

ISSN 0971-8656

Seshaiyana



ENVIS RP on
Indian estuaries, mangroves, coral reefs and lagoons
Quarterly Newsletter

Volume 29

Issue 3

Third quarter (Oct.-Dec.), 2021



(Credit: Photo credit: Jason flower)

Vibrant shallow reef with *Caulerpa racemosa*

ENVIS RESOURCE PARTNER

Ministry of Environment, Forest and Climate Change, Government of India
New Delhi - 110 003

CENTRE OF ADVANCED STUDY IN MARINE BIOLOGY
FACULTY OF MARINE SCIENCES
ANNAMALAI UNIVERSITY
PARANGIPETTAI - 608 502, TAMIL NADU
E-mail: casmb@envis.nic.in
Website: <http://casmbenvis.nic.in>

Seshaiyana

Quarterly newsletter of ENVIS RP on Indian estuaries, mangroves, coral reefs and lagoons

Volume 29

Issue 3

Third quarter (Oct.-Dec.), 2021

ISSN 0971-8656

Chief Editor and ENVIS RP Co-ordinator

Prof. Dr. M. Srinivasan

Director and Dean

Editors

Prof. Dr. L. Kannan

Former Director & Honorary Professor

Prof. Dr. S. Ajmal Khan, Honorary Professor

ENVIS Team

Dr. T. Lenin: Programme Officer

Mrs. L. Vijayalakshmi: Information Officer

Mr. B. Senthilkumar: I.T. Officer

Mr. A. Subramanian: Data Entry Operator

Mr. R. Nagarajan: Office Assistant

Faculty Team

Dr. P. Anantharaman, Professor

Dr. G. Ananthan, Associate Professor

Dr. T. Ramanathan, Associate Professor

Dr. K. Sivakumar, Associate Professor

Dr. B. Deivasigamani, Associate Professor

Dr. P. Sureshkumar, Assistant Professor

Sponsored by

Ministry of Environment, Forest and Climate Change
Government of India, New Delhi

Instructions to Authors

We welcome research and popular articles, notes, news, snippets and cartoons or any scientific information on our core subjects—estuaries, mangroves, coral reefs and lagoons.

The articles should not exceed five typed pages in double space.

Figures should be clear for good reproduction.

References should be limited and cited in the text by name and year. *Council of Science Editors Style* may be referred to for listing references at the end.

E-mail your articles in MS Word 2003 or 2007 to:

casmb@envis.nic.in

or

Send both the hard and soft copies to:

ENVIS RP Co-ordinator

Centre of Advanced Study in Marine Biology

Faculty of Marine Sciences

Parangipettai - 608 502, Tamil Nadu, India

We invite comments, suggestions and constructive criticism on the current issue of *Seshaiyana* from the authors and readers.

Disclaimer

Authors are solely responsible for the scientific facts and copyright permissions if they have reproduced materials or quoted lines from other sources. They are also responsible for the publication of the same in the past or in the future.

CONTENTS

Phytochemical Analysis and Antibacterial Activity of the Seaweed, <i>Caulerpa racemosa</i> Against Clinical Pathogens	1
Activities of Environmental Information System Resource Partner	6
Florida Manatee in a Fresh Water Spring	7

Editors' Desk

Dear Readers,

Greetings from the ENVIS RP of CAS in Marine Biology, Annamalai University.

*The research article in the issue describes about the bio-active potential of the seaweed *Caulerpa racemosa* and the capability of the seaweed in the synthesis of novel antimicrobial compounds.*

Further, the newsletter showcases the activities of ENVIS RP viz. National Children's Day, National Pollution Control Day and Webinar on Biodiversity.

In addition, this issue has news on Florida Manatee in a fresh water spring.

We request all the readers to contribute articles to Seshaiyana. We also invite your comments and valuable feedback to improve the contents of the newsletter.

Editors

Phytochemical Analysis and Antibacterial Activity of the seaweed, *Caulerpa racemosa* Against Clinical Pathogens

