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Synthesis, Biological and *In silico* Analysis of Silver Nanoparticles Isolated from Ethanolic Extract of Filamentous *Algae Cladophora* glomerata

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Abstract

Cladophora glomerata is found in fresh-water algae that provides habitat and food for numerous organisms like fishes. *Cladophora glomerata* was collected from bore water bodies of Bilaspur regions, Chhattisgarh, India. The sample was dried, made as powder, extracted with ethanol and synthesized with $AgNO_3$ to obtain silver nanoparticles (AgNP). The obtained silver nanoparticles were confirmed using visible regions of spectra in colorimeter. The supernatant was tested for enzymatic and antimicrobial activities. The extract has shown good activity with Albumin and Hemoglobin proteins. The X-ray gelatin degradation was also conducted and the gelatin was degraded showing enzymatic reaction. The AgNP from ethanolic extract of *Cladophora glomerata* also formed zone of antimicrobial zone of inhibition (30 mm) with *E. coli*. The standard (Penicillin) showed zone of inhibition of 44mm and the aqueous extract shown zone of inhibition of 25mm based on antimicrobial test with *E. coli*. Analysis using *in silico* techniques has shown better activity for Transphytol compared to β-Carotein, Eckol, Linioc acid and β-sitosterol as antibacterial agent against AB5 toxin from *E. coli*.

Keywords: Biochemical analysis; *Cladophora glomerate;* Silver nanoparticals; Synthesis

Introduction

Since times, Medicinal algae and several other microbial species are being used as medicinal sources in control of several human diseases [1,2]. The sources contain medicinal compounds as micro and macro elements and molecules that act as metabolic compounds for living species. Algae are an important species that are controlling the life systems in the atmosphere. There is lot of importance of the algae from the usage as food to the control of earth's environment [3]. It is to be known that the first macro and complex species on the earth may be algae. The earth is evolved with water and exogenous particles like algae and fungal spores in the beginning. The aqueous globe with spore forms are gelatinous filaments present in water formed symbiotic association with fungal forms formed lichens [4]. Lichens float on water and formed earth's crust and in turn of evolution other species of life were formed. The biochemical studies in algae have shown good amount of carbohydrates [5]. The development studies like production of biofuels, using as food material, medicinal product for cancer and diabetes, absorbent material studies are conducted. Anti-mosquito repellents and room fresheners from *Kaempferia galanga* compounds and the base of lichen material have been shown great importance. The material can also be used as cosmetics due to presence of secondary metabolites. (Figure 1)

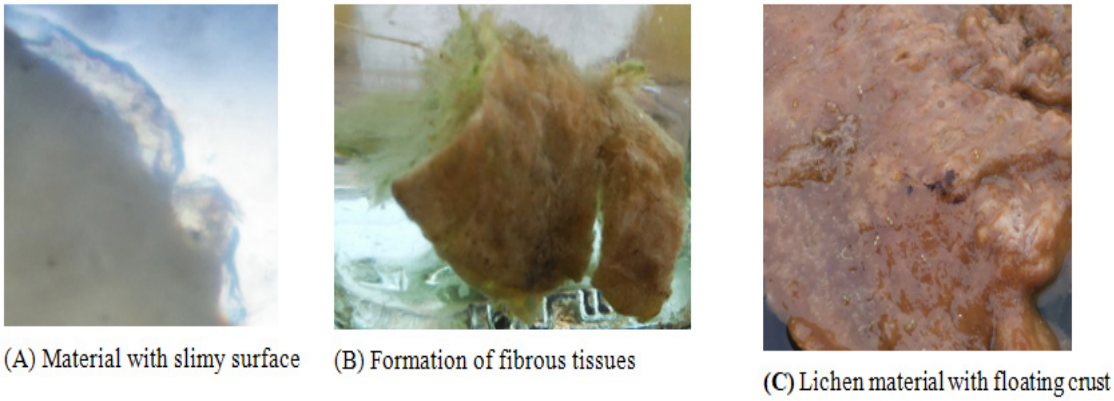


Figure 1: *Cladophora glomerata* showing environmental protection system.

