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RESEARCH ARTICLE

Green synthesis of silver nanoparticles and anti microbial activity from *Eclipta alba* – An important Ethno medicinal herb of Kurnool District, Andhra Pradesh, India

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Abstract

The medicinal herb *Eclipta alba* leaves are extensively using by the ethnic groups of Kurnool district, Andhra Pradesh, India to cure leucoderma. Biological synthesis of silver nanoparticles was carried out from leaf aqueous extract of *Eclipta alba* 10 ml leaf extract was mixed to 90 ml of 1 mM aqueous of $\text{Ag}(\text{NO}_3)_2$ and was heated at 60-80°C for 20 min. The color change of aqueous solution into dark brown color. For characterization using UV-Vis spectrophotometer, AFM and TEM. AFM, UV-Vis spectrophotometer and TEM showed the formation of silver nanoparticles with spherical shape and average size 25.02 nm. SNPs have good antimicrobial activity against different microorganisms.

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Introduction

The globe endowed with a rich variety of life forms and the teeming millions of these living organisms been well-knit by the laws of nature. 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 (Venkatasubbaiah *et al.*, 2013). In this connection a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic, Siddha and Unani.

WHO is encouraging the traditional drugs because of its less side effects and matter of low cost and easy availability (WHO, 2000). WHO also has organized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines (Patwardhan *et al.*, 2004).

The medicinal herb *Eclipta alba* (L). Hassk. Known for its medicinal values in alternative systems of (Ayurveda, Unani, Siddha, Homeopathy and Chinese) holistic health and herbal medicine (Neeraja and Margaret, 2012). It is commonly known as Bhringaraj belongs to the family Asteraceae. 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. It has been used as a remedy for skin diseases, good hair tonic, expels intestinal worms and cures asthma.

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 *E. alba* leaves to treat leucoderma. Leucoderma is a skin disorder in human for a number of reasons depigmentation occurs due to auto immune disorder (Lerner and Nordlund, 1986) or lacking of pigments due to absence of melanocytes (Aaron *et al.*, 1986).

E. alba. leaf powder contains a variety of important chemical compounds. The herb consists Wedelolactone and Demethylwedelolactone which processing potent antihepatotoxic property (The wealth of India, 2003). Anti-viral, anti-bacterial, anti-oxidant, anti-cancer and hair growth activity (Neeraja and Margaret, 2012).

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 (Mohanpuria *et al.*, 2008). Silver a noble metal is known to improve the immunity since ancient times, $\text{Ag}(\text{NO}_3)_2$ was using for

biosynthesis of nanoparticles by using leaf aqueous extract of *E. alba*. The possibility of using plant materials for the synthesis of nanoscale metals was initially reported by Gradea-Torresdey *et al.*, 2002 and 2003. SNPs have particular properties that may perhaps have numerous applications in the field of dentistry, clothing, catalysis, mirrors, optics, phytochemistry, electronics and food industry (Rai *et al.*, 2009).

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* (Patil *et al.*, 2011).

In this study, ethno-medico botanical studies, qualitative analysis of phytochemical constituents by using leaf extract of *E. alba* and biological synthesis of SNPs and screening of SNPs for microbial efficacy.

Material and Methods

Plant material

The fresh leaves of *E. alba* was collected in July 2012 from Nagaloty reserve Forest, Kurnool District of Andhra Pradesh, India. The leaves were cleaned, finally distilled water dried at room temperature and ground to fine powder.

Preparation of extract

25 g of leaf powder of *E. alba* was taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 min at 100°C 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 leaf extract was used for preliminary phytochemical screening. The qualitative analysis of secondary metabolites was carried out by using the methods (Peach and Tracey, 1956) for flavonoids, (Gibbs, 1974) for steroids, alkaloids and phenols, (Ayoola *et al.*, 2008) for triterpenoids and glycosides, (Treare and Evans, 1982) for tannins, anthraquinones, leucoanthocyanins and emodins, (Kumar *et al.*, 2009) for saponins, (Paris and Moyses, 1969) 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 AgNO₃ 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 leaf aqueous extract of *E. alba*. The reduction of pure Ag²⁺ ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5th 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), and TEM.

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 (Systronic 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).

TEM analysis

TEM observations were carried out on a H-7100 electron microscope (Hitachi, Tokyo, Japan), the particle distribution were determined using the UTHSCSA image Tool version 3.00 programme.

