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A study on the antibacterial activity of green synthesized silver Nano particle and crude extracts of some selected sea weeds.

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Abstract- **In order to synthesize alternative antibacterial drug from natural sources ,this work was carried to synthesize silver nano particles from sea weed and antibacterial activity of crude extracts of the sea weed using methanol, ethanol and acetone was determined their influence on fish pathogen. The crude extract using methanol, ethanol, and acetone were concentrated using rotary vaccumevoprator. The extract with silver nano particles was synthesized from 1mM silver nitrate using magnetic stirrer. The characterization of nano particles was done by, UV-vis spectroscopy, FTIR and SEM analysis. The antibacterial efficacy of the crude sea weed extracts and extracts with Silver nano particles was tested by disc diffusion method. The finding of the results were, extracts with silver nano particles shown maximum zone of inhibition against all bacterial pathogen and for crude extracts the zone of inhibition was varied among all species but among the all acetone extracts shown maximum zone of inhibition.**

Keywords: Fishpathogen,Silvernanoparticle,Disc diffusion method

INTRODUCTION

Seaweeds are an important cash crop. It is a mairnealga. Among the marine flora and fauna marine algae are rich sources of diverse bio active compounds with various biological activities. Marine organisms such as marine algae are source material for structurally unique natural product with pharmacological and biological activities(Schuartsmanetal.,2001).Metabolites from green, brown, and red marine algae may be useful for inhibiting bacteria, virus, fungi and other epibionts. Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms(Hernandez et al.,2008;Drorehreet al.,2009;Ebyet al.,2009;Panaceket al.,2009).Silver nanoparticles find use in many antibacterial applications ,the action of metal on microbes is not fully known It has been hypothesized that silver nanoparticles can cause cell lysis or growth inhibition via various mechanisms(Kim et.al;2007;Prabhu and Poulose,2012).The lethality of silver for bacteria can also be in part explained by thiol group reactions that inactivate enzymes(Chen and Schluesenal,2008; Feng et al.,2000). The present study was aimes at that of, Synthesis of silver nano particles using seaweed extracts, Application of synthesized nanoparticles against some species of bacteri, a Produce crude extract of seaweeds from methanol ,ethanol and acetone, Application of crude extracts against some species of bacteria, Compare the antibacterial activity of crude and silver nano particle sea weed exstracts.

MATERIALS AND METHODS:

Collection of sea weeds:

Samples which are healthy fully grown and submerged under water livings are collected from Hare island Tuticorin. The sample was picked with hand and immediately washed with sea water to remove foreign particles and sand epiphytes. Then it was transported to laboratory and washed thoroughly with fresh water to remove salt on the surface of the sample. Then the sea weeds were spread on blotting paper to remove water and dried in a shade.

See weed species: Sargassummuticum, Turbinaria Ornata, Ulva lactuca, Padinaantillarum, Gracilaria Corticata, Eucheumadenticulatum.

Collection of micro organisms:

The tested microorganisms with MTCC number 7073-Vibriosps,1739-Aeromonas hydrophila,3629-Yersinia enterocolitica,8076-Pseudomonas aeruginosa,and7770-Flavobacterium sps.MTCC was obtained from IMTECH, Chandigarh, India and the culture was maintained as glycerol stocks (40% W/V) at-20°C for routine work.

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Preparation of extracts by rotary vaccumevoprator extraction method:

The powdered samples were extracted by using rotary vaccumevoprator apparatus, using methanol, ethanol, acetone as the solvent for extraction. 25g of the sample and 250ml of the solvent were taken for extraction. The apparatus was run for 4hr and syrupy extracts were collected. Then the extract was stored in cold storage for further use.

Biosynthesis of sivernano particles:

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5 gm of seaweed powder was mixed in 100 ml distilled water and the mixer was allowed to standat 100°C for 10 min and filter out the seaweed extract using Whatman no.1 filter paper. To aclean and surface sterilized burette, 50 ml of seaweed extract was taken and allowed to fall indrops to the beaker containing 100 ml of 1 mM of silver nitrate. The beaker was kept on hotmagnetic stirrer at 70°C. The change in colour from colourless to brown colour was taken for visible confirmation of formation of silver nanoparticles.(Plates.1 and 2).Then the sample was subjected tofurther characterization UV-vis spectroscopy,FT-IR,SEM-Analysis.