Green Algae

Green algae are a large group of algae containing two groups cholrophyta and charophyta that are placed as a three-separate division of families Mesostigmatophyceae, chlorokybophyceae and spirotaenia [6,7]. The green algae show colonial, unicellular flagellates or multicellular filaments with coccoid and septate forms of living systems containing more than 8000 species. The chlorophyll was acquired from ingested green algae and conduct photosynthesis for few fungal species that are often communicate with algal species in nature as lichens. The few filamentous green algae live independently on water bodies, dead organisms/cells, humid soils and rocks. Most of the photosynthetic eukaryotes like green algae have served as model experimental organisms for understanding the mechanisms of water permeability and ionic changes of membranes, salt tolerance, turgid regulation, osmoregulation, generation of action potentials and cytoplasmic streaming. Green algae are eukaryotes organisms containing chlorophyll a and b as major photosynthetic pigments comprising between 9000-12000 species [8]. Blue-green algae are actually sometimes grouped as bacteria known as fresh water green algae that are included in charophytes. They are closely related to higher plants than marine green algae belonging to chlorophyta. Most green algae are aquatic and are found commonly in marine and fresh water habitats. Some algae are symbiotic with fungi giving lichens. Food reserves are starch, fats or oils like high plants. Green algae are thought to have progenitors from the high green plants with many components like organic beta carotene and other food supplements which are very much useful as health diet and control of several chemicals.

Cladophora glomerate

Cladophora glomerata is a sessile chlorophyta widely distributed in lakes and running waters used as an indicator of pollutant, particularly available with heavy metals like Ni, Fe, Cr, Mn, Cu, Pb, Cd and Co [10,11]. The bore waters contain rich amounts of salts with TDS of around 550-600 mg/L in Chhattisgarh regions like Bilaspur. *Cladophora* is a genus of reticulated, filamentous Ulvophyceae green algae containing many species with great variation in their appearances, habitat, age and environmental conditions. The *cladophora* species show major alterations to benthic conditions that are linked particularly with increase phosphorous loading in the surrounding. The genus *cladophora* is cosmopolitan distributed and is generally harmless growth is important and food for many species and aquatic animals, buffer of sequestration of nutrients in water and protection of aquatic organisms from several solar UV radiations and mobile radiations [12]. Currently, algae and their extracts are commonly used in natural cosmetic raw materials due to biological active compounds in filament containing carbohydrates, polyphenols and fatty acids that is useful in cosmetics for the glory of skin. *Cladophora glomerata* is a filamentous green macro alga with rectangular shape to thalli, green and branched genus present in marine and fresh water habitats. These algae contain rich concentration of bioactive elements like phenol compounds saturated and unsaturated fatty acids, sterols and terpenoids with rich concentration of carbohydrates. Hence, the algae are worth to continue studies in new directions of research as a potential source of cosmetic raw material and antimicrobial agents. (Figure 2)

Figure 2: *Cladophora glomerata* as lichen algae and non-toxic species.

Nanoparticles

Nanoparticles are the particles measuring between 1-100 nm in size with a surrounding interfacial layer [13-15]. The interfacial layer is an integral part of nanoscale like silver fundamentally affecting all of its properties. The term nanoparticle is equally referred to inorganic materials. nanoparticles are associated with modern science showing great scientific interest that bridge between bulk materials and atomic or molecular structures. The properties of materials change as their size approaches the nanoscale and as the percentage of the surface in relation to the percentage of the volume of a material becomes significant. The chemical processing and synthesis of high-performance technological components are useful in private, industrial and military sectors. There are several methods creating nanoparticles that include gas condensation, chemical perspiration, ion implantation, pyrolysis and hypodermal synthesis. Scientific search on nanoparticles is intense as they have many potential applications in physics, optics, electronics and medicine [16]. Nanoparticles are studied as dietary plan supplements for delivery of biologically active substance like mineral elements. Nanomaterials are also being invented as potentially drug delivery substance. Growth factors or other biomolecules can be conjugated to nanoparticles to aid target delivery.

Silver nanoparticles

Silver nanoparticles are the nanosized particles that measures between 1-100 nm and containing of silver coat [17]. Numerous shape of silver nanoparticle can be constructed depending on the application of the most common method are nanoparticle synthesis fall under the category of wet chemistry of the nucleation of particles taken in solution. This nucleation's occur when a silver ion complex usually silver nitrate is reduced to colloidal silver in the presence of a reducing agent. Researchers have explored the use of silver nanoparticles as carriers for delivering various payloads such as small drug molecules and large biomolecules to specific targets. Once the Silver Nanoparticles (AgNPs) have sufficient time to achieve its target, release the active molecule of payload could potentially have triggered by an internal or external stimulus. The targeting and accumulation of nanoparticles may provide high payload, concentrations at specific targets sites and could minimize side effects.