Microorganisms

Pure cultures of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* species of bacteria and *Paecilomyces varioti*, *Penicillium rubrum* and *Aspergillus 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. Leaves along with the seeds of *Foeniculum vulgare* L. (1:1 ratio) are boiled in coconut oil (100 ml) and the oil extract applied daily in the morning hours for a week to treat the leucoderma infection. This ethno medicinal information was cross checked with Ayurvedic physicians, Sri Venkateswara Ayurvedic college, Tirupati, Andhra Pradesh, India for authentication. In this regard *E. alba* leaves are extensively using in Ayurvedic medicines to treat leucoderma.

The secondary metabolites screening of *E. alba*. Showed that the leaf extract is rich in alkaloids, coumarins, flavonoids, lignins, saponins, steroids, tannins and triterpenoids and lacking of anthocyanins, anthraquinones, emodins, leucoanthocyanins glycosides, phenols and reducing sugars (**Table-1**).

Table-1: Secondary metabolites of leaf extract of *Eclipta alba*

S. No.	Secondary metabolites	leaf 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 antistomachic, diarrhoea and anti-inflammatory, anti cancer and anti oxidative. It also known to possess antiviral and anti fungal (Fairbarin, 1959 and Tripathi and Rastogi, 1981) and antimicrobial properties (Takahasi *et al.*, 2004). The presence of bioactive compounds indicates the medicinal values of the plant. Steroids and triterpenoids possess anti bacterial activity (Reddy *et al.*, 2003). Tannins possess astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial activities (Killender and More, 2010). Similar chemical constituents were also found in *Shorea tumbuagaia* (Ankanna and Savithramma, 2011), *Thespesia populnea* (Bhumi *et al.*, 2013), in *Curcuma longa* (Venkatasubbaiah *et al.*, 2013) and in *Psoralea corylifolia* (Venkatasubbaiah and Savithramma, 2013) *Holarrhena Pubescens* (Venkatasubbaiah and Savithramma, 2013).

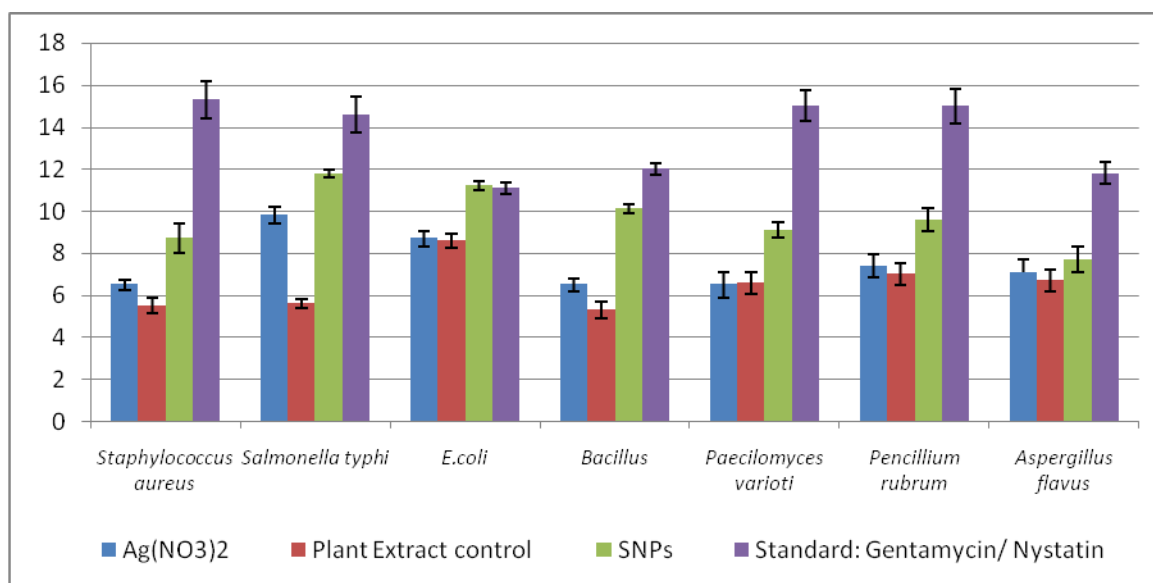
In the present study SNPs were synthesized by using leaf aqueous extract of *E. alba* rapidly with in 10 min was able to be followed by color change. The fresh suspension of *E. alba* was yellow in color. However, after adding of Ag (NO₃)₂ the sample turned to dark brown color. The color change in aqueous solution is due to the surface-Plasmon resonance (SPR) phenomena (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 leaf

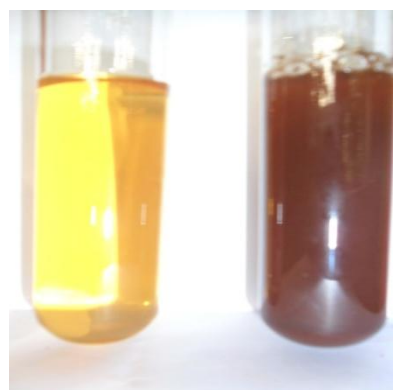
extract *E. alba*. have the characteristic absorbance peaks at 240 and 380 nm respectively (**Fig-1(b)**). This illustrated the presence of homogenous distribution of hydrosol SNPs after 10 min of stirring (Shameli *et al.*, 2012).

