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ORIGINAL ARTICLE

An *In Vitro* Evaluation of Cyanobacterial Extract and its Mediated Synthesized Silver Nanoparticles as a Topical Gel for Candidal vaginitis Control

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ABSTRACT

Candidiasis, a yeast infection, results from Candida overgrowth, affecting the vagina. Approximately 75% of women experience vulvovaginal candidiasis infection at least once in their lifetime. This research addresses candidal vulvovaginitis through a comprehensive approach. Candida albicans pathogens are meticulously collected from diagnosed patients, and metabolites are extracted from Synechococcus spp. Simultaneously, silver nanoparticles are synthesized from the same algae. These materials undergo thorough evaluation for their anticandidal activity using standardized assays, and a topical gel is formulated with the synthesized silver nanoparticles. The research adheres to ethical guidelines, obtains necessary approvals, and integrates safety measures. In parallel, cyanobacterial cultures are explored for bioactive compound production. Ethyl acetate extraction yields a bioactive compound, and silver nanoparticles (AgNPs) synthesized from algae are characterized. AgNPs, inhibiting germ tube formation, outperform the compound in antifungal plate assays. Selected for a topical gel, AgNPs exhibit superior anticandidal activity over a commercially available gel (ketoconazole). Characterization techniques, including UV-Vis spectroscopy, FTIR analysis, XRD results, and EDS, provide insights into the properties of the synthesized AgNPs. Results indicate the potential of the AgNPs-mediated gel as an alternative treatment for candidal vulvovaginitis, presenting a promising avenue for further research and clinical application in fungal infection management. These findings contribute valuable insights to the scientific community's understanding of potential therapeutic interventions for Candidal vulvovaginitis. The multifaceted approach, integrating advanced techniques, ethical considerations, and safety measures, aligns with the broader objective of advancing knowledge and developing innovative approaches to combat fungal infections.

Keywords: *Candidal vulvovaginitis; Candida albicans; Synechococcus spp.; Silver nanoparticles; Anticandidal activity; Topical gel; Bioactive compounds; Fungal infection management*

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INTRODUCTION

Candidiasis is an infection caused by a yeast (a type of fungus) called Candida. Candida normally lives inside the body (in places such as the mouth, throat, gut, and vagina) and on skin without causing any problems. Sometimes Candida can multiply and cause an infection if the environment inside the vagina changes in a way that encourages its growth. Candidiasis in the vagina is commonly called a "vaginal yeast infection." Other names for this infection are "vaginal candidiasis," "vulvovaginal candidiasis," or "candidal vaginitis" (1). Vulvovaginal candidiasis (VVC) is a disease caused by abnormal growth of yeast-like fungi in the

mucosa of the female genital tract, classified by the World Health Organization as a sexually transmitted disease of frequent sexual transmission (2).Vulvovaginal candidiasis infection affects 75% of women at least once in their lifetime (3). Vaginitis is an inflammation of the vagina. Vaginitis affects women of all ages but is most common during the reproductive years. A change in the balance of the yeast and bacteria that normally live in the vagina can result in vaginitis. This causes the lining of the vagina to become inflamed. Vulvovaginal candidiasis is a common problem. The majority of infections are caused by *Candida albicans*, but there is increased awareness of the role of yeasts other than *C. albicans*. It is important to identify these other yeasts because they tend to be less susceptible to the commonly used topical and oral azole antifungals and are associated more frequently with recurrent infection than *C. albicans* (4). Vaginitis is characterize by vaginal symptoms, including discharge, odor, itching, irritation, or burning (**Figure 1**). Most women have at least one episode of vaginitis during their lives, making it the most common gynaecologic diagnosis in primary care. Studies have shown a negative effect on quality of life in women with vaginitis (5).