Phytochemical Analysis

Phytochemicals are chemical compounds present in plants contains chlorophyll that is used to thrive the predators, competitors or pathogens [18]. These algal-derived compounds show therapeutic activities such as anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, and antimicrobial properties. The main groups of phytochemicals are carotenes, flavones and anthocyanins. Many primary and secondary metabolites produce from several green algae that remove toxic materials from the diseased humans [19]. Green algae are members of the division Chlorophyta that are considered to be very closely related to plants**.**

Enzyme hydrolysis by X-ray film method

The gelatin is a protein that was crosslinked, insoluble and can swell in biofluids [20]. X-rays films generally used in medical applications in diagnosis which are made of a plastic sheet (polyester film) coated with a thin coating of gelatin that was impregnated with silver grain. Nowadays various research studies have been conducting using proteases that play a crucial role in the recycling of exposed X-ray films [21]. Protease, referred to as proteolytic enzymes are present in plants/ animals/ microbes that hydrolyze and digest, depolymerize or degrade or decompose protein. Human skin gelatinase shows no or little reactivity towards

the protein substrates like hemoglobin or casein [22]. Penicillin G (PenG) shows enzymatic hydrolysis with proteins like alginate, agarose, gelatin, and chitosan [23]. Algal proteases along with hemoglobin or albumin break down the gelatin protein that serves as the support medium for the suspension when it reacts with X-ray films. If the gelatin is destroyed on x-ray film, the black silver grains will slough off, leaving a clear plastic sheet of x-ray film.

Antibacterial activity

Most bacteria are the species that require metabolic machinery like plant or animal cells or nutrients for growth and reproduction [24]. Some species are pathogenic that grows as model organisms in humans. *E. coli* is a model organism that is non-pathogenic at lower concentrations but become pathogenic has higher concentrations. Antibacterial activity provides control of bacteria using extracts/ metabolites from higher / lower living species. In general, Mueller Hinton Agar (MHA) will be used as nutrient medium for the growth of bacteria in Antibacterial activity test.

In silico analysis

In silico analysis in Bioinformatics is a method that analyses mechanisms and control of biological compounds on diseased proteins using computers [25,26]. The present research is conducted for the prediction of the proposed compounds from *Cladophora glomerata* as antimicrobial agents. AB5 toxins are very important virulence factors present in several major bacterial pathogens that destroy eukaryotic target cells by inactivating essential enzyme complexes. AB5 is a Heat-Labile Enterotoxins and cytotoxins present in clinical strains of *Escherichia coli* that causes Diarrhea [27].

Materials and Methods

The green algae are collected from tanks and water stagnant regions produced from bore waters of Bilaspur (CG) regions of India. The TDS of the water was measures as 550 mg/L. The algal material produces oxygen attached to the fungi (forming lichens) and produce pungent smell. These soft lichens form blockages in the water pipes and stops water flow. The association as commensally forms of algae and fungi is very important in environmental protection, food for living species and control of temperatures in atmosphere [28].

Sample collection

The fresh Algae are slimy and whitish green color before formation of Lichens. The fresh material was collected and was observed in Microscopy at 100X magnification (Figure 3).

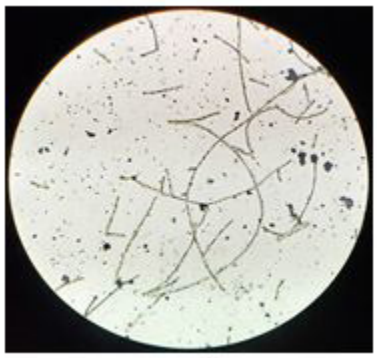


Figure 3: Microscopic observation of Green algae.

Extraction Process

The material of *Cladophora glomerata* contains slimy material is collected as biomass, washed three times with distilled water and dried under sunlight has been shown in (Figure 4).

Figure 4: Fresh and dried samples of *Cladophora glomerate.*

The dried samples are made powder using motor and pestle. The powder of *Cladophora glomerata* is stored in refrigerator for further exaction and experimentation.

About one gram of powder was mixed in 10 ml of distilled water and prepared aqueous extract of *Cladophora glomerata.* The Aqueous extract of *Cladophora glomerata* was separated using centrifugation at 4000 rpm for 20 minutes (concentration Aqueous extract of *Cladophora glomerata* is 100 mg/ml).

About one gram of powder was mixed in 10 ml of ethanol and prepared ethanolic extract of *Cladophora glomerata*. The Ethanolic extract of *Cladophora glomerata* was separated using centrifugation at 4000 rpm for 20 minutes (concentration Aqueous extract of *Cladophora glomerata* is 100 mg/ml).