Table-2: Antimicrobial activity of SNPs isolated from leaf extract of *Eclipta alba*

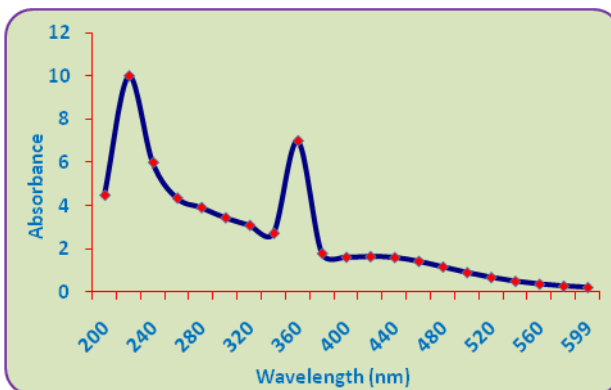
S.No	Microorganisms	Inhibition zone in mm			
		Ag(NO ₃) ₂	Plant Extract control	SNPs	Standard: Gentamycin/ Nystatin
Bacterial species					
1.	<i>Staphylococcus aureus</i>	6.5±0.23	5.5±0.35	8.70±0.70	15.3±0.86
2.	<i>Salmonella typhi</i>	9.8±0.41	5.6±0.20	11.8±0.18	14.6±0.86
3.	<i>E.coli</i>	8.7±0.36	8.6±0.33	11.2±0.22	11.1±0.26
4.	<i>Bacillus</i>	6.5±0.29	5.3±0.37	10.1±0.22	12±0.28
Fungal species					
5.	<i>Paecilomyces varioti</i>	6.5±0.61	6.6±0.51	9.1±0.38	15±0.74
6.	<i>Pencillium rubrum</i>	7.4±0.55	7±0.52	9.6±0.55	15±0.81
7.	<i>Aspergillus flavus</i>	7.1±0.61	6.7±0.52	7.7±0.61	11.8±0.51

Graph-1: Antimicrobial activity of SNPs isolated from leaf extract of *Eclipta alba*

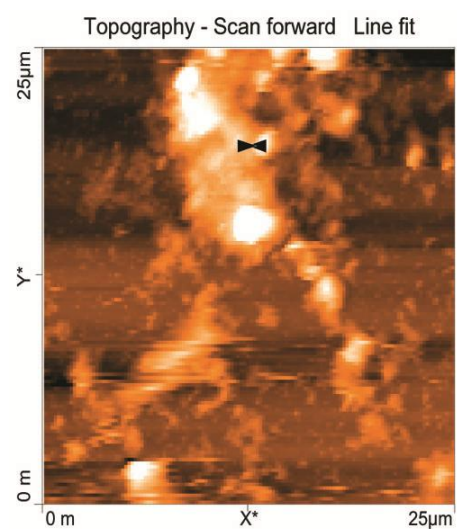




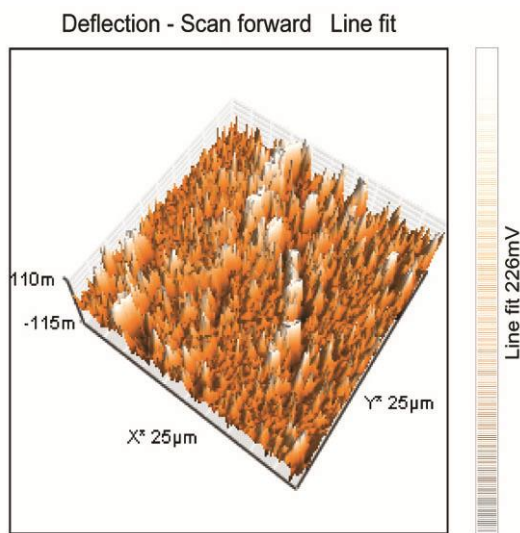
Control (1) (a) Treated (2)