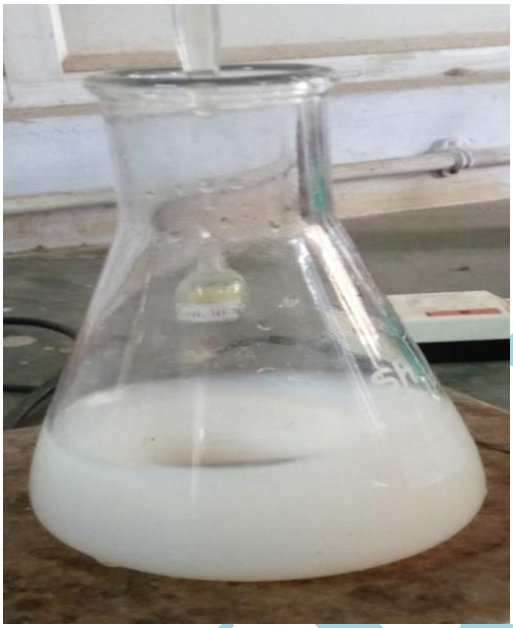

Plate1.Before synthesis,of Ag NPs

Plate.2. After synthesis of Ag NPs

Discs diffusion method

The antibacterial activity of crude extracts of seaweeds against three species of bacteria namely Vibriosps, Aeromonashy drophilla, Yersinia enterocolitica,. Pseudomonas aeruginosa, and Flavobacteriumsps were determined by well diffusion method (Bauer et al., 1966). The circular well of 3 mm diameter and 25 µl holding capacity was prepared using well cutter. Streak plate method was performed to seed pathogenic bacterial culture on the agar plates. Using the loop which had been flamed, cooled and dipped in the inoculums, continuous horizontal streaks were made in the solid agar plates. Different fractions of the seaweed extracts were then added to the wells. The clear labels of sample were marked on the plate. The plates were then incubated at 37 °C for 24 hours.

Statistical Analysis:

Statistical analysis was done by, Mean, Standard deviation, One way ANOVA

Results:

The results for antibacterial activity of sea weed crude and Ag NPs extratcts show higher zone of inhibition in extracts with Ag NPs .

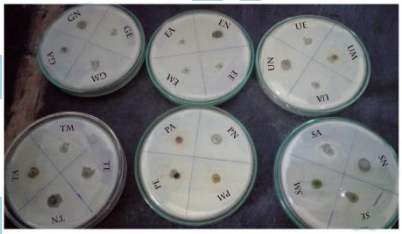

Plate:3.Showing antibacterial activity of seaweed extracts againstVibirioSps.(Table1,Fig1)

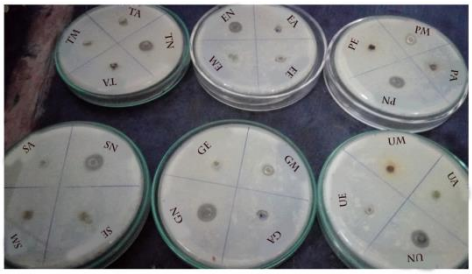

Plate4.:Showing antibacterial activity seaweed extracts against Aeromonashydrophila(Table2,Fig2)

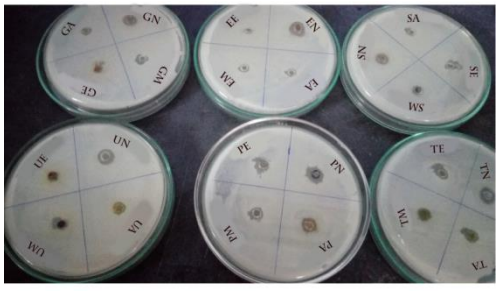

Plate5.:Showing antibacterial activity of seaweed extracts against Yersniaenterocolitica(Table3,Fig3)

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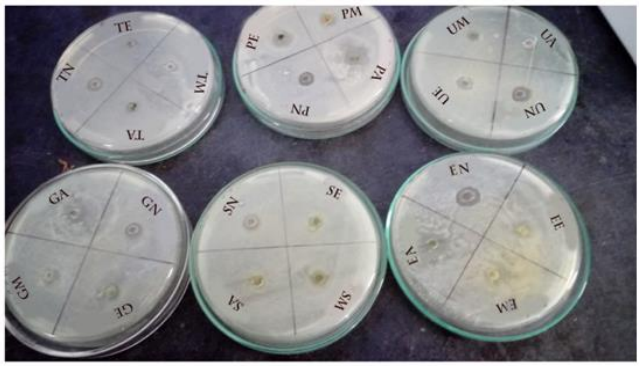
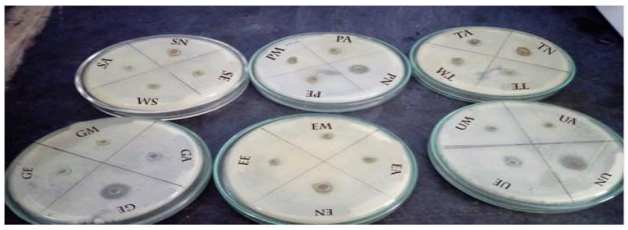