About one gram of powder was mixed in 10 ml of ethanol was prepared. 5 ml of the extract was added to 100ml of $1X10^{-3}$ M aqueous Silver Nitrate solution at room temperature. The solution was kept in sunlight for 2 hours in summer season (around 47 degree centigrade). The yellow color of solution changes to black suspension mixture after 2 hours. The AgNP from ethanolic extract of *Cladophora glomerata* was separated using centrifugation at 4000 rpm for 20 minutes (concentration AgNP of ethanolic extract of *Cladophora glomerata* is 100 mg/ml).

The extracts of AgNP, ethanolic and aqueous are shown from left to right respectively in A and B of (Figure 5). (Figure 5 (C)) is AgNP, ethanolic, control and aqueous extracts after centrifugation respectively (from left to right).

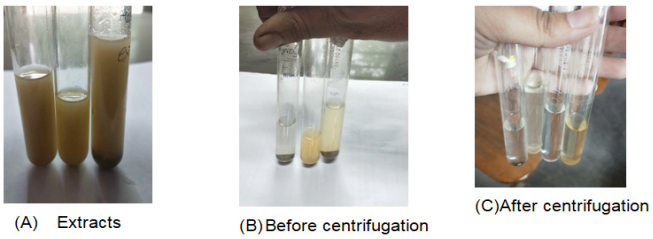


Figure 5: Extraction Samples used in present study.

Calorimetric Analysis

Due to the side effects of the present-day antimicrobial compounds, naturally occurring antibacterial compounds can be derived from microorganisms are need for developing the newer antimicrobial compounds through delivery and protective system like nanoparticles. Calorimetry provides insight into the stability and active forms of nanophase materials and increases surface energy of the molecules. The initial step of such a spontaneous process is strongly exothermic is considered as a driving force to from low entropy bimetallic nanoparticle showing low OD values in colorimeter

Phytochemical activity

Plants always contains common source of medicaments in traditional preparations or as pure bioactive principles. The identification of plants or plant extracts could be used as drugs, or that could replace some pharmaceutical preparations are being purchased and imported from different countries.

Ethanolic extract of *Cladophora glomerata* was used for preliminary screening for phytochemicals such as carbohydrates (Molisch's test), protein (Biuret test), amino acid (Ninhydrin test), tannins, flavinoids, terpenoids, phlobatanins, etc.

Test for Amino Acids

To 2 ml of the algal extract, 2 ml of Ninhydrin reagent is to be added and keep the solution in hot water bath for about 15 minutes. The formation of purple color indicates the presence of amino acids in the sample.

Test for Proteins

To 2 ml of the algal extract, 2 ml of Biuret reagent is to be added. An appearance of violet color ring indicates the presence of protein.

Test for Carbohydrates

To 2 ml of algal extract add 2 drops of Molisch reagent and mix the solution. Nearly 2ml of Concentrate H_2SO_4 is to be added drop by drop from the sides of the test tube. A reddish violet color ring appearance at the junction of two layers immediately indicates the presence of carbohydrates.

Test for Flavonoids

To 5 ml of algal extract of dilute ammonia solution, a few drops of Conc.H_2SO_4 are to be added. A yellow colored solution confirms the presence of flavonoids and will disappears on long standing.

Test for Tannins

To 5 ml of algal extract of extract, few drops of 1% of lead acetate are to be added. A yellow colored precipitate formed in the test tube shows the presence of tannins.

Test for Terpenoids

To 2 ml of algal extract add 2 ml of chloroform and 3 ml of Conc. H_2SO_4. Formation of a monolayer of reddish-brown coloration of an interface shows a positive result for the terpenoids.

Test for Phlobatinins

To 2 ml of algal extract add 1% aqueous HCl and boiled for few minutes. A red precipitate formed and deposited in the test tube is an evidence for the presence of phlobatinins.

Test for Phenols

About 2 ml of algal extract, 3 ml of ethanol and a pinch of $FeCl_3$ is to be added. The formation of greenish yellow color solution indicates the presence of phenols.

Test for Quinones

To 2 ml of algal extract, 3 ml of Conc. HCl is to be added. Formation of green color solution indicates the presence of quinones.

Slide method and Microscopy

The extracts are mixed with Proteins like Albumin respectively and observed for formation of coagulation. The sample was observed under optical microscope at 100X magnification.

Enzyme hydrolysis by X-ray film method

Approximately 10 µl of protein activator (Algal extract) was mixed with 10 µl of protein (Albumin or Haemoglobin of 0.5 mg/ml) and was spotted onto a stripe of the X-ray film. 10 µl of Penicillin was mixed with 10 µl of 0.1 M (pH 7.0) phosphate buffer as the control and was spotted on to the X-ray film. The above inhibitor, protein and buffer mixtures were incubated of X-ray film at 37°C for 10 minutes. After 10 minutes, wash the film under tap water gently without touching other objects for the zone of gelatin hydrolysis. The non-protein activity will be visualized as thick color without gelatin hydrolysis and protein activity will be shown as zone of gelatin hydrolysis.

