthesized using acetone and methanol extracts (Table 2). The ethyl acetate extract based AgNP's showed highest zone of inhibition 29.2 mm noted against MRSA. Activity when compared with reference drugs used in the study, was found subtle low with the activity of Dicloxacillin (29.8mm) and high with Clindamycin (28.5mm). On the other side the Ag NP's synthesised using acetone extract also possessed antibacterial activity with the zone of inhibition 21.5 mm noted against MRSA. Whereas, the Ag NP's synthesised using methanol extract showed moderate activity with zone of inhibition 13.5mm recorded against MRSA (Table 2). Following to MRSA, the AgNP's synthesized using ethyl acetate extract are also showed significant antibacterial activity against MMSA and VRSA with zone of inhibition 26.0 and 22.5mm respectively. On the other hand AgNP's synthesized using acetone extract was showed moderate antibacterial activity against MRSA and least activity against MMSA and VRSA with zone of inhibition 21.7, 15.5 and 13.2mm respectively (Table 2). The AgNP's synthesized using methanol extract was also found average antibacterial activity against MMSA, MRSA, VRSA with zone of inhibition 16.3, 13.5, 17.3mm respectively (Table 2).

Table 2:- Antibacterial activity of AgNP's synthesized from *Strychons nux vomica* root extracts.

Test strains	Ethyl acetate (Ag NP's)			Acetone (Ag NP's)			Methanol (Ag NP's)			CLD[#]	DCL[#]
	25	50	75	25	50	75	25	50	75	10	10
MMSA	15.1 ± 0.3	19.8 ± 0.5	26.0[*] ± 0.1	7.1 ± 0.2	11.0 ± 1.3	15.5 ± 0.5	7.2 ± 1.0	12.1 ± 1.1	16.3 ± 0.1	27.3 ± 0.5	30.9 ± 1.1
MRSA	16.7 ± 0.2	23.1[*] ± 0.5	29.2[*] ± 0.1	8.4 ± 1.1	15.3 ± 0.3	21.7[*] ± 1.3	7.1 ± 0.5	10.2 ± 0.1	13.5 ± 0.5	28.5 ± 0.2	29.8 ± 0.5
VRSA	10.3	16.6	22.5[*]	9.0	11.4	13.2	10.1	14.4	17.3	28.4	28.7

	± 1.2	± 0.1	± 1.3	± 0.5	± 1.1	± 0.1	± 0.5	± 1.2	± 1.0	± 0.1	± 0.5
CLD- Clindamycin, DCL- Dicloxacillin, #Concentration of antibiotics (µg/mL), #Concentration of Ag NP's (mg/mL), *P<0.05, n=4											

Discussion:-

The selected clinically resistant *Staphylococcus* species susceptibility was found significant towards AgNP's of *Strychons nux vomica* leaf and root extracts. Among these root AgNP's exhibited significant activity. Moreover, the AgNP's of root synthesized using ethyl acetate competed to exhibit highest antibacterial activity with reference drug Clindamycin, and almost equals when compare with Dicloxacillin against MRSA at 75 mg/mL. However, MMSA and VRSA susceptibility was slightly varied with reference to Clindamycin and Dicloxacillin. The ability of hydrogen bonding and subsequent solubilisation of bioactive compounds is an important characteristic of ethyl acetate to establish the pharmacological activities (Theodore, 1978). The synthesis of AgNP's using crude extracts of plant material is a well known method for enhancing the antibacterial activity. This might probably due its surface-to-volume ratio characteristic which absorbs bioactive compounds and carry them to the target site for their action. By the literature review we noticed that the most of the antibacterial activity of *Strychons nux vomica* was confined to the seeds, leaves and stems rather than the root. Therefore, this study holds great importance in the development novel antibacterial drugs against multidrug resistant Staphylococcus species from the leaves and root of *Strychons nux vomica*.

Conclusion:-

Based on our data of present investigation, we conclude that silver particles synthesized using ethyl acetate root extract exhibited significant antibacterial activity. However, further evaluation is necessary to use this root sliver nanoparticles as novel drug against Staphylococcus infections.

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Conflict of Interest

Authors do not have any conflict of interest

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