Plate 6:Showing antibacterial activity of seaweed extracts against Pseudomonasaeruginosa(Table4,Fig4)



Pate7: Showing antibacterial activityof seaweed extracts against Flavobacteriumsps(Table5,Fig5)

SM – Methanol extract of S.muticum
SE – Ethanol extract of S.muticum
SA – Ethanol extract of S.muticum
SN – AgNPs extract of S.muticum

PM – Methanol extract of P.antillarum
PE – Ethanol extract of P.antillarum
PA – Acetone extract of P.antillarum
PN – AgNPs extract of P.antillarum

TM – Methanol extract of T.ornata
TE – Ethanol extract of T.ornata
TA – Acetone extract of T.ornata
TN – AgNPs extract of T.ornata

GM – Methanol extract of G.Corticata
GE – Ethanol extract of G.Corticata
GA – Acetone extract of G.Corticata
GN – AgNPs extract of G.Corticata

EM – Methanol extract of E.denticulatum
EE – Ethanol extract of E.denticulatum
EA – Acetone extract of E.denticulatum
EN – AgNps extract of E.denticulatum

UM – Methanol extract of U.lactuca
UE – Ethanol extract of U.lactuca
UA – Acetone extract of U.lactuca
UN – AgNPs extract of U.lactuca

FT-IR Result:
FI-IR has emerged as a valuable tool for understanding the involvement of biological groups in metal reactions. This technique was applie to determine the functional groups that were present in the algal biomass.

The FT-IR spectrum recorded for SNPsynthesized using Sargassum muticum produced the following result. A number of bands were observed in the regions 31636.59, 2122.74, 3338.03, and 3810.07, 1636.59. The peak at 3810.07 cm -1 may be assigned to the vibrational modes of acohols of these molecules. The peak at 3338.03 cm -1 show the presence of phenols and aleohols of these molecules. The peak at 2122.74 my be assigned to C C stretch. The band at 1636.59 showed a peak showing the presence of alkense with C-C C symmetric stretch. The results also indicate the presence of functional group in the algal bio-mass which may have participated in the synthesis of SNP. (Fig6)
The FT-IR spectrum of Turbinaria ornata seaweed showed the peaks in the following regions 1636.08, 2102.81, 3270.00 and 3854.47 cm -1

The peak at 1636.08 cm-1 may be assigned to the presence of amides with N-H bend or C=O stretch which was responsible for the absorption of IR vibarations, next peak was observed at 2102.8 cm-1may be assigned to C≡C Stretch. The band at 3270.00 cm-1 showed the presence of C≡C-H group and the peak at 3854.47 showed be presence of alcohol group.These bands are desired from the functional group which are the ligands of nano particles. (Fig.7)

The FT-IRspectrum of Padina antellarum seawed showed the peaks in the following regions 1635.87, 2117.83, 2389.09, 3284.26, 3854.01, 3955.78 cm-1

The peak at 1635.87 cm-1 may be assigned to the presence of N-H bend C=O stretch which was responsible for the absorption of FT-IR vibrations. The peak at 2117.83 showed the presence of -C≡C- stretch. The band at 2389.09 cm-1 indicate the presence of C≡C stretch bond. The peak at 3284.26 cm-1 showed the presence of O-H stretch and hydrogen bond. The peak at 3854.01 and 3955.78 showed the presence of alcoholic groups. The functional groups present in these bands indicate that may be synthesize SNP. (Fig.8)

The FT- IR spectrum of Ulva lactua seawed showed the peak at 1108.84, 1635.88, 2114.27, 2390.06, 3270.51 and 3852.73 cm-1

The peak at 1108.84 cm-1 may be assigned to the presence of C-O stretch. The peak at 1635.88 cm-1 showed the presence of primary amines N-H bend. The band at 2114.29 showed the presence of -C≡C- stretch. The vibrations at 2390.06 assigned to the presence of C≡C stretch. The peak at 3270.51 showed the presence of O-H stretch and the band at 3852.73 was responsible for the presence of alcoholic group. The functional, groups present in all the bands may be responsible for the synthesis of SNP in algal biomass. (Fig.9)
The FT- IR spectrum of the sea weed, Padina antillarum showed the peak at 1635.87, 2117.83, 2389.09, 3284.26, 3854.01 cm-1

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Then band 1635.87 cm-1 responsible for the presence of N-H bend or C=O stretch. The peak at 2117.83 cm-1 assigned to the presence of C stretch. The peak at 2389.09 cm-1 was the presence of C≡C stretch. The band at 3284.26 was responsible for O-H stretch. The peak at 3854.01 was assigned to the presence of alcoholic group. These functional groups may be responsible for the synthesis of silver nano particles.(Fig..10)

