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VALIDATION AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *STRYCHNOS NUX-VOMICA*– AN IMPORTANT ETHNOMEDICINAL PLANT OF KURNOOL DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT
Strychnos nux-vomica seed are extensively using by the ethnic groups of Kurnool district, Andhra Pradesh, India to cure vitiligo. Biological synthesis of silver nanoparticles was carried out from seed aqueous extract of Strychnos nux-vomica 10 ml seed extract was mixed to 90 ml of 1 mM aqueous of $Ag(NO_3)_2$ and was heated at 60-80^{0}C for 20 min. The color change of aqueous solution into dark brown color. For characterization using UV-Vis spectrophotometer and AFM. AFM and UV-Vis spectrophotometer showed the formation of silver nanoparticles with spherical shape and average size 41.72 nm. SNPs have good antimicrobial activity against different microorganisms.

KEY WORDS
Medicinal plant; Silver nanoparticles; Atomic Force Microscope (AFM); Inhibition zone; Secondary metabolites; Anti microbial efficacy.

INTRODUCTION
India is one of the twelfth mega biodiversity hotspot in the world. Eastern Ghats are one among them, which are characterized by different wild medicinal flora. Nallamalai hills as a part of Eastern Ghats in Kurnool District of Andhra Pradesh, India. Mainly four ethnic groups (Chenchu, Sugali, Yerukala and Yanadi) are inhabited in this region [1].In this connection a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic, Siddha and Unani[1].

WHO is encouraging the traditional drugs because of its less side effects and matter of low cost and easy availability [3].WHO also has organized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines [4].

The medicinal herb *Strychnos nux-vomica* known for its medicinal values in alternative systems of (Ayurveda, Unani, Siddha, Homeopathy and Chinese) holistic health and herbal medicine. It is belongs to the family Strychnaceae. The paste prepared by mincing fresh plants has got an anti-inflammatory effect and may be applied to insect bites, stings, swellings and other skin diseases.

Skin diseases are commonly occurring among the rural masses due to poor hygienic conditions, poor sanitation facilities and contaminated water etc., the traditional healers of these ethnic groups are extensively using *Strychnos nux-vomica* seed to treat leucoderma. Leucoderma is a skin disorder in humen for a number of reasons depigmentation occurs due to auto immune disorder [5] or lacking of pigments due to absence of melanocytes[6].

Strychnos nux-vomica seed powder contains a variety of important chemical compounds. It consis Strychnine, Brucine, Pseudostrychnine, Pseudobrucine, Secoxyloganin, Caffeic acid, P-

Hydroxybenzoic acid, P-Hydroxyphenylacetic acid, Uvaol, Lupeol, 11-Oxo-α-Amyrin palmitate, Maltol [7]. Nanoparticles are gaining much importance especially in medicinal field. Synthesis of metal nanoparticles receives great attention due to their unusual optical, chemical, phytochemical and electronic properties [8]. Silver a nobel metal is known to improve the immunity since ancient times, $Ag(NO_3)_2$ was using for biosynthesis of nanoparticles by using seed aqueous extract of *Strychnos nux-vomica.* The possibility of using plant materials for the synthesis of nanoscale metals was initially reported by [9, 10]. SNPs have particular properties that may perhaps have numerous applications in the field of dentistry, clothing, catalysis, mirrors, optics, phytography, electronics and food industry [11].

At present extensive work has been done to develop new drug from natural products because of the resistance of microorganisms to the existing drug. The pathogens like *E. coli, Bacillus, Salmonella typhi* and *Staphylococcus aureus* ([12].

In this study, ethno-medico botanical studies, qualitative analysis of phytochemical constituents by using seed extract of *Strychnos nux-vomica* and biological synthesis of SNPs and screening of SNPs for microbial efficacy.

MATERIAL AND METHODS

Plant material

The fresh seed of *Strychnos nux-vomica* was collected in June 2013 from Nagaloty reserve Forest, Kurnool District of Andhra Pradesh, India. The seed were cleaned, finally distilled water dried at room temperature and ground to fine powder.

Preparation of extract

25 g of seed powder of *Strychnos nux-vomica* was taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 min at 100^0C on water bath. Then plant material extracts were collected in separate conical flask by standard filtration method and stored in refrigerator for further use.