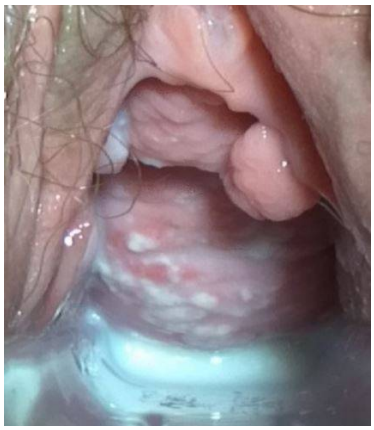

Figure 1: Candida vaginitis infection in female genital

In recent research interest metal nanoparticles have been synthesized extensively for a variety of applications and gaining enormous research attention in various areas such as chemistry, physics, life science, material science, medical science, nanomedicine and engineering due to size and shape tune able properties (6). Nanoparticles possess unique optical, magnetic, electronic and catalytic properties with their distinctive feature of size and shape (7-9). Biological methods for synthesizing nanoparticles have shown better results compared to chemical and physical techniques due to time consumption, low cost and most importantly it is more eco-friendly compared to the chemical and physical techniques (10,11). Nanoparticles of metals are the most potential agents as they show excellent antibacterial activities due to their large surface area-to-volume ratio, which is getting up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the growth of resistant strains (12). Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine (13). In the biosynthesis of nanoparticles, biological organisms like bacteria, fungi, actinomycetes, yeast, algae and plants were utilized as reducing agent or protective agents (14, 15). Algae used in the biosynthesis of nanoparticles have recently attracted the attention of many scientific researches due to their rapid growth, to their biomass formation and to their different biological activities by nature Algae are also used as “bio-factories” for synthesis of metallic nanoparticles. Green synthesis of silver nanoparticles by algae extract shows more advantageous over other biological processes are bacteria and fungi, because it eliminates the cell culture maintaining process, and also it more suitable for large scale production of silver nanoparticles. Cyanobacteria are excellent bio-systems for the intracellular or extracellular production of NPs. However, the bio fabrication of metallic NPs using cyanobacteria has not been well characterized. This research initiative encompasses a comprehensive investigation into addressing candidal vulvovaginitis through a multi-faceted approach involving *Candida albicans* and *Synechococcus* spp. The first phase involves the collection of *Candida albicans* from individuals diagnosed with candidal vulvovaginitis. Subsequently, metabolites will be extracted from *Synechococcus* spp. through carefully controlled cultivation and extraction procedures. These extracted metabolites will be employed in the synthesis of silver nanoparticles from *Synechococcus* spp., utilizing a green synthesis approach. The next critical step entails a comparative assessment of the metabolic extract and silver nanoparticles against *Candida albicans*, evaluating their anticandidal activity through rigorous in vitro experiments and associated assays. Following the determination of efficacy, the synthesized silver nanoparticles will be incorporated into a topical gel, with the gel's composition optimized for stability and efficient delivery. The final stage of this research involves the application of the formulated gel in experimental setups to analyze

its anticandidal activity against *Candida albicans*, employing diverse assays for a comprehensive evaluation. Throughout these processes, strict adherence to ethical guidelines, safety protocols, and meticulous documentation will be maintained. This research aims to contribute valuable insights into potential treatments for candidal vulvovaginitis, laying the groundwork for the development of innovative therapeutic interventions.

MATERIAL AND METHODS

Sample collection

Candida albicans were collected from the Candidal vulvovaginitis patients of Arupadai Veedu Medical College and Hospital, Puducherry, India and the cultures were maintained by department of Microbiology Alagappa university, Karaikudi, Tamilnadu, India. Cyanobacteria were collected from the National Facility for Marine Cyanobacteria (NFMC) from Tiruchirappalli, Tamilnadu, India.

Cultivation of Cyanobacteria

Cyanobacteria cultivated in ASN III medium and the temperature was maintained at 27±2°C for 30 days 16:8 h light: dark cycle in nutrient medium.

Preparation of Cyanobacterial Extract

The dried powdered biomass was extracted with ethyl acetate by freeze thaw method. The cultivated algae were collected after it attain complete growth then the mixture was centrifuged at 10,000 rpm for 15 minutes at 25-30°C. The supernatants were collected after centrifugation the equal volume of supernatant that contain bioactive compound and ethyl acetate was added mixed vigorously in order to separate the bioactive compound in the organic phase after that collect the organic phase extract it with rotary evaporator the collected crude extract was then lyophilized and dissolved in deionized water to make the desired concentration.

Preparation of AgNPs extracts

Shade-dried algal biomass is boiled with distilled water, filtered, and centrifuged to obtain an extract used as a reducing agent. Mixing 5 ml of this extract with 45 ml of 1 mM AgNO3 forms silver nanoparticles, monitored by color change and UV-visible spectrum. Controls validate the process. After saturation, nanoparticles are isolated by centrifugation, washed thrice with deionized water to remove impurities, and dried at 55°C for 5 hours. This method offers a green synthesis route for silver nanoparticles using algal biomass as a reducing agent, with precise control and efficient isolation of nanoparticles for various applications.

Characterization of synthesized silver nanoparticles

The UV-Visible spectroscopic analysis was performed by UV-Vis spectrophotometer taking a spectra from 200–800 nm for each sample. Silver nanoparticles were characterized by FTIR in the range of 4000–450 cm−1 using dried powder of silver nanoparticles. Samples for analysis was prepared at ambient conditions and mixed with KBr. X-ray diffraction. Elemental analysis was examined by Energy Dispersive x-ray analysis performed using Tescan VEGA 3SBH with Bruker easy EDS.

Antifungal plate assays (well diffusion method)

Antifungal activity was assessed using the agar well diffusion test. Muller Hinton agar (MHA)/potato dextrose agar was prepared and poured into petri dishes, then inoculated with test microorganisms. Wells were made in the agar, and different concentrations (25-125 µg/ml) of Synechococcal algal extract and silver nanoparticles from algal biomass were added. Plates were incubated at 37°C for 24 hours. Inhibition zones around wells indicated antifungal activity. Controls lacked nanoparticles or ethyl acetate. Results represent the average of triplicate analyses, providing insight into the effectiveness of algal-derived substances against fungal pathogens.

Anticandidal activity conformation

Broth micro dilution assay will be used to confirm the anticandidal activity. The ethyl acetate extract and AgNPS from *Synechococcus* spp., will be dissolved in dimethyl sulfoxide. The concentration ranged will be varied from 25-125 micro litre/ml. The sabouraud dextrose broth/agar will be added and washed cell suspension of *candida* species to tube. Further, tube will be incubated at room temperature in shaker at 150 rpm for 48 hrs.

Biomass will be separated by centrifugation for 5 min at 5000 rpm at 37°C. The pellet will be resuspended of broth and incubated at room temperature. Inhibition activity of extracts and synthesized AgNPs from Cyanobacteria will be recorded.