Antimicrobial activity

Antibacterial activity test was carried out by well diffusion method. The 10 mm wells on *E. coli* inoculated agar plates were filled with 10 µl of the samples (Control, standard, aqueous extract of *Cladophora glomerata*, ethanol extract of *Cladophora glomerata* and AgNP ethanolic extract of *Cladophora glomerata*) at the concentration of 100 mg/well. Sterile distilled water was added as control. Penicillin (Antibiotic) was used (100 mg/well) as positive reference standard. The Muller Hinton agar plates were incubated at 37°C for 24 hours. The inhibitory activities of the samples were quantitatively assessed by observing the presence or absence of the inhibition zones and the zone diameters (including well size of 10 mm). The AgNPs have excellent antimicrobial property may be due to their extremely large surface area.

In silico analysis

As per the reference of Fabrowska et al, 2015 [29], the freshwater green macroalgae *Cladophora glomerata* is a natural raw material available in Chhattisgarh with rich in various biologically active compounds like β-carotein, Eckol, Linioc acid, β-sistosterol and Transphytol. The ligands are designed using Chemsketch software of ACD/Labs v10.02 and receptor (AB5 toxin of *E. coli* with pdb id 2IY9) is retrieved from PDB database. The QSAR studies are conducted with HyperChem v8.0.1 software. Both the molecules are docked using iGEMDOCK v2.1 for screening better compounds from the selected ligands as antimicrobial agents.

Results

The useful medicinal properties from *Cladophora glomerata* materials usually results due to the combination of primary and secondary metabolites present in the filaments. In algae, proteins, fatty acids, polysaccharides, and amino acids are present as primary metabolites. Phenolic compounds, sterols, pigments, vitamins, and other bioactive agents are present as secondary metabolites [30]. These metabolites have great potential in pharmaceuticals, nutraceuticals, and cosmeceuticals. The medicinal procedures of green algae are exclusive to particular algal groups or species like *Cladophora glomerata* and are constant with the perception as the combination of secondary products in a specific plant are often taxonomically distinctive.

The basic idea of colorimeter that absorption depends on sizes, shape, fabrication, state of agglomeration or aggregation of nanoparticles, but in more simply your range varies with the size, larger NP's contributes visible (400-780 nm). The absorbance has occurred at 480 nm wavelength and have great slope specifies the formation of nanoparticles (Figure 6).

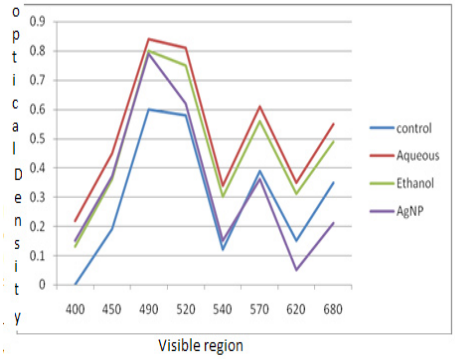


Figure 6: Calorimeter reading at different wavelengths.

Phytochemical analysis

The phytochemical analysis for Ethanolic AgNP extract of *Cladophora glomerata* has shown good amount of carbohydrates followed by Flavonoids, terpenoids, phenols, aminoacids and proteins (Table 1).

Phytochemical	Reaction
Amino acids	+
Protein	+
Carbohydrates	+++
Flavinoids	++
Tannins	-
phlobatanins	-
Terpenoids	++
Phenols	++
Quinones	-
Note: - Absence, + Less concentration, ++-medium concentration, +++-high concentration	

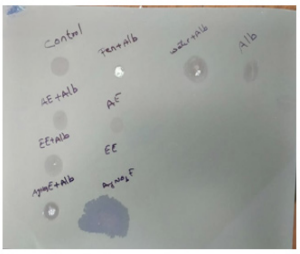
Table 1: Phytochemical analysis for Ethanolic AgNP extract of *Cladophora glomerate.*

Carbohydrates are the basic source of energy to the complex species like humans. Proteins and amino acids are the building bodies of life provide structural component. Flavonoids, a group of bioactive compounds having variable phenolic structures that are beneficial effects on health and act as a secondary antioxidant defense system. Terpenoid perform basic functions in growth and development. The algae are more specialized due to terpenoids showing chemical interactions and protection in the biotic and abiotic environment

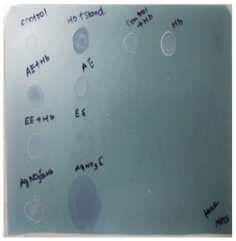
Enzyme hydrolysis by X-ray film method

The standard penicillin with albumin had shown the complete degradation of gelatin showing protein degradation activity. The control was not shown the inhibition with proteins with X-ray photographic film. AgNP with proteins like Hemoglobin or Albumin has also shown degradation of gelatin similar to penicillin. Proteins are coagulated with penicillin, Ethanolic and Ethanolic AgNP extracts of *Cladophora glomerata*. Hemoglobin or albumin mixed with water not shown any protein degradation activity (Figure 7).