Phytochemical screening

10 ml seed extract was used for preliminary phytochemical screening. The qualitative analysis of secondary metabolites was carried out by using the methods [13] for flavonoids, [14]for steroids, alkaloids and phenols, [15] for triterpenoids and glycosides, [16] for tannins, anthraquinons, leucoanthocyanins and emodins, [17] for saponins, [18] for reducing sugars and anthocyanins.

Preparation of 1 mM Silver nitrate solution

1 molar silver nitrate stock solution was prepared by 1.7 g of $AgNO_3$ was dissolved in 10 ml distilled water. 1 mM solution was prepared by 1 ml of 1 M solution was made up to 100 ml with 99 ml of distilled water. This solution was stored in amber colored bottle for further use.

Synthesis of silver nanoparticles

SNPs were synthesized by using seed aqueous extract of *Strychnos nux-vomica.* The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5^{th} h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Vis Spectrophotometer. The size and shape of SNPs were confirmed with AFM (Nanosurf ®, AG, Switzerland; Product: BTO2089, VI, 3RO),

UV-Vis spectra analysis

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3 hrs. after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was carried out by using UV-Vis spectrophotometer (Systronics type 118).

AFM analysis

The silver nanoparticles extracted by the above protocol were visualized with an Atomic Force Microscope (AFM). A thin film of the sample was prepared on a glass slide by dropping 100 μl of the sample on the slide and was allowed to dry for 5 min, the slides were then scanned with the AFM (Nano surf ® AG, Switzerland, Product: BTO2089, 3RO). Nanosurf ® Easyscan-2 software was used for the AFM Analysis (VIT, Vellore, Tamil Nadu).

Microorganisms

Pure cultures of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* species of bacteria and *Paecilomyces varioti, Pencillium rubrum* and *Aspergilus flavus* species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India.

Antimicrobial activity

The antimicrobial activities of SNPs were carried out with paper disc diffusion method using nutrient agar medium and potato dextrose agar medium for bacterial and fungal cultures respectively. Zones of inhibition for control, SNPs and silver nitrate were measured after 24 h and 7 days and compared with standard drugs Gentamycin and Nystatin for bacterial and fungal growth respectively. The experiments were repeated thrice and mean values of inhibition zone diameter were presented.

RESULTS AND DISCUSSION

The ethnic groups (Chenchu, Sugali, Yerukala and Yanadi) of Kurnool District, Andhra Pradesh, India. Traditional healers of these groups have staunch confidence to treat leucoderma skin disease. Dry seeds ground, made into ash, mixed with neem oil and applied as an external application to treat leucoderma. This ethnomedicinal information was cross checked with Ayurvedic physicians, Sri Venkateswara Ayurvedic College, Tirupati, Andhra Pradesh, India for authentication. In this regard *Strychnos nux-vomica* seeds are extensively using in Ayurvedic medicines to treat leucoderma[2].

The secondary metabolites screening of *Strychnos nux-vomica* Showed that the seed extract is rich in alkaloids, flavonoids, tannins and triterpenoids, glycosides, lignins, steroids and lacking of anthocyanins, anthraquinones, coumarins, emodins, fatty acids, leucoanthocyanins, phenols and reducing sugars and Saponins, (**Table-1**).

Table-1: Secondary metabolites of seed extract of *Strychnos nux-vomica*

S. No.	Secondary metabolites	Seed extract
1.	Alkaloids	++
2.	Anthocyanins	-
3.	Anthraquinones	
4.	Coumarins	-
5.	Emodins	-
6.	Fatty acids	-
7.	Flavonoids	++
8.	Glycosides	+
9.	Lignins	+
10.	Leuco anthocyanins	-
11.	Phenols	-
12.	Reducing sugars	-
13.	Saponins	-
14.	Steroids	+
15.	Tannins	++
16.	Triterpenoids	++

Note: '+' indicates presence, '++' indicates presence of more amounts, '-' indicates absence

Flavonoids, are medically used as antistomatic, diarrhoea and anti-inflammatory, anti cancer and anti oxidative. It also known to possess antiviral and anti fungal [19, 20] and antimicrobial properties[21]. The presence of bioactive compounds indicates the medicinal values of the plant. Steroids and triterpenoids possess anti bacterial activity [22]. Tannins possess astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial activities [23]. Similar chemical constituents were also found in *Shorea tumbuggaia* [24], *Thespesia populnea* [25], in *Curcuma longa* [26] and in *Psoralea corylifolia* [27, 28].