Introduction

Marine ecosystem is the largest aquatic system on the planet. Its size and complexity make it difficult to deal with as a whole. Marine natural products encompass a wide variety of chemical classes, including terpenes, polyketides, acetogenins, peptides and alkaloids of varying structures and a multitude of compounds of mixed biosynthesis. Approximately 5000 compounds have been isolated from organisms living in shallow waters upto 900 m depth of the sea (Sandell *et al.*, 2004). Seaweeds are marine macroscopic algae without true root, stem and leaves. They form one of the commercially important marine living renewable resources. Edible seaweeds are widely consumed, especially in Asian countries as fresh, dried or as ingredients in prepared foods. From the view point of foodstuff sources, seaweeds provide with high nutritional compounds, fatty acids and free amino acids (Norziah and Ching, 2000; Yoshie *et al.*, 2000). As seafood, seaweeds are a rich source of minerals, especially micro and macronutrients necessary for human nutrition (Wong and Cheung, 2000). Presently, infectious diseases are responsible for high morbidity and mortality rate and are considered a public health problem because of their frequency and severity. For the treatment of these diseases, people often use synthetic drugs. But, bacteria have developed a resistance mechanism to fight against most of the synthetic antibiotics. The multidrug resistance of microbes is due to indiscriminate use of commercial antimicrobial medicines. Hence search for new antimicrobial substances is in progress from several medicinal plants and seaweeds (Alagesaboopathi and Kalaiselvi, 2012).

Materials and methods

Collection of seaweeds

The green seaweed *Caulerpa racemosa* was collected from the coast of Ramashwaram, Tamil Nadu, India.

Preparation of seaweed extracts

The collected *C. racemosa* samples were cleaned and the necrotic parts were removed. Samples were washed with tap water to remove any associated debris

and shade dried at room temperature ($28\pm 2^{\circ}\text{C}$) for 5-8 days. The samples after drying were weighed and then chopped. The chopped samples were finely powered using a clean motor and pestle. The finely powered samples were weighed and 5 g of samples was dissolved in organic solvents, ethanol and chloroform and kept for 48 hours at room temperature and mixed at regular intervals. After 48 hours, the samples dissolved in each solvent were filtered using Whatman filter paper to separate the filtrate for further use in antimicrobial testing of *C. racemosa* samples.

Test microorganisms

Pure cultures of *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were used as the test microorganisms for antibacterial testing.

Preparation of inoculum

From the 24 hours incubated nutrient agar slant of each test organism, a loop full of the bacteria was inoculated in nutrient broth at pH 7.4 so as to activate the bacterial strain used as test organism. The broths were kept for incubation at 37°C for 24 hours so that the microorganism can reach the log phase. A nutrient broth was maintained as control without inoculating the test organism.

Antibacterial activity test

Antibacterial activity was assayed using the agar well diffusion test. Muller Hinton Agar Medium (MHA) was prepared; pH was maintained at 7.4 and then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. A sterile cotton swab was used for spreading the test organism from the 24 hours inoculated broth evenly on the MHA plates. Different concentrations of the seaweed extract were added to the respective wells on the MHA plates. Concentrations of 20, 60, 80 and 100 μl were placed in the wells and allowed to diffuse at room temperature for 30 minutes. The extract loaded plates were kept for incubation at 37°C for 24 hours. After incubation, a clear zone was observed around the well which showed the presence of antibacterial active compounds in the algal extracts. Diameters of the zone of inhibition were measured in mm (including the diameter of the well).

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) profiling was done for the seaweed extracts in solvent system of ethanol and chloroform in proportions of 6:4. The plates developed light pink spot in both the solvent systems when the TLC plate was sprayed with ninhydrin. The plate showing pink spots indicated the presence of amino acids and peptides.

FT-IR analysis

Approximately 5 mg of sample mixed with 1000 mg of dried KBr was subjected to a pressure of 5×10^6 pa and made into clear pellet of 3 mm diameter with 1mm thickness. Nicolet Avatar-360 FTIR Spectrometer with KBr beam splitter and air-cooled DTGS detector was used to capture absorbance spectra. The baseline approach was used to calculate the peak's absorption of light intensity. All sharp bands' frequencies were consistent at 0.01 cm^{-1} (Abu *et al.*, 1991).

Phytochemical screening

Phytochemical analyses were carried out for all the extracts as per the standard methods. The crude extracts and the extracts obtained from fractionation of the crude methanolic extracts were screened for the presence/absence of phytochemicals such as steroids, flavonoids, tannins, saponins, alkaloids, terpenoids and glycosides using the method described by Harborne (1985).

Results

Antibacterial property of aqueous extracts of the seaweed (*C. racemosa*) was analyzed by Disc diffusion method. In this present study, five bacterial pathogens such as *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were used as test organisms and Ampicillin as positive control. To evaluate the antibacterial efficacy of ethanol and chloroform extracts, the zone of inhibition (mm) was measured after 24 hours of incubation and compared with the control (Tables 1 and 2).

Table 1. Antibacterial activity of ethanol extract of *C. racemosa* against clinical pathogens.