(A)Reaction of Albumin in slide method



(B) Reaction of Albumin in X-ray film method

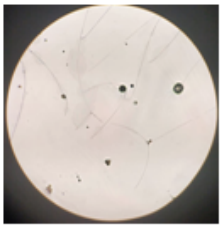


(C) Reaction of Hemoglobin in X-ray film method

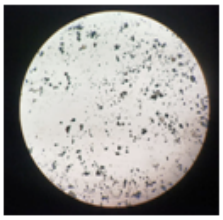
Figure 7: Enzyme hydrolysis by X-ray film method.

The microscopic observation as shown in figure 8 shown the particulate coagulation factures in aqueous and ethanolic extracts of *Cladophora glomerata* with albumin but AgNP formed clear particular sized brown material with lack of coagulating material under 100X microscopy. (Figure 8)

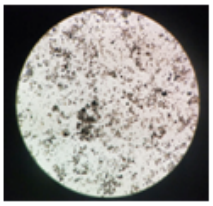
(A)Control+Albumin



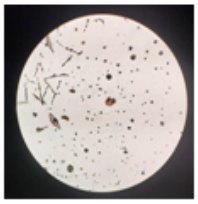
(B)Aqueous Extract + albumin

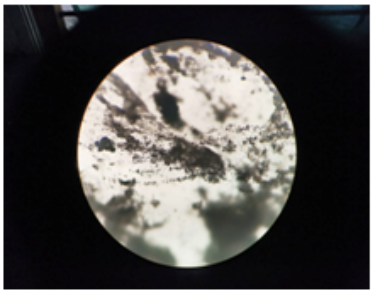


(C)Ethanol Extract+Albumin



(D)AgNP+Albumin





(E)Penicillin +Albumin

(F)Control (Distilled Water)

Figure 8: Microscopic examination at 100X for Albumin reaction.

Antimicrobial activity

Based on the antimicrobial activity conducted for 4 samples (Control, Aqueous extract, Ethanolic extract and Ethanolic AgNP extract), the penicillin (44 mm Zone) against *E. coli* has shown good activity at 100 mg/ml. The Ethanolic AgNP (30 mm), Ethanolic (25 mm) extracts followed by Aqueous extract (12 mm) has also shown good antimicrobial activity (Figure 9), (Table 2).

Figure 9: Antimicrobial activity.

Sample	Zone of Inhibition (in mm)
Penicillin	44
Control	0
Aqueous	12
Ethanol	25
Ethanolic AgNPs	30

Table 2: Antimicrobial activity.

In silico analysis

The ligands like Carotein, Eckol, Linioc acid, Sistosterol and Transphytol proposed from *Cladophora glomerata* by Fabrowska et al, 2015 (Figure 10) was designed using Chemsketch software (Figure 11).

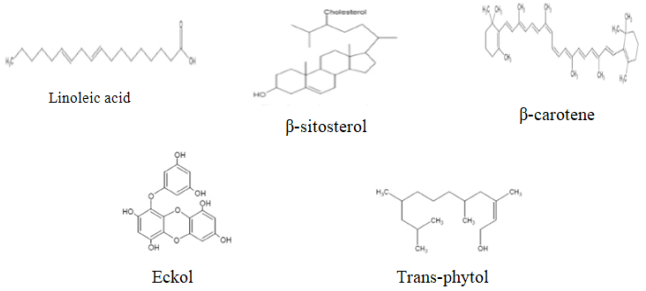


Figure 10: Ligands proposed by Fabrowska et al, 2015 from *Cladophora glomerate.*

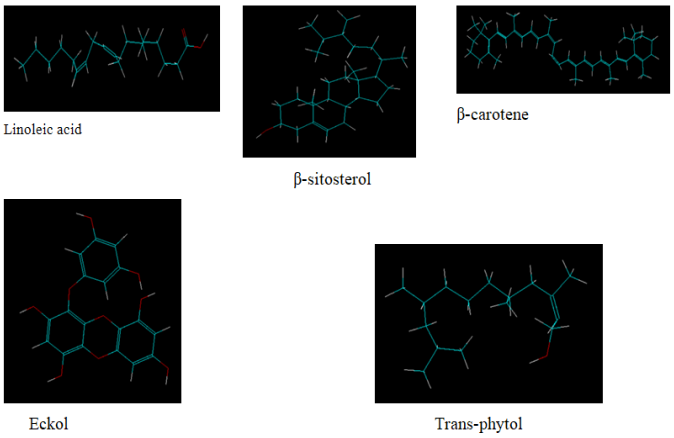


Figure 11: Ligands designed and optimised using ChemSketch.