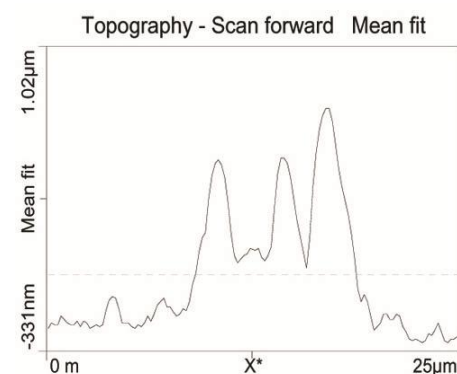
(b)



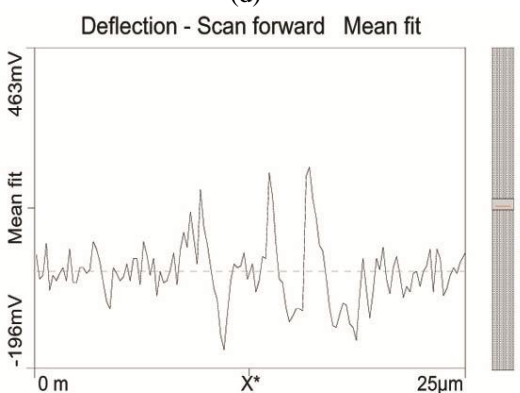
(c)



(d)



(e)



(f)

Parameter:

-- Scan group --

Length = 25 nm

Scan direction = Up

-- Feedback group --

Setpoint = 20nN

P-Gain = 10000

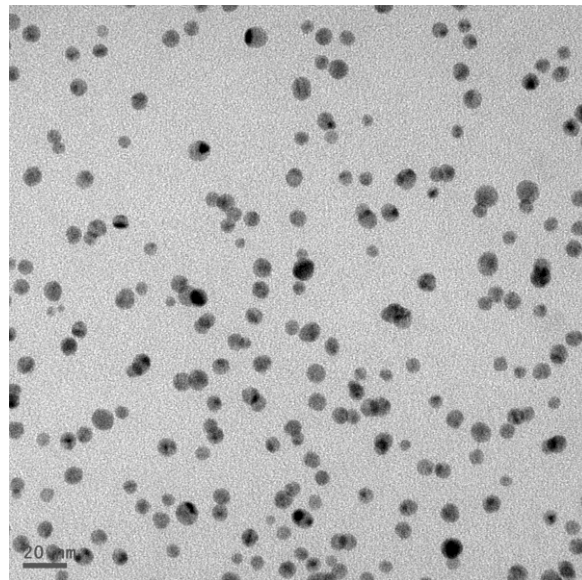
Deflection offset = 0.0%

Software ver. = 3.0.2.4

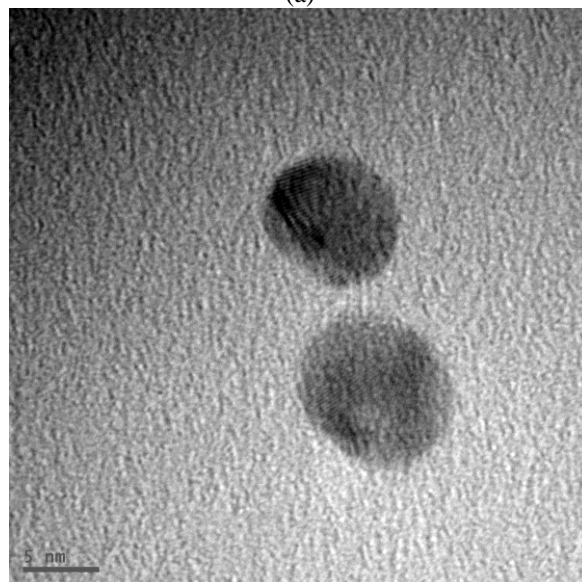
Firmware ver. = 3.1.3.5

Fig-1: The color change of leaf extract of *Eclipta alba*

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



(a)



(b)