In the present study SNPs were synthesized by using seed aqueous extract of *Strychnos nux-vomica* rapidly with in 15 min was able to be followed by color change. The fresh suspension of *Strychnos nux-vomica* was cream in color. However, after adding of Ag $(NO_3)_2$ the sample turned to dark brown color. The colour change in aqueous solution is due to the surface- Plasmon resonance (SPR) phenomina (Fig-1). The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrums of colloidal solutions of SNPs synthesized from the seed extract *Strychnos nux-vomica*have the characteristic absorbance peaks at 260 and 430 nm respectively **(Fig-1(b))**. This illustrated the presence of homogenous distribution of hydrosol SNPs after 15 min of stirring [29].

It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape of nanoparticles in aqueous suspension [30]. The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions same results observed in *Boswellia ovalifoliolata* stem seed [31]. Silver nanoparticles have free electrons, which give rise to an SPR absorption bonds [32], due to the combined vibration of electrons of metal nanoparticles in resonance with the light waves [33, 34]. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles.

The size and shape of SNPs was detected by using AFM (Atomic Force Microscope). Size of SNPs was 41.72 nm, spherical in shape **(Fig-1(d)**). The result obtained in this study is interesting because it can serve as a foundation in terms of identification of potential forest plants for synthesizing SNPs.

The seed extract of *Strychnos nux-vomica* SNPs showed highest percentage of bacterial inhibition zones both gram-positive (*Staphylococcus* (10.4±0.28), *E.coli* (9.8±0.23**)** and *Bacillus* (9.5±0.52) and gram-negative (*Salmonella* (8.9±0.22**)** and (**Table-2**), (**Graph-1**) and (**Fig-3**). the results were compared to that of standard antibacterial antibiotic Gentamycin. The antifungal activity was studied and the results were compared to that of antifungal antibiotic Nystatin. The result showed that *Pencillium rubrum* (9.3±0.55**)** has sensitive followed by *Paecilomyces varioti* (8.3±0.40) and Aspergillus *flavus* (8.1±0.42). The maximum toxicity was observed in SNPs of *Strychnos nux-vomica*use that $Ag(NO_3)_2$. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction.

The results were compared to that of standard antibiotics Gentamycin / Nystatin anti bacterial and antifungal respectively. Standard drugs (Gentamycin / Nystatin), showed higher inhibition zones, because these are highly purified forms which may be cost and leads to side effects in high dosage, whereas the SNPs are biologically synthesized form with less in cost, eco-friendly, safe and pollutant free with less or no side effects.

In general, gram-positive bacteria appeared to be more tolerant to silver than gram-negative cells. The cell wall of gram-positive bacteria contains multiple layers of peptidoglycon compared to the cell wall of gram-negative bacteria. Thus, gram-positive bacteria may allow less Ag^+ to reach the cytoplasmic membrane than gram-negative bacteria [35]. The SNPs are also reported to be nontoxic to human and most effective against bacteria, viruses and other eukaryotic micro-organisms at very low concentrations and without any side effects [36].

The results showed seed extract of *Strychnos nux-vomica* SNPs could be used as an eco-friendly antimicrobial agent in the control of bacterial and fungal diseases. Biologically synthesized SNPs are less in cost, eco-friendly, safe and pollutant free with less or no side effects.

CONCLUSION

The present study includes the treatment of leucoderma using *Strychnos nux-vomica* by the ethnic groups. Phytochemical screening indicates that the plant part is a good source for bio active principle for pharmacognostic and pharmaceutical industries. The SNPs prepared by using the aqueous seed extract of *Strychnos nux-vomica.* The aqueous silver ions exposed to the extracts, the synthesis of SNPs were confirmed by the change of color of plant extracts. These environmentally benign SNPs were further confirmed by using UV-Vis spectroscopy finally the size and shape of the SNPs was characterized by AFM analysis. The results indicated that SNPs have good antimicrobial activity against different microorganisms due to the cumulative effect of secondary metabolites or active molecules present in the plant extract of selected medicinal plant used by ethnic groups of Kurnool district of Andhra Pradesh, India to cure skin diseases. It is confirmed that SNPs of *Strychnos nux-vomica*are capable of rendering antimicrobial efficacy and hence has a great potential in the preparation of drugs used against bacterial and fungal diseases.