Germ tube inhibition assay

A germ tube is an outgrowth produced by spores of spore- releasing fungi during germination. The germ tube differentiate, grows and develops by mitosis to create somatic hyphae. A germ tube test is a diagnostic test in which a sample of fungal spores are suspended in animal serum and examined by microscopy for the detection of any germ tube. Dried extracts and synthesized AgNPs from Cyanobacteria will be taken in the test tube and it will be resuspended in DMSO. *Candida* culture is inoculated in the human/sheep serum or

Bovin serum and incubated for three hrs at room temperature. The growth of germ tube will be observed under the microscope . Control tubes were maintained similarly without extract treatment and observed for comparison of germ tube formation

Formulation of topical gel: Polymer (like Carbopol 934p or HPMC) and purified water were taken in a beaker and allowed to soak for 24 h. To this required amount of drug (2 gm) was dispersed in water and then Carbopol 934p or HPMC was then neutralized with sufficient quantity of Triethanolamine. Glycerine as a moistening agent, methylparaben and Propylparaben as preservatives was added slowly with continuous gently stirring until the homogenous gel was formed. The evaluation process for the topical gel commenced with the determination of the percentage yield by weighing the container before and after filling it with the gel formulation, providing insights into the practical yield achieved. Drug content analysis involved transferring 10 grams of the gel into a 250 ml volumetric flask with 20 ml of alcohol, stirring, and adjusting the volume to 100 ml for subsequent spectrophotometric measurement at 260 nm to ascertain the concentration of the active ingredient. pH determination was carried out by measuring the pH of 50 grams of gel in a beaker to ensure compatibility with skin infections. Spreadability assessment involved measuring the diameter of 1 gram of gel between horizontal plates after 1 minute, with a standardized weight tied to the upper plate. Extrudability was tested by filling collapsible tubes with the gel and assessing its ease of dispensation. Viscosity estimation, crucial for application and stability, was conducted using a Brookfield viscometer DVII model with a T-Bar spindle and helipath stand.

Activity of the prepared topical gel against the C*andida albicans*

After preparing topical gel the gel is analysed for the anticandidal activity by agar well method. In agar well method the test pathogens were swabbed in the Muller Hinton agar plates and then two wells were created in the plate using well puncture and the both commercially available topical ointment and prepared ointment were filled in the well and these plates were incubated at 25ºC for 24hours and after incubation the plates were observed for zone of inhibition.

RESULTS

Characterization

UV–visible absorption spectrometer

Synechococcus spp.*,* it is well known that Ag nanoparticles exhibit light yellowish to brown color (**Figure 2**). The bio fabrication of silver nanoparticles could be ascertained using a visual marker represented by chromatic change of the reaction substrate, since the color transition from green to brown imply the biotransformation of Ag + ions to Ag synthesis silver nanoparticles. **Figure 3** illustrate the formation of *Synechococcus* spp., AgNPs surface plasmon resonance (SPR) peak at 400nm with intensity of 2.6 nm at the addition of 1mM $AgNo_3$ to *Synechococcus* spp., aqueous extract. At lower concentrations, the SPR band is broad and it is due to large anisotropic particles. A smooth and narrow absorption band at 400 nm is observed which is characteristic of almost spherical nanoparticles. Nanometal showed conspicuous spectral characteristic according to the SPR due to mutual vibrations of the free electrons resonance with light wave which influenced by each of size and shape of the synthesized NPs.

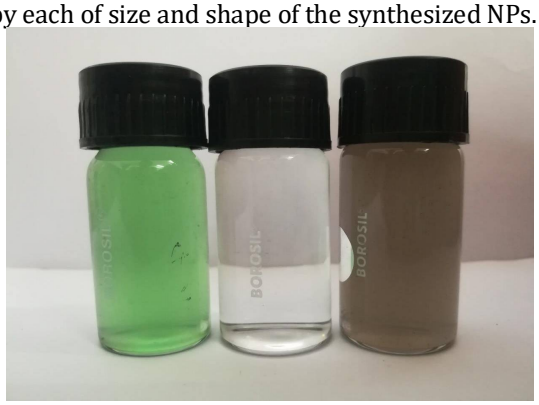

Figure 2. AgNPs changes color from green to brown after the addition of silver nitrate solution

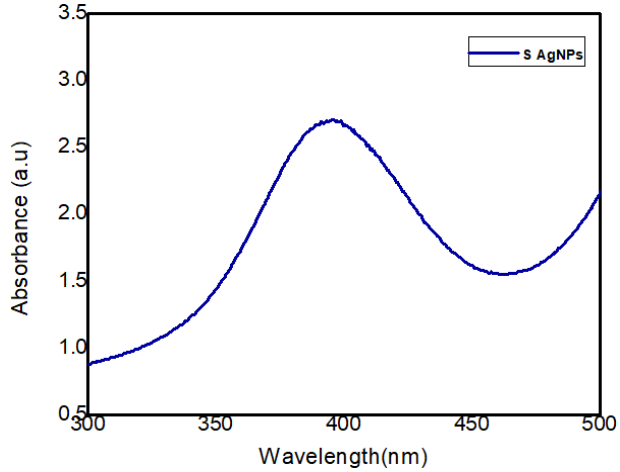

Figure 3. UV – visible spectra of Aqueous AgNO3 with *Synechococcus* spp., extract

XRD

Furthermore, evidence for biosynthesis of silver nanoparticles and the development of single phase compound and the crystalline structure of the silver nanoparticles was confirmed by X-ray diffraction (XRD) method. The XRD pattern of synthesized AgNPs was observed and compared with the standard powder diffraction card of (JCPDS). The green synthesized silver nanostructure by employing algae extract of *Synechococcus* spp., and its intense diffraction peaks due to AgNPs are clearly observed at **figure 4.** These peaks are represent to the (111), (200), (220), (311) plans of pure AgNPs respectively. X-ray diffraction confirmed that Face centre cubic of green synthesized NPs. In addition, the acquired reflections are sharp with good intensity which confirms that the structures of synthesized nanoparticles as well as crystalline.