Fig-2: TEM image of Topography of SNPs of *Elcipta alba*
(a) 20 nm, (b) 5 nm

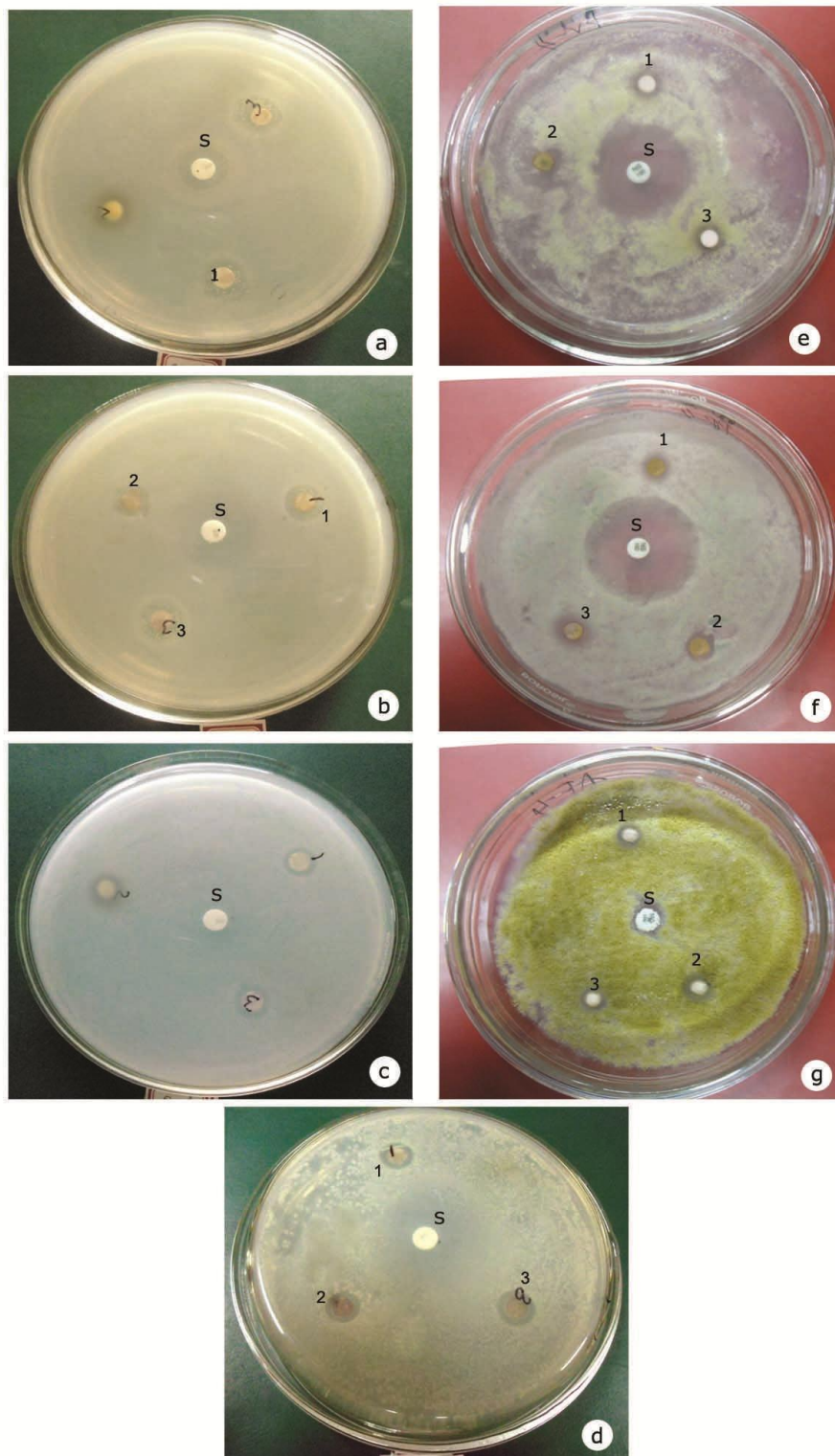


Fig-3: Antimicrobial activity of leaf extract of *Eclipta alba*

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

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

1-Ag(NO₃)₂, 2-plant extract, 3-SNPs, S-Gentamycine/ Nystatin

It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape of nanoparticles in aqueous suspension (Wiley *et al.*, 2006). 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 bark (Ankanna *et al.*, 2010) Silver nanoparticles have free electrons, which give rise to an SPR absorption bands (Noginov., 2006), due to the combined vibration of electrons of metal nanoparticles in resonance with the light waves (Nath *et al.*, 2007 and Dubey *et al.*, 2009). 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 25.01 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. TEM in **Fig-2 (a&b)** indicates the size of SNPs was 20 nm and 5 nm respectively.

The leaf extract of *E. alba* SNPs showed highest percentage of bacterial inhibition zones both gram-positive {(*Staphylococcus* (8.7±0.70) and *Bacillus* (10.1±0.22))} and gram-negative {(*Salmonella* (11.8±0.18) and *E.coli* (11.2±0.22))}, (**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* (9.6±0.55) has sensitive followed by *Paecilomyces varioti* (9.1±0.38) and *Aspergillus flavus* (7.7±0.61). The maximum toxicity was observed in SNPs of *E.alba* use that Ag(NO₃)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 (Kawahara *et al.*, 2000).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 Jeong *et al*, 2005.

The results showed leaf extract of *E.alba*. 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 *E. alba* 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 leaf extract of *E. alba*. 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 and TEM 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 *E. alba* 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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