S. No.	Bacterial strains	Concentration of seaweed extract				Positive control (Ampicillin)
		30 µg/µl	40 µg/µl	60 µg/µl	80 µg/µl	
1	<i>E. coli</i>	3mm	5mm	10mm	11mm	8mm
2	<i>K. pneumoniae</i>	4mm	5mm	8mm	10mm	10mm
3	<i>S. aureus</i>	Nil	5mm	9mm	11mm	3mm
4	<i>B. subtilis</i>	3mm	5mm	11mm	13mm	4mm
5	<i>P. vulgaris</i>	Nil	4mm	5mm	8mm	5mm

A

Ethanol extract concentration varied from 20 to 80 µg/µl and the higher zone of inhibition was noted at 80 µg/µl for all pathogens. It denoted that the higher concentration of seaweed extract is more active against the clinical pathogens.

Table 2. Antibacterial activity of chloroform extract of *C. racemosa* against clinical pathogens.

S. No.	Bacterial strains	Concentration of seaweed extract				Positive control (Ampicillin)
		20 µg/µl	40 µg/µl	60 µg/µl	80 µg/µl	
1	<i>E. coli</i>	Nil	Nil	5mm	7mm	8mm
2	<i>K. pneumoniae</i>	Nil	Nil	Nil	5mm	10mm
3	<i>S. aureus</i>	Nil	Nil	Nil	Nil	3mm
4	<i>B. subtilis</i>	Nil	Nil	8mm	12mm	4mm
5	<i>P. vulgaris</i>	Nil	Nil	5mm	7mm	8mm

A

Chloroform extract concentration varied from 20 to 80 µg/ml where the higher zone of inhibition was noted at 80 µg/µl for all the pathogens. It denoted that the higher concentration of seaweed extract is more active against the clinical pathogens.

Thin Layer Chromatography (TLC)

TLC is one of the valuable methods to detect the phytochemistry of the biological samples. TLC profiling of seaweed ethanol extract and chloroform extract were tested. In the TLC plates, the extracts were partitioned in the pre-coated silica gel. The plate was sprayed with ninhydrin solution which resulted with light pink spots due to the presence of amino acids. The fractions on the plates varied depending on the solvent system (Polar to Non polar) and the running distance by the component present in the solvent extracts.

Phytochemical screening test

Phytochemical assay of algal extracts showed the presence of compounds such as alkaloids, phytosterols, phenolic ferric chloride, flavonoids, saponins, and aminoacids.

FTIR analysis

Major chemical constituents of the *C. racemosa* extracts through FTIR analysis revealed the presence of main chemical group viz. phenols namely nitro compounds, aromatics, alkanes, 1^o and 2^o amines, alkyl halides which have inhibitory effect. Peak assignments for N-O asymmetric stretch, N-O Asymmetric Stretch, C-C Stretch, C-H bend, N-H Wag, C-Cl Stretch, C-Br Stretch were also observed (Table 3).

Table 3. Band assignments of the FTIR spectra of *C. racemosa*

S. No.	Wave number (cm ⁻¹)	Functional group	Peak assignments
1	1521.84	Nitro compound	N-O asymmetric stretch
2	1489.05	Nitro compound	N-O Asymmetric Stretch
3	1473.62	Aromatics	C-C Stretch (in -ring)
4	1456.26	Alkanes	C-H bend
5	669.30	1°/2° amines.	N-H Wag
6	582.50	Alkyl halides	C-Cl Stretch
7	547.78	Alkyl halides	C-Br Stretch

A Discussion

Seaweeds are important marine and estuarine plants distributed throughout the world. The biomass as well as species composition of seaweeds largely depend on season, population structure and several other ecological factors (Thakur *et al.*, 2008). The exact mechanism and the compounds responsible for their antimicrobial activity are still unclear. Many studies suggest that the higher phenol content of marine macro algae may affect the bacterial growth and their metabolism; they could show antimicrobial properties according to their constitution and concentration (Regunant *et al.*, 2000; Alberto *et al.*, 2001).

Marine organisms including seaweeds are a source of new genes and exploitation of which is likely to yield new drugs. Seaweeds are found to be good sources of proteins, carbohydrates, vitamins and minerals in human nutrition. Seaweeds contain carbohydrates which are different from those of higher land plants, in addition to higher protein content (Arasaki and Arasaki, 1983). Seaweeds show greater variations in their nutrient contents, which are related to several environmental factors, such as water temperature, salinity, light and nutrients (Dawes, 1998).