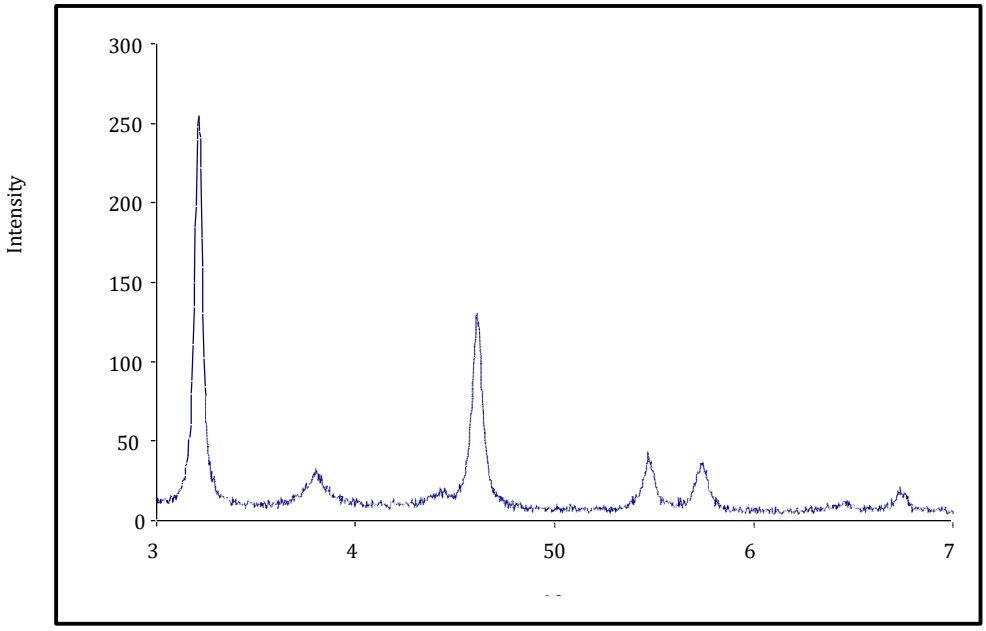

Figure 4. XRD analysis of *Synechococcal* AgNPs

FTIR

FTIR spectra were recorded for *Synechococcus* spp., extract and synthesized AgNPs nanoparticles to identify the possible biomolecules responsible for the reduction of AgNO3 into AgNPs. FT-IR spectrum of *Synechococcus* spp., extract shows different major peaks positioned at 3843,3679, 3436,2923,1653,1546,1384,1107,621cm^{-1}(**figure 5**). The peaks 3843 and 3679 shows the presence of -OH group alcohol, peaks on 3436 shows –OH Stretching, peak on 2923 shows the presence of Stretching vibration of C-H, 1653 shows – C=O Stretching and the peaks on 1384,1107 and 621 shows the presence of-CH3 bending, C- X(F) and –C-Br respectively.

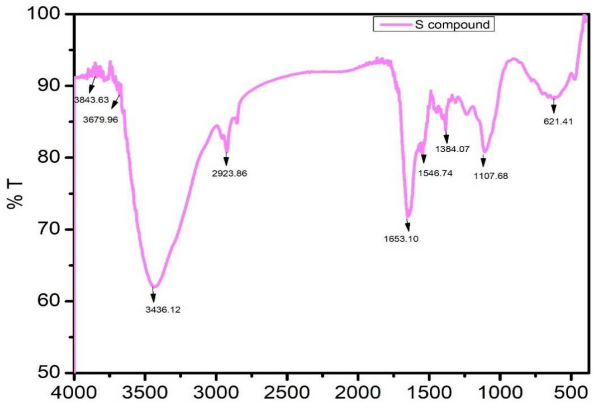

Figure 5. FTIR spectra of compound from *Synechococcus* spp.

The FT-IR spectrum of *Synechococcal* AgNPs nanoparticles shows different major peaks positioned at 3678, 3429, 2922, 2852, 1653, 1545, 1384, 1105, 621, 473 cm^{-1}(**figure 6**). The peaks on 3679 shows the presence of OH alcohol group in it, peak on 3429 shows OH stretching, 2852 shows the Stretching vibration of CH2, peak on 1653 shows –C=O Stretching, 1545 shows –C=C aromatic ring, peak on 1384 shows the -CH3 bending, 1105 shows the presence of C-X(F) and the peaks on 621 and 473 shows the presence of –C-Br and –C-I bonds respectively. The absorbance bands were due to the vibration effect of potential biomolecules.