The FT- IR Spectrum of Gracilaria corticata showed the peak at 1634.94, 2082.56, 2388.52, 3269.63 and 3852.40 cm-1. The peak at, 1634.94 cm-1 may be assigned to the presence of primary amines -N-H bend. The band at 2082.56 cm-1 showed the presence of -C≡C- stretch. The band at 3269.63 assigned to the presence of O-H stretch and 3852.40 cm-1 showed the presence of alcoholic group. The functional group present in these bands may be responsible for the synthesis of SNP in algal biomass. (Fig..11)

The FT- IR Spectrum of the sea weed, Eucheuma denticulatum, showed the peak at, 1635.31, 2389.55, 3270.29 and 3852.23 cm-1. The band at 1635.31 cm-1 assigned to the presence of N-H bend or C=O stretch. The peak at 2389.55 cm-1 was responsible for the presence of C≡C stretch. The peak at 3270.29 cm-1 indicate the presence of O-H stretch and 3852.23 cm-1 showed the presence of alcoholic group. The functional groups present in these bands are responsible for the synthesis of SNPs. (Fig..12).

UV-Result

The synthesized silver nano particles was confirmed by the formation of brown colour. The reduced brown coloured molecules of Sargassum muticum (Fig..13), Turbinaria Ornata (Fig.14.), Ulva lactuca (Fig15), Padina antillarum (Fig.16), Gracilaria Corticata(Fig.17) ands Eucheuma denticulatum(Fig.18) was analysed by UV-VIS Spectrophotometer.The nano particles from sea weeds showed the peak at 400nm.

SEM – Result :

This study showed the surface morphology of the silver nano particles synthesized by S. muticum, T. ornata, U.lactuca, P.atillarum, G.corticata, E.denticulatum confirmed the presence of Ag nano particles. From the image, (Plates.9,10,11,12,13and14) we conclude that the nano particles were clustred. The particles are in different shapes

Statistical analysis:

One way ANOVA was carried out between the antibacterial activity of crude and reduced silver nano particles.They were found to be significantly($P<0.05$) different. (Tables.6,7,8,9and10)

Table.1:showing antibacterial activity of crude sea weed extracts and extracts with Ag NPs against Vibrosps

	S.muticum	*T.ornata*	*U.lactuca*	*P.antillarum*	*G.corticata*	*E. denticulatum*
METHANOL	1.5±0.7	1.5±0.5	1±0.5	1.5±0.5	1±0.5	1.2±0.6
ETHANOL	3.5±0.5	2.5±0.5	1.1±0.7	1.5±0.5	1±0.5	1.5±0.5
ACETONE	2.5±0.5	2±0.5	1.8±0.7	1±0.5	0.8±0.2	1.2±0.7
Ag NPs	5±1	4.5±1.3	2.5±0.5	2.5±0.5	3.8±0.7	2.8±0.7

Table. 2:showing antibacterial activity of crude sea weed extracts and extracts with Ag NPs against Aeromonashydrophila

	S.muticum	*T.ornata*	*U.lactuca*	*P.antillarum*	*G.corticata*	*E. denticulatum*
METHANOL	2±1	1±0.5	1.5±0.7	1.5±0.7	2.5±0.5	2.8±0.7
ETHANOL	1±0.5	0.8±0.2	1.5±0.5	1.1±0.7	1.5±0.8	2.5±0.5
ACETONE	1.1±0.7	0.8±0.2	1.5±0.5	1.5±0.5	2.5±0.5	1.5±0.5
Ag NPs	3.5±0.5	3.8±0.7	5.8±0.7	3.8±0.7	5±1	2.5±0.5

Table .3:showing antibacterial activity of crude sea weed extracts and extracts with Ag NPs sagainstYesinia enterocolitica

	S.muticum	T.ornata	U.lactuca	P.antillarum	G.corticata	E.denticulatum
METHANOL	1.5±0.5	2.5±0.5	1.5±0.5	1.8±0.7	1.1±0.7	0.8±0.2
ETHANOL	1.5±0.5	2±0.4	1±0.5	1.5±0.5	0.8±0.2	1±0.5
ACETONE	1±0.5	2.5±0.5	2.5±0.5	1.8±0.7	2±0.5	1.5±0.5
Ag NPs	3±1	3.3±0.7	4.6±0.5	3.8±0.7	3.3±0.7	4±0.5

Table .4:showingantbacterial activity of crude sea weed extracts and extracts with AgNPs against Pseudomonas aerugInosa

	S.muticum	*Tornata*	*U.lactuca*	*P.antillarum*	*G.corticata*	*E. denticulatum*
METHANOL	1.5±0.5	0.8±0.2	1.6±0.5	0.8±0.2	1.5±0.5	1.1±0.7
ETHANOL	1.1±0.2	0.8±0.2	2.3±0.2	2.0±0.4	2.5±0.5	2±0.5
ACETONE	1.3±0.5	1±0.5	0.8±0.2	1.5±0.5	2±0.4	3±0.7
Ag NPS	1.5±05	2.5±0.5	3.8±0.7	1.8±0.7	3.8±0.7	4.5±0.5