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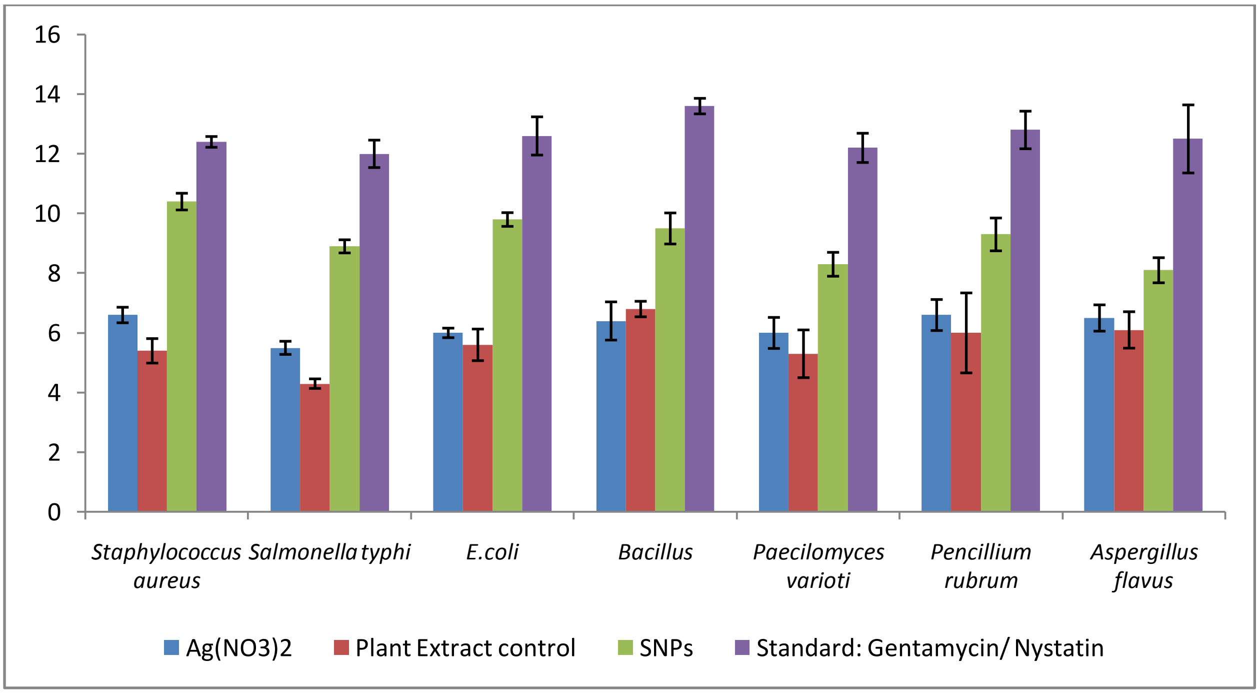
Table-2: Antimicrobial activity of SNPs isolated from seed extract of *Strychnos nux-vomica*

S.No	Microorganisms	Inhibition zone in mm			
		$Ag(NO_3)_2$	Plant Extract control	SNPs	Standard: Gentamycin/ Nystatin
	Bacterial species				
1.	*Staphylococcus aureus*	6.6±0.26	5.4±0.41	10.4±0.28	12.4±0.18
2.	*Salmonella typhi*	5.5±0.22	4.3±0.16	**8.9±0.22**	12±0.46
3.	*E.coli*	6±0.16	5.6±0.53	9.8±0.23	12.6±0.64
4.	*Bacillus*	6.4±0.64	6.8±0.26	9.5±0.52	13.6±0.26
	Fungal species				
5.	*Paecilomyces varioti*	6±0.52	5.3±0.80	8.3±0.40	12.2±0.49
6.	*Pencillium rubrum*	6.6±0.52	6±1.34	9.3±0.55	12.8±0.63
7.	*Aspergillus flavus*	6.5±0.44	6.1±0.61	8.1±0.42	12.5±1.14

Graph-1: Antimicrobial activity of SNPs isolated from seed extract of *Strychnos nux-vomica*