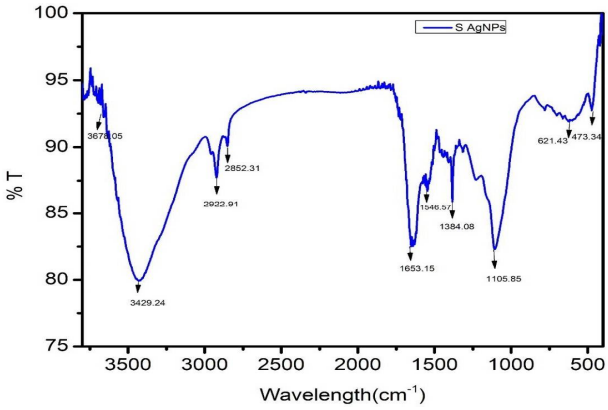

Figure 6. FTIR spectra of AgNPs synthesized from *Synechococcus spp.*,

EDS

The energy dispersive x-ray spectrum of AgNPs affirmed the incidence of sharp spectral signal in the silver region which was approximately in a range of 0.2- 3Kev for the absorption of nanocrystalite. The silver particle occurrence in EDS was higher (2.3 Kev) with the trace of metal namely silver and chlorine (**Figure 7**). EDS analysis strongly declared the presence of elemental silver signals.

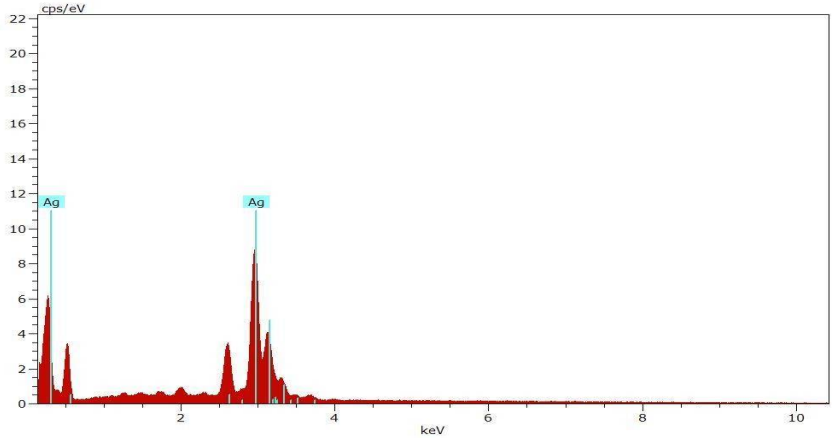

Figure 7: Energy dispersive analysis of AgNPs from *Synechococcus* spp.

Antigerm tube test

4 isolate from the vulvovaginitis patients were collected and out of 4 , three candida sps., showed germ tube positive (**figure 8(a)**)when grown in foetal bovine serum. These were confirmed as candida albicans. This is one of the most rapid, simple and effective way to screen yeast mold shift inhibitor is germ tube inhibitory study. In the present study we identified that *Synechococcus* spp., extract and algae mediated AgNPs completely inhibits germ tube formation in *C. albicans* until the end of incubation period. Both the compound and AgNPs were completely inhibit the germ tube formation (figure 8(b)) hence can be used in prevention of invasive infections.

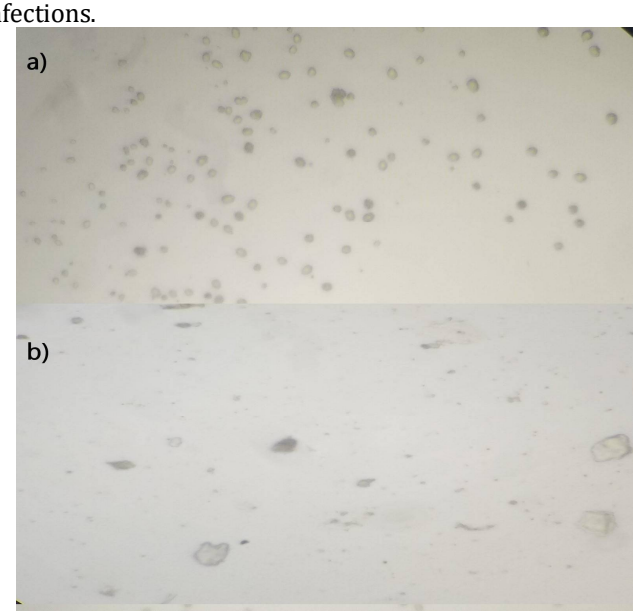

***Figure 8 a) germ tube formation by candida albicans b) germ tube inhibition by the Synechococcus spp.,* mediated synthesis AgNPs**

Antifungal plate assay

In the present study, the antifungal activity of green synthesized silver nanoparticles were tested against test fungal pathogens with various concentrations (25, 50, 75,100,125 µg/ml) and the results are shown in **Table 1&2** and **Figure 9&10**. The results of antifungal activity with a zone of inhibition. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. When silver nanoparticles enter the bacterial cell, it forms a low molecular weight region in the centre of the bacteria to which the bacteria conglomerates thus protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain cell division finally leading to cell death.