The AB5 toxin of *E. coli* with pdb id 2IY9 is retrieved from PDB database and the structure was shown in (Figure 12.)

Figure 12: AB5 Toxin of *E. coli* with pdb id 2IY9.

Transphytol has less molecular surface area and mass compared to penicillin may show good diffusing capacity in living cells as Nano materials. Transphytol and Eckol have shown low polarizability compared with Penicillin may show better ligand activity based on QSAR properties (Table 3).

Ligands	Partial charge	Surface area (Approx.) $^{\circ}A^2$	Surface area (Grid) $^{\circ}A^2$	Volume $^{\circ}A^3$	Hydration energy kcal/mol	LogP	Refractivity $^{\circ}A^3$	Polarizability $^{\circ}A^3$	Mass (in amu)
β-carotein	0	1073.27	978.9	1664.31	-0.17	2.75	90.88	38.60	480.44
Eckol	0	**599.39**	**505.64**	867.34	**-46.96**	**-3.91**	**9.6**	**4.64**	372.29
Linioc acid	0	855.91	513.23	869.67	-3.50	5.58	72.4	23.02	248.20
β-sitosterol	0	610.65	617.74	1085.26	-1.95	3.44	73.16	30.64	368.35
Transphytol	0	**521.63**	**508.36**	878.84	**-9.01**	**-10.4**	**25.60**	**12.38**	**240.43**
Penicillin	0	546.59	525.11	863.03	-8.53	7.61	49.64	25.59	317.25
Silver	0	81.71	81.71	70.04	0	0	0	0.30	107.87

Table 3: QSAR studies with selected ligands.

The ligand Transphytol has shown better activity compared to penicillin. The ligands β-carotein and Linioc acid has antibacterial shown less activity than penicillin (Table 4). The docked poses of AB5 toxin with Transphytol has shown in (Figure 13).

Ligands	Energy (in kcal/mol)	Active site
β-carotein	-84.59	phe69,ser70,phe71,pro77,phe78,glu131
Penicillin	-87.53	lys210, lys211,his203,pro204,arg217,asn245,lys298
Eckol	-99.3	glu34, met37,ser38, thr40,lys42
Linioc acid	-73.6	arg175, gly142,glu143,lys145,asp174
β-sitosterol	-86.8	tyr202, his203, arg217, lys210, lys211,asp213, arg217,asn245
Transphytol	**-107.03**	lys210, lys211,asp213, asn245, lys298, arg299, leu302, arg319

Table 4: Docking studies with selected ligands with AB5 toxin of *E. coli.*

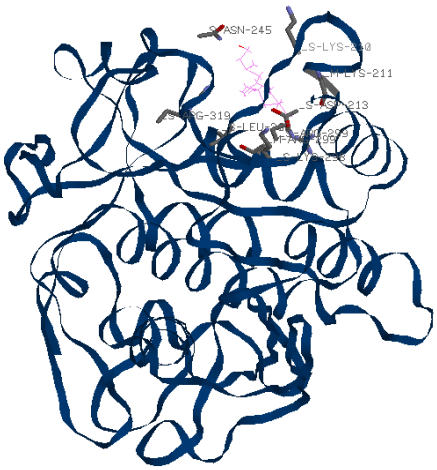


Figure 13: Docking pose of AB5 with Transphytol.

Hence the phytocompounds like Carotein, Eckol, Linioc acid, Sistosterol and Transphytol present in *Cladophora glomerata* may be used as biomass, as food and medicine sources alternate to plants.

Discussion

Algae are ubiquitous, protein-rich and carbohydrate-rich due to having chlorophylls a, b, c 1 and c 2 [31,32]. Green algae occupy an important position in the biological world use water as electron donor and releases rich amount oxygen in the environment [33]. *Cladophora* has ecological functions that are beneficial and characterized as an ecological engineer. The algae are useful from wastewater remediation that is attached to renewable biofuel production [34]. Lot of water bodies and rivers flow from Chhattisgarh state of India and if any decrease I lakes and rivers may show increase in the temperatures due to decrease of algae.