Table .5: showing antibacterial activity of crude sea weed extracts and extracts with Ag NPs against Flavobacteriumsps

	S.muticum	*T.ornata*	*U.lactuca*	*P.antillarum*	*G.corticata*	*E.denticulatum*
METHANOL	1.5±0.5	2.1±0.2	0.8±0.2	2.5±0.5	2±0.5	2.8±0.7
ETHANOL	1.3±0.5	1.5±0.5	1.1±0.7	0.6±0.2	1±0.5	1.1±0.7
ACETONE	0.6±0.2	1.8±0.7	0.8±0.2	1.5±0.5	0.8±0.2	1.5±0.5
Ag NPS	2.5±0.5	2.6±0.2	4.8±0.7	2.5±0.5	4.5±0.5	2.5±0.5

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TABLE:6.SHOWING ONE WAY ANOVA FOR ANTIBACTERIAL ACTIVITY OF SEA WEED EXTRACTS AGAINST Vibiriosps

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	Level of significance
Between Groups	18.17333	3	6.057778	9.343616	0.000457	3.098391	P<0.05
Within Groups	12.96667	20	0.648333				
Total	31.14	23					

TABLE 7.SHOWING ONE WAY ANOVA FOR ANTIBACTERIAL ACTIVITY OF SEA WEED EXTRACTS AGAINST A.hydrophila

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	Level of significance
Between Groups	28.42833	3	9.476111	15.05339	2.33-05	3.098391	P<0.05
Within Groups	12.59	20	0.6295				
Total	41.01833	23					

TABLE .8:SHOWING ONE WAY ANOVA FOR ANTBACTERIAL ACTIVITY OF SEA WEED EXTRACTS AGAINST Y.enterocolitica

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	Level of significance
Between Groups	20.77458	3	6.924861	22.50158	1.2806	3.098391	P<0.05
Within Groups	6.155	20	0.30775				
Total	26.92958	23					

TABLE .9:SHOWING ONE WAY ANOVA FOR ANTIBACTERIAL ACTIVITY OF SEA WEED EXTRACTS AGAINST P.aerusginosa

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	Level of significance
Between Groups	10.46458	3	3.488194	5.105297	0.008735	3.098391	P<0,05
Within Groups	13.665	20	0.68325				
Total	24.12958	23					

TABLE 10:SHOWNG ONE WAY ANOVA FOR ANTIBACTERIAL ACTIVITY OF SEA WEED EXTRACTS AGAINSY Flavobacteriumsps

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	Level of signifcance
Between Groups	17.71458	3	5.904861	11.44168	0.000138	3.098391	P<0.05
Within Groups	10.32167	20	0.516083				
Total	28.03625	23					

FIG.1.Antibacterial activity of sea weed crude extracts and extracts with Ag NPs against...

Fig.2 :Antibacterial activity of sea weed crude extracts and extracts with Ag NPs against *Aeromonas hydrophila*

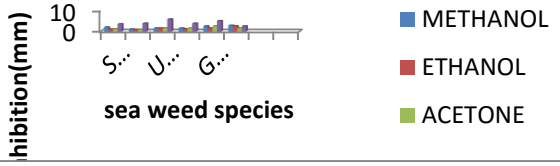

Fig 3:Showing antibacterial activity of sea weed crude extracts and extracts with Ag NPs *against Yersinia enterocolitica*...

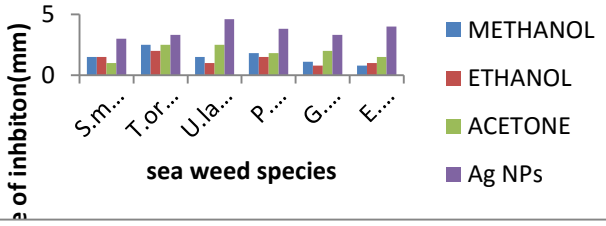

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Fig .4: Showing antibacterial activity of sea weed crude extracts and extracts with Ag NPs against *Pseudomonas aeruginosa*