Table: 1 Anticandidal plate assay activity (Zone of Inhibition) of *Synechococcus* spp., AgNPs against *Candida albicans* and *Candida tropicalis*

Bacterium name	Zone of Inhibition of *Synechococcus* spp., AgNPs (mm)				
	25µg/ml	50µg/ml	75 µg/ml	100µg/ml	125 µg/ml
***Candida albicans* AMB C1**	8	10 ±0.3	13 ±0.3	15 ±0.3	17 ±0.1
***Candida albicans* AMB C2**	13 ±0.1	15 ±0.2	16 ±0.2	18 ±0.3	20 ±0.2
***Candida albicans* AMB C3**	8 ±0.2	9 ±0.2	10 ±0.5	15 ±0.2	18 ±0.1
Candida tropicalis	5±0.2	8±0.1	10±0.2	13±0.3	13±0.1

Table: 2 Anticandidal plate assay activity (Zone of Inhibition) of compounds from *Synechococcus* spp., against *Candida albicans* and *Candida tropicalis*

Bacterium name	Zone of Inhibition of Compound from *Synechococcus* spp., (mm)				
	25µg/ml	50µg/ml	75 µg/ml	100µg/ml	125 µg/ml
***Candida albicans* AMB C1**	7	8 ±0.3	12 ±0.3	13 ±0.3	15 ±0.1
***Candida albicans* AMB C2**	5 ±0.1	7 ±0.1	8 ±0.2	15 ±0.1	16 ±0.2
***Candida albicans* AMB C3**	8 ±0.3	10 ±0.1	12 ±0.2	13 ±0.2	15 ±0.1
Candida tropicalis	10±0.2	13±0.1	15±0.2	16±0.1	18 ±0.3

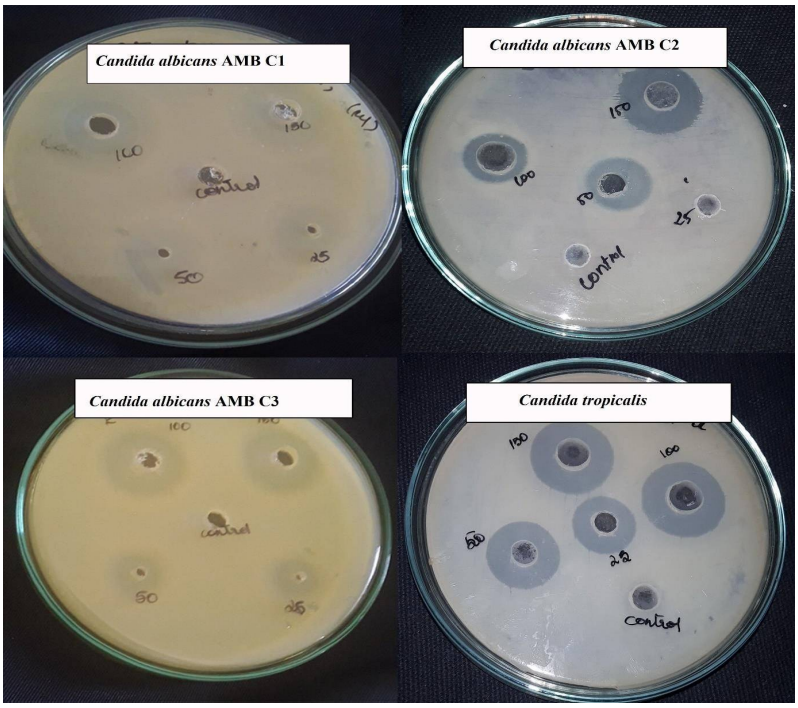

Figure 9. Zone of Inhibition observed (mm) with different concentration (25, 50, 75, 100, 125 µg/ml) of bioactive compound from *Synechococcus* spp.,

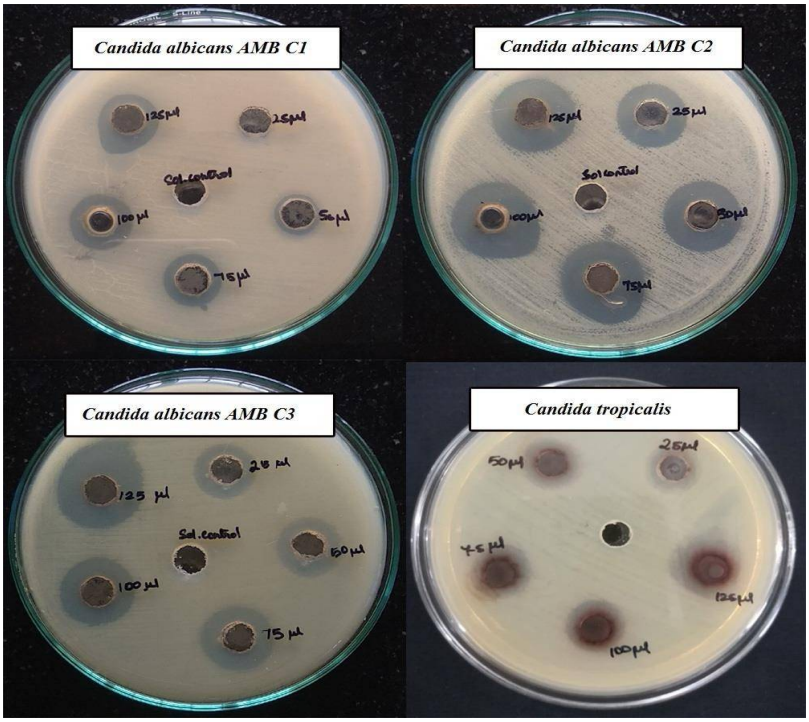

Figure 10. Zone of Inhibition observed (mm) with different concentration (25, 50, 75, 100, 125 µg/ml) of AgNPs from *Synechococcus* spp.,

Anticandidal activity

The result for the extract derived from *Synechococcal*'s and AgNPs synthesized from algal biomass for its antifungal activity against *C. albicans* strains were determined using the MIC in micro dilutions. The MIC of *Synechococcus* spp., extract was 60 µg/ml and Minimum inhibitory concentration of *Synechococcus* spp., AgNPs nanoparticles 80 µg/ml, inhibiting the growth of all tested fungal strains and complete growth was observed the in control medium containing only test fungi (without drug).