Figure 14: Chhattisgarh state.

Arpa, Mahanadi, Sheonath, Hasdeo, Indravati, Kelo, Son, Rehar, and Kanhar are some of the main rivers present in the state of Chhattisgarh (Figure 14) of India providing a lifeline to the socio-economic development of the state. About 44% of the state is covered with forests. Most of the land, lakes and rivers contains rich and verities of algae that the left as waste without usage in neither food nor natural sources.

The phytochemical analysis of green seaweed *Cladophora glomerata* revealed the presence of compounds in varying concentration like Carbohydrates, Alkaloids, Tannin, Flavonoids, Phenols, Steroids, Saponins, Volatile oils, Fixed oils, Fats, Gums, Mucillage, and Proteins. *Cladophora glomerata* has shown good antibacterial activity against *Pseudomonas aeruginosa* [35]. *P. aeruginosa* was found to be the most resistant among all the bacteria (*B. subtilis, S. aureus, P. mirabilis* and *S.typhimurium*) [36]. The present work has found carbohydrates, Flavonoids, terpenoids, phenols, aminoacids and proteins in Ethanolic AgNP extract of *Cladophora glomerata.*

A potent extracellular fibrinolytic enzyme is present in the Ethanolic AgNP extract of *Cladophora glomerata* under growth conditions. The enzyme showed a versatile proteolytic activity against several protein substrates including gelatin, hemoglobin and egg albumin.

The nanocellulose preparation is very useful by algae due to having renewability, low toxicity, bio-degradability, biocompatibility, easy availability and low-cost properties [37]. Some proteinaceous molecule such as a peptide and/an enzyme that confer a metal binding from algae (treating the surface with a metal binding peptide) shows reducing fouling properties [38]. Enzymatic decomposition of gelatin layers on X-ray films can be achieved by enzymes like protease K, trypsin, chymotripsin, etc [39,40]. Phytocompounds present in algae shows different interactions with various proteins and enzymes and control several diseases. Polymyxin B and silver NPs shows potential antimicrobial treatment against Gram-negative bacteria and a Very low hemolytic activity of silver nanoparticles has been previously reported [41].

Diatom include ß-carotene, ct-cryptoxanthin, fucoxanthin, lutein, trans-phytol, and plastoquinone-9 that shows good antibacterial properties [42]. Red alga Cystoclonium purpreum also shown antibacterial constituents like α-carotene, trans-phytol, plastoquinone-9, ubiquinol-9, lutein and fucoxanthin [43]. Transphytol and a Glycolipid possessed good activity against both COX-1 and COX-2 [44] (Figure 15). The pathway shows electron transfer activity, cytochrome-c oxidase activity, oxidoreductase activity, proton transmembrane transporter activity and catalytic activity as molecular functions.

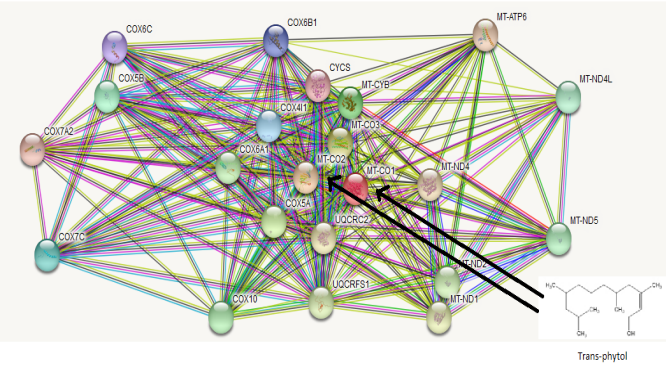


Figure 15: Mechanism of control of Trans-phytol.

Phytol is an acyclic diterpene alcohol that is used as a precursor for vitamin E and vitamin K1 [45]. The trans-phytol

obtained by saponification of chlorophyll. Trans-phytol was found to potently inhibit estrogen biosynthesis. Bioactive compounds from algae isolated from this species include cholesterol, ergosterol, stigmasterol, B-sitosterol, transphytol, xylomacin, and stigmasteryl galactoside shows antimicrobial properties against human pathogenic species.

Conclusion

Green algae *Cladophora glomerata* is available in fresh bore waters of Bilaspur Chhattisgarh, India has good composition of phytochemicals and is non-toxic. The Ethanolic AgNP extract of *Cladophora glomerata* has good protein activity containing enzymes. The Ethanolic AgNP extract of *Cladophora glomerata* has also shown good antibacterial activity against *E. coli*.

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