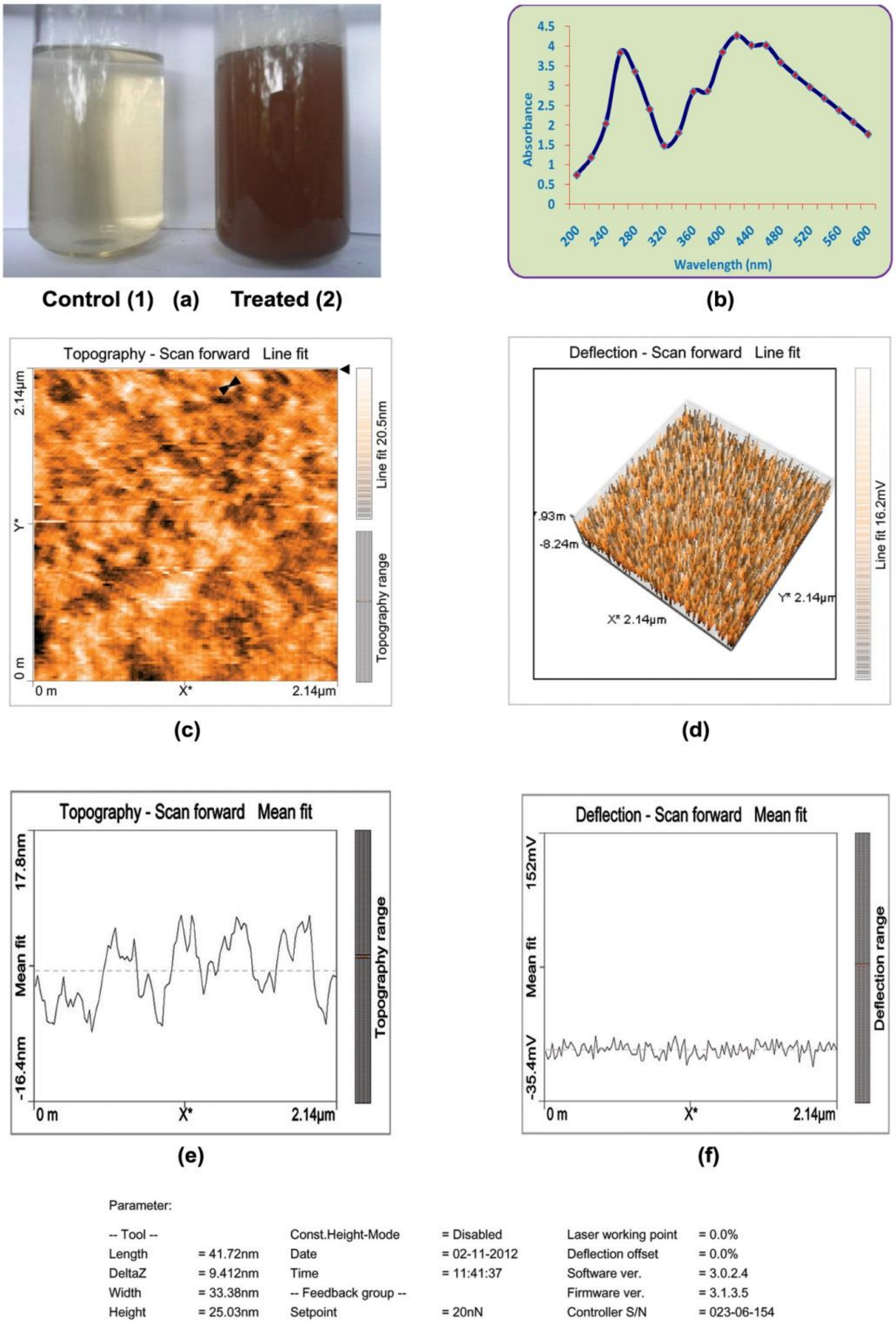


Fig-1: The color change of seed extract of *Strychnos-nux-vomica*

(1) Plant extract without silver nitrate (2) leaf extract with 1 mM silver nitrate; b) UV-Vis spectroscopy of synthesized of silver nanoparticles, (c, e & f) AFM of Topography of SNPs (d) Three dimensional structure of SNPs