Evaluation of topical gel

As algae mediated synthesized AgNPs showed good results than the bioactive compound so that the AgNPs was chosen. Topical gel is prepared with the algae synthesized AgNPs nanoparticles because it showed promising and better activity than the bioactive compound produced by the algae, since silver itself had its antimicrobial property so AgNPs from algae enhanced its activity from the bioactive compound produced by the algae. And gel is prepared by mixing Carbopol 934p with the 2 gram of AgNPs (drug) with the sufficient quantity Triethanolamine along with the glycerine (moistening agent) and methylparaben as preservative. The properties of gel such as pH, Spreadability, extrudability, viscosity and its percentage yield were evaluated and presented. The formulation shows a viscous gel appearance it is homogenous, with a brown color, and has the characteristic AgNPs odour. The formulation remained homogeneous without precipitate formation, coalescence. The pH initially presented values around 3.0 and were corrected to 4.8 by addition of sodium hydroxide within the pH range ideal for Vaginal use (4.5-4.0) viscosity were observed as 535,000 cps. Spreadability is an important property of local formulation to assure uniform application, dosage transfer and therapeutic efficacy. The diameter (6.26±0.55 g.cm/sec) found in the test to formulation are indicative of good Spreadability, being the formulation with glycerine better. The formulation showed good extrudability.

Activity of the prepared topical gel against the *Candida albicans*

Before the gel formulation development, the *Synechococcus* spp., mediated synthesized AgNPs was submitted to antifungal test to evaluate its capacity to inhibit the growth of yeast in the Candida genus, which is responsible for Vaginal Candidiasis. The sample showed activity, and the Candida albicans and Candida tropicalis strain were the most sensitive to AgNPs. Prepared topical gel from synthesized AgNPs were analysed for its activity by agar plate assay, where the prepared gel and commercial antifungal agent (ketoconazole) were compared for its potential activity against the test pathogens. The result of antifungal activity with the zone of inhibition was shown in Table 5 and Figure 11.

Table: 3 Comparative analysis of zone of Inhibition of topical gel from *Synechococcus* spp., AgNPs and commercially available topical anticandidal gel (mm)

Bacterium name	Zone of Inhibition of topical gel from *Synechococcus* spp., AgNPs (mm)	
	AgNPs gel	Commercial gel
***Candida albicans* AMB C1**	15 ±0.1	15 ±0.2
***Candida albicans* AMB C2**	17 ±0.1	14 ±0.1
***Candida albicans* AMB C3**	18 ±0.2	15 ±0.1
Candida tropicalis	16±0.1	14±0.1

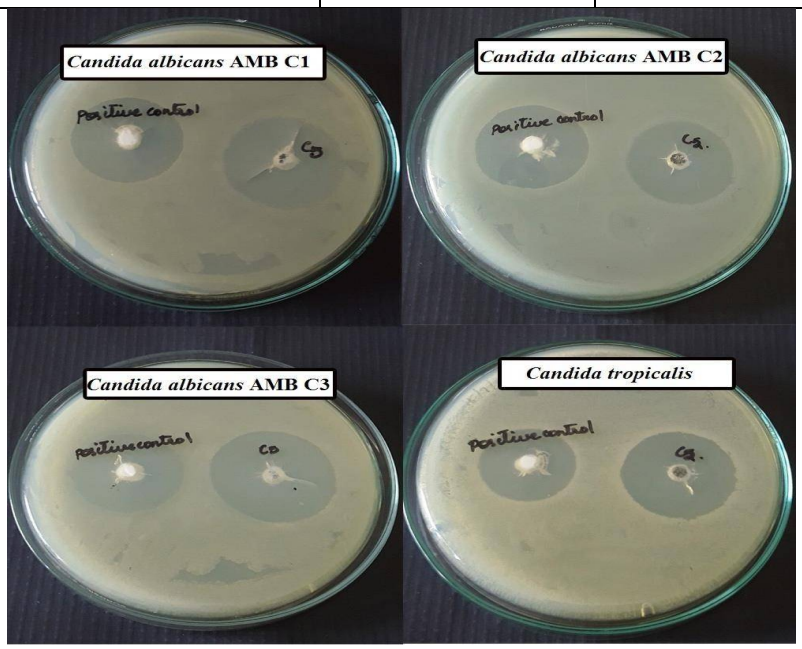

Figure 11. Zone of Inhibition observed (mm) with commercially available gel and prepared topical gel from *Synechococcus* spp., AgNPs