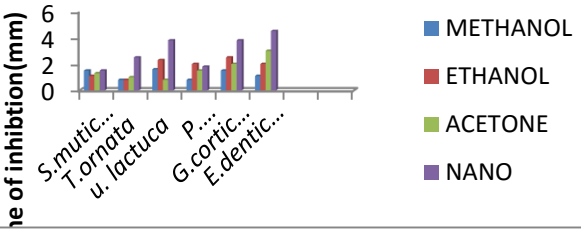
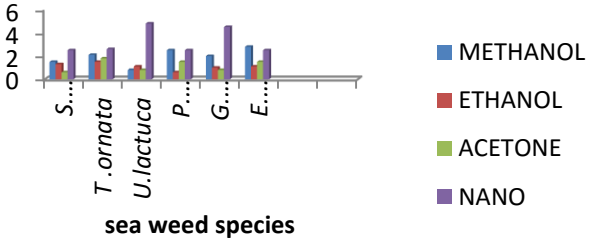


Fig.5:Antibacterial actvity of sea weed crude extracts and extracts with Ag NPs against *flavobacterum sps*



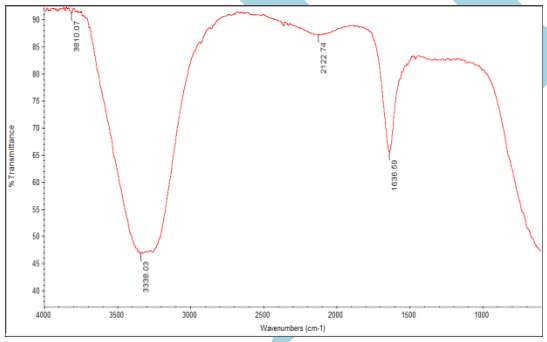


Fig 6: Showing FT-IR spectrum of S. muticum

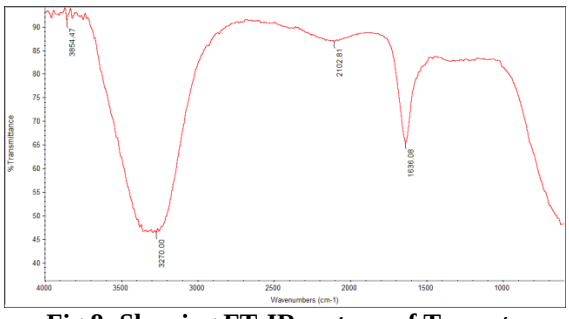


Fig.8 :Showing FT-IR sectrumof T.ornata

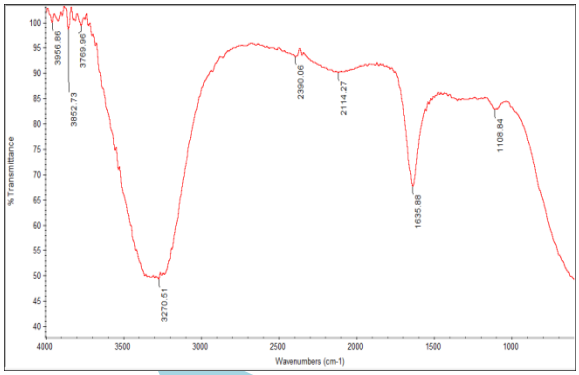


Fig .9: Showing FT-IR spectrum of U.lactuca

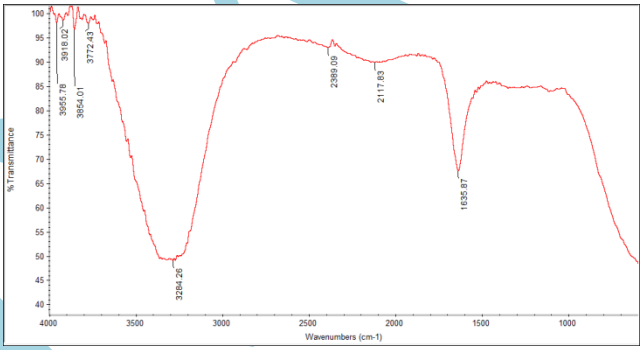


Fig.10 :Showing FT-IR spectrum of P.antillarum

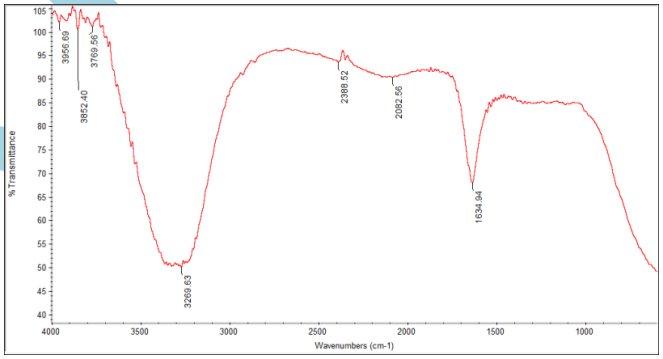


Fig.11:showing FT-IR spectrum of G.corticata

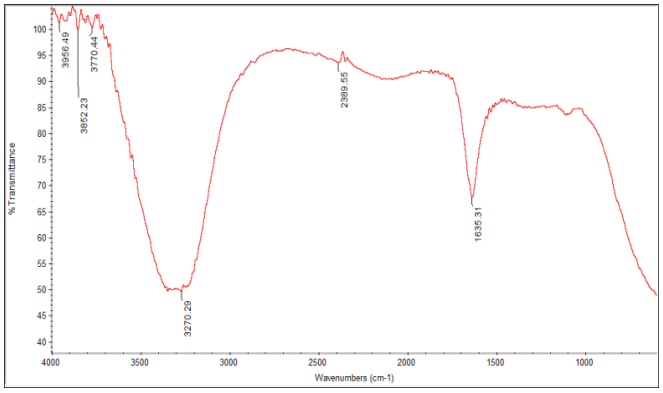


Fig.12: Showing FT-IR spectrum of E.denticulatum

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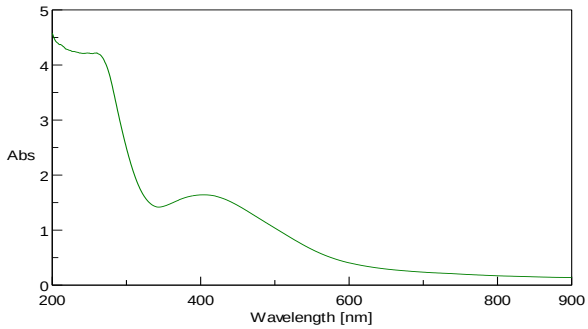