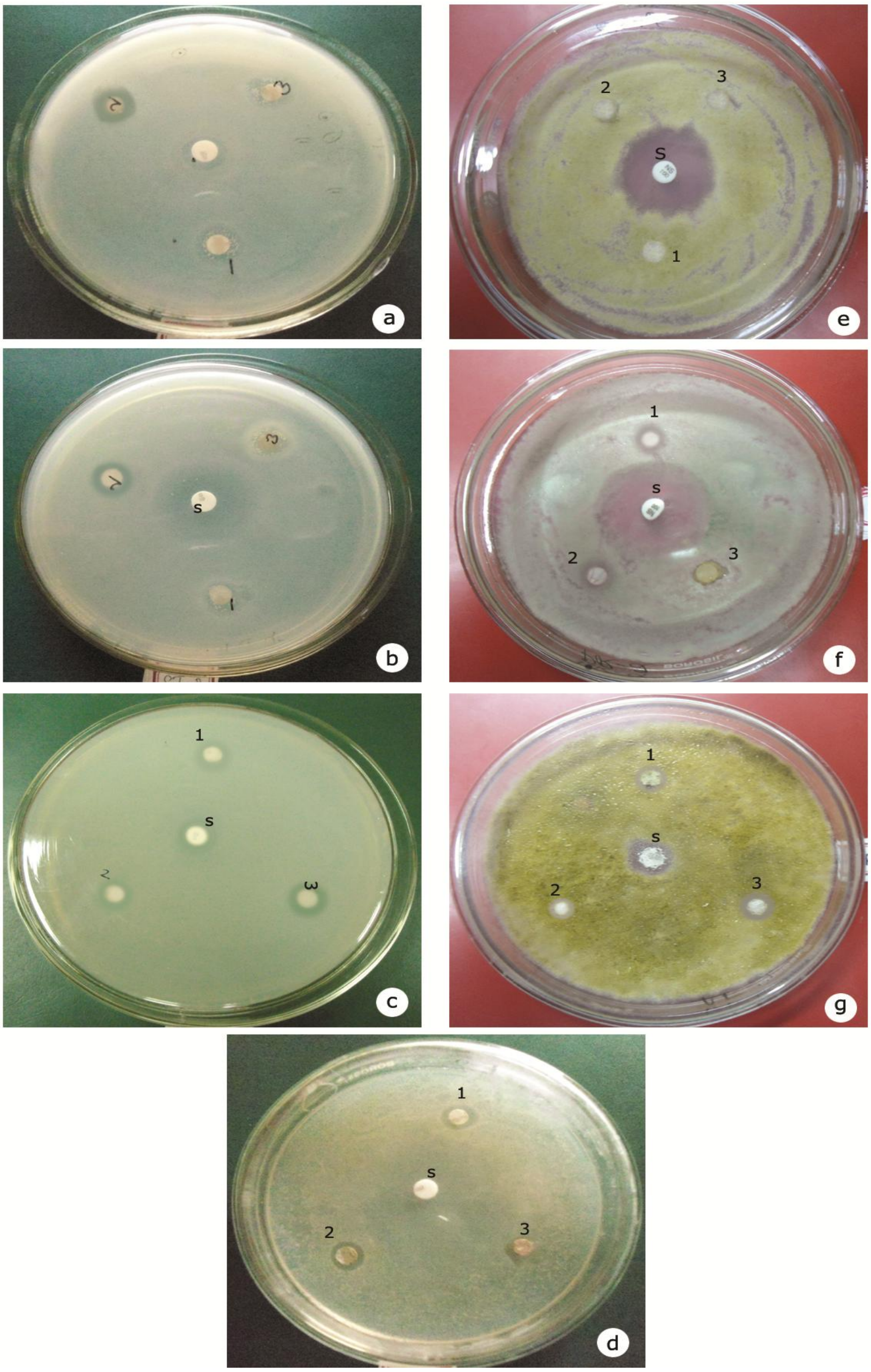


Fig-3: Antimicrobial activity of seed extract of *Strychnos-nux-vomica*

a. *Staphylococcus aureus, b. Salmonella typhi,* c. *Bacillus,* d. *Escherichia coli*

e.*Paecilomyces varioti* , f. *Pencilium rubrum,* g. *Aspergillus flavus*

1-Ag$(NO_3)_2$, 2-plant extract, 3-SNPs, S-Gentamycine/ Nystatin

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