DISCUSSION

After synthesis of silver nanoparticles it was characterized where similarly, (16). Synthesized silver nanoparticle using Aloe Vera extract taking 24 hrs. of reaction time in the presence of ammonia which enhances the nanoparticle formation. In addition, the acquired reflections are sharp with good intensity which confirms that the structure of synthesized nanoparticles are well crystalline. Our findings match with the reports suggest by Govindaraju *et al.* (17) .The presence of peak at 3416 cm-1 could be ascribed to O-H group in polyphenols or proteins/enzymes or polysaccharide (18, 19) . A small peak positioned at 2924 cm-1 may due to CH- stretching of alkanes. A sharp intense band observed at 1631 cm-1 can be due to the stretching vibration of the (NH) =O group. The observed band at 660 cm is due to Alpha - glucopyranose rings deformation of carbohydrate (20). Silver could be used as an antibacterial agent for many infectious disease at ancient time and before the emergence of antibiotics (21). Spherical shape with size reduced silver ions has the increased contact area so that it can eliminate the bacterial growth. Activity of silver nanoparticle has similar effect as silver ions (22). Positively charged silver ions may attach with negatively charged cell membranes of microbes by electrostatic attraction (23). Silver nanoparticles from the pits in the cell wall and damage the cell permeability (24) and induce the proton leakage caused by ROS in the membrane (25, 26) resulting in cell death. *Kim et al.* (27) demonstrate the silver nanoparticle inhibit the conidial germination of fungi. Finally, the silver nanoparticles have a great potential to control the spore producing fungi. Among the pharmaceutical forms used in the local treatment of conditions and pathologies in the vulvovaginal area are ointments, creams, foams and gels. The vagina surface is lined with squamous epithelial cells and mucus produced by several glands. Local vaginal products are used to treat infections, vaginitis, conditions of endometrial atrophy, and contraception by the use of spermicides. These products must be free of pathogens such as bacteria and fungi, and should be packed in specific tubes and packages, and applied to the vagina through specific tips (28). Carbomers may be used in formulations as emulsifiers, suspenders, solid agglutinating agents, and viscosity modifiers. They can be dispersed in water to form acidic colloidal solutions of low viscosity and viscous gels when neutralized (29). At the concentrations of 0.5-2% in water, Carbomers are used as gelling agents added to pharmaceuticals, solvents such as propylene glycol, antimicrobials, and stabilizers, without interference in their theological properties (28). Carbomers 980 was chosen in this study as the gel base for the incorporation of the geopropolis extract because it presents excellent viscosity parameters, compatibility with several active complexes, bio-adhesive properties, thermal stability, good patient accept ability, and excellent organoleptic characteristics (30,31). The acidic nature of carbopol causes the formulation pH to decrease, resulting in reduced ionization of carboxylic groups and increased winding of polymer molecules. This fact reflects a marked reduction in viscosity, gel strength, and muco adhesion (32); therefore the formulations tested in the present study required pH correction and consequently viscosity correction. In the specific case of products for vaginal application site in order to prevent it's interference in normal vagina physiological processes or in the unbalance of inherent micro biota (32). The higher the viscosity of formulations for local administration, the better the bio-adhesive mechanism at the application site when compared to less viscous formulations . The final viscosity in the gel formulation proposed in the present study was specified as the consequence of the amount of Neutralizing agent applied for pH correction*, i.e.,* decreasing pH along with the viscosity. The resulting viscosity seemed ideal when compared to other proposed formulations (33), promoting good Spreadability and robustness on the phase separation tests. Substance or bioactive complexes with apolar characteristics, incorporated in to gels, will have a greater affinity for the receptor fluid (mucosae) than for the polymer base, contributing to an improved release. Thus, during the development of formulations incorporated in to gels, their affinity for the base must be considered to predict the release profile of an active agent from a carrier (37). Cyanobacterial cultures were collected from NFMC, where the collected strain where subjected for the production of bioactive compound and extracted using the ethyl acetate and the ethylacetate extact were subjected to the Anticandidal activity on the other hand silver nanoparticles were synthesized from the algae and were analysed for Anticandidal activity and further the synthesized nanoparticles were characterized by UV-Vis spectroscopy where the absorbance band was found at 400nm and FTIR analysis shows the capping of silver ions to biomolecules present in the algal extract and where the XRD results shows the (111), (200), (220), (311) plans of pure AgNPs respectively. EDS analysis strongly declared the presence of elemental silver signals. In the germ tube inhibition assay AgNPs completely inhibit the germ tube formation by the *Candida albicans* and in the antifungal plate assay the observed highest zone of inhibition (20±0.2) for the synthesized AgNPs and the highest zone of inhibition for the compound was found to be (18±0.3). After analysing the efficacy of compound and AgNPs it is observed that AgNPs shows better results than compound and therefore it is selected for the preparation of topical gel and the prepared topical gel is analysed for Anticandidal activity where it is compared with the commercially available antifungal gel (ketoconazole) is compared for its efficacy and the results showed that AgNPs mediated topical gel showed excellent activity than the commercially available gel. Finally the prepared topical gel can be used as an alternative for the treatment of Candidal vulvovaginitis.

CONCLUSION

Synechococcus spp., was subjected for the production of bioactive compound and the *Synechococcus* spp., mediated synthesised AgNPs are also prepared and the AgNPs were characterized by UV, FTIR, XRD, EDS analysis and the synthesized AgNPs and the produced compound were analysed for the Anticandidal activity where the collected pathogens *Candida albicans* were isolated from the patient of candidal vulvovaginitis. In that AgNPs showed promising activity towards *Candida albicans* and also topical gel was also prepared and compared its efficacy with the commercially available antifungal agent namely ketoconazole where in the plate assay it showed excellent activity against the *Candida albicans*. Thereby concluding that prepared gel from the *Synechococcus* spp., mediated synthesised AgNPs shows promising and better activity against the pathogen *Candida albicans* which causes disease namely candidal vulvovaginitis.

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