FIG.13.Showing the UV visible spectrum of Sargassummuticum

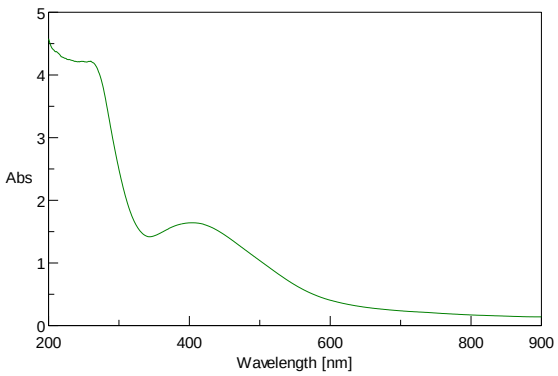

Fig.17. Showing the UV visible spectrum of G.corticata

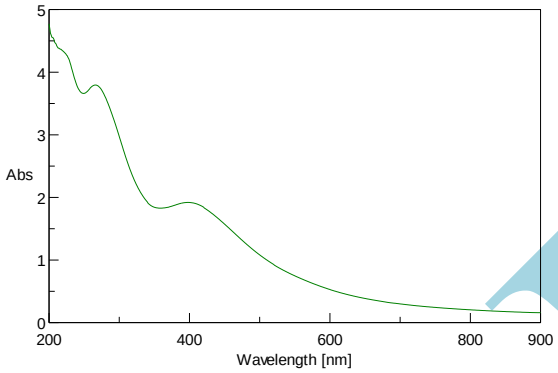

Fig.14. Showing the UV visible spectrum of T. ornata

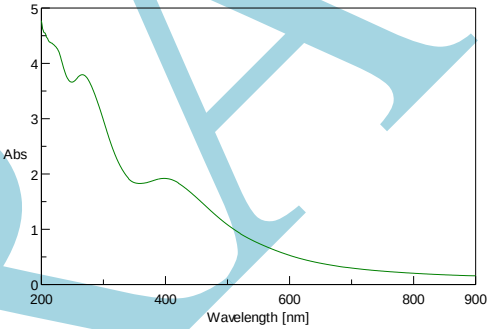

Fig.18.Showing the UV visible spectrum of E.denticulatum

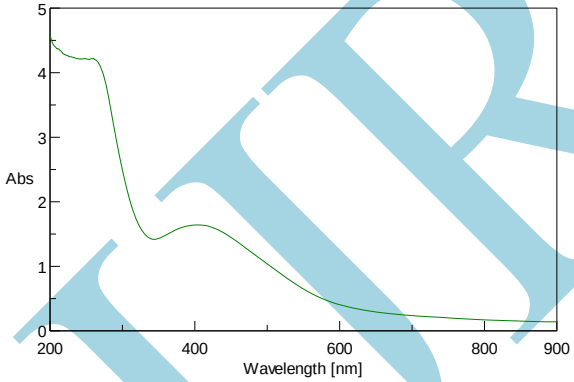

Fig.15.Showing the UV visible spectrum of U.lactuca

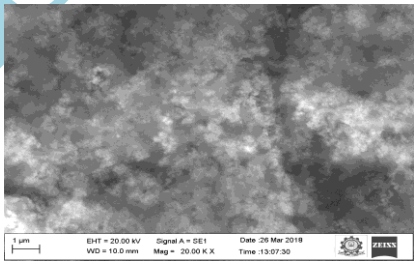

Plate9.:showing SEM image of S.muticum

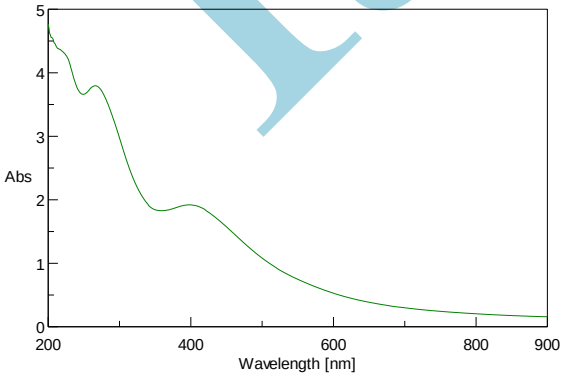

Fig.16. Showing the UV visible spectrum of P.antillarum

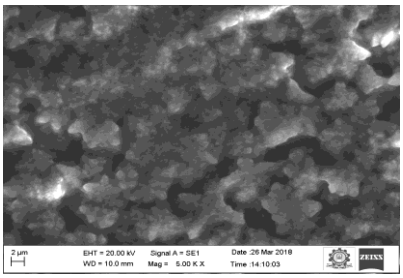

Plate.10:showing SEM image of T.ornata

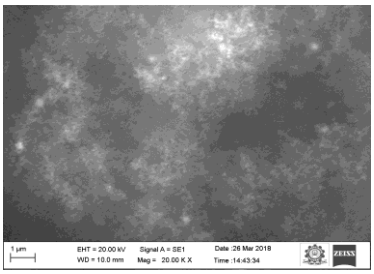

Plate.11:showing SEM image of Ulva lactuca

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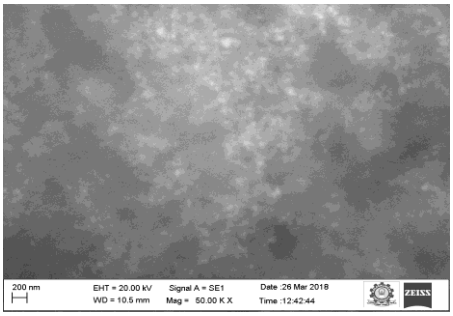

Plate.12 : showing SEM image of P.antillarum

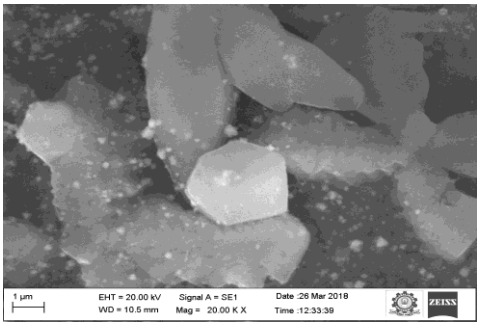

Plate.13 : showing SEM image of G.corticata

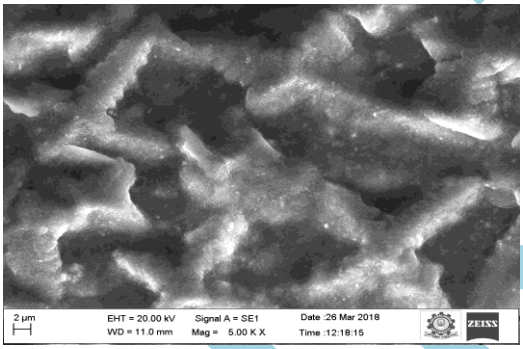

Plate.13 : showing SEM image of E.denticulatum

DISCUSSION AND CONCLUSION:

There are many studies revealed the antibacterial activity of sea weed extracts.The present study reveals that the antibacterial effect of Ag NPs extracts was high when compared with crude extracts.The silver nano particles in different sizes were obtained from the reaction medium which can be directly put forward to various biomedical applications because of the green technology procedure in their synthesis. This is the route in which there is no involvement of any toxic or hazardous reducing agents, capping or dispersing agents. The present study is simple, safe, less expensive, non-toxic and eco-friendly as compared to the toxic chemical process. Marine algae could thus be used as an efficient choice of the cost intensive conventional methods in the development of value-added products for biomedical and nanotechnology based industries.

In conclusion, the bio-synthesis of silver nano particles six different types of seaweeds have been demonstrated. This green synthesis of SNPs has many advantages such as case with which the process can be scaled up, economic viability etc. Applications of such Nano particles are medical and other applications make this method potentially use feel for the large scale synthesis of other inorganic nano materials. Toxicity studies of Ag-Nps on fish pathogen open a door for a new range of antibacterial agents.

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