Seaweeds are known for rich secondary metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industries (Eluvakkal *et al.*, 2010). The zone of inhibition noted varied according to different solvents. Present study revealed that the higher concentration of seaweed extract only is more active against the clinical pathogens tested. Inhibition of *B. subtilis* was higher in both chloroform and ethanol extracts of *C. racemosa*. FTIR analysis of the seaweed extracts revealed the presence of the main chemical groups of phenols namely alkyl halides and aliphatic amines.

Summary and conclusion

Present study concludes that the inorganic solvent extraction was suitable to verify the antimicrobial properties of *C. racemosa*. The investigation on the antibacterial activity of the crude extracts of *C. racemosa* showed that the ethanol extracts showed promising antimicrobial activity when compared to that of chloroform extracts, suggesting that the seaweeds could serve as useful source of new antimicrobial agents. Present study also justifies the claimed uses of seaweeds in the traditional system of medicine to treat various infectious microbial diseases. This study also encourages cultivation of valuable seaweeds at large scale to increase the economic status of the seaweed farmers in the country as seaweeds yield economic, health and environmental benefits.

References

- Abu, G. O., R. M. Weiner, J. Rice and R. R. Colwell, 1991. Properties of an extracellular adhesive polymer from the marine bacterium *Shewanella colwelliana*. *Biofouling*, 3: 69–84.
- Alagesaboopathi, C. and N. Kalaiselvi, 2012. Antimicrobial activities of the root, stem and leaf extracts of *Argemone mexicana* L. *International Journal of Bioscience*, 2(5): 61–68.
- Alberto, M.R., M.E. Faryas and M.C. Manca de Nadra, 2001. Effect of gallic acid and catechin on *Lactobacillus hilgardii* growth and metabolism of organic compounds. *Journal of Agricultural Food Chemistry*, 49: 4359–4363.
- Arasaki, S. and T. Arasaki, 1983. *Vegetables from the sea*. Jaban Publication International, Tokyo, 196pp.
- Dawes, A.C.T., 1998. Marine content of edible marine seaweeds food. 79: 23–26.
- Eluvakkal, T., S.R. Sivakumar and K. Arunkumar, 2010. Fucoidan in some Indian brown seaweeds found along the coast of Gulf of Mannar. *International Journal of Botany*, 6(2): 176–181.
- Harborne, J.B., 1985. *Phytochemical methods*. Chapman and Hall, London, 60–66.
- Norziah, M.A. and C.Y. Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chemistry*, 68: 69–76.
- Regunant, C., A. Bordons, L. Arda and N. Roze, 2000. Influence of phenolic compounds on the physiology of *Ocenococcus oeni*. *Journal of Applied Microbiology*, 88: 1065–1071.
- Sandell, K.C., Peterson, D. Harmody, P.J. McCarthy, S.A. Pomponi and J.V. Lopez, 2004. A molecular systematic survey of sponge derived microbes. *Bollettinoddei Musei e Degli: Institute Biologici dell Universita di, Genova*, 68: 579–585.

Thakur, C.M., C.R.K. Reddy and B. Jha, 2008. Seasonal variation in biomass and composition of seaweeds standard along portokha, North West coast of India. *Journal of Earth System Science*,117:211–218.

Wong, K.H., and P.C.K. Cheung, 2000. Nutritional evaluation of some subtropical red and green seaweeds. Part-I proximate composition, amino acid profiles and some physic-chemical properties. *Food Chemistry*, 71: 475–482.

Yoshie, Y., W. Wata, D. Petillo and T. Suzuki, 2000. Distribution of catechism in Japanese seaweeds. *Fisheries Science*, 66(5):998–1000.

G. Hemapoojavalli^{1*}, C. Thiyagarajan^{2*} and S. Bragadeeswaran^{3*}

¹Department of Microbiology, TBAKC, Kilakarai, Tamil Nadu 623 517, India

²Department of Microbiology, Srimad Andavan Arts and Science College, Tiruchirappalli, Tamil Nadu 620 005, India

³CAS in Marine Biology, Faculty of marine Sciences, Annamalai University, Parangipettai, Tamil Nadu 608 502,

Corresponding Author: poojagopal14@gmail.com

Annamalai University

Activities of Environmental Information System Resource Partner (ENVISRP)
Centre of Advanced Study in Marine Biology (CASMB)

National Children's Day – 14.11.2021

Every year, November 14 is celebrated as Children's Day in India to commemorate the birth anniversary of India's first Prime Minister, Jawaharlal Nehru. The day is dedicated to increase awareness about the rights, care and education of children. It is also a day of offering tribute to Nehruji who loved children and considered them the real strength of a nation and foundation of society.

ENVISRP, CAS in Marine Biology along with Government High School, Mudasalodai, celebrated the Children's Day on November 14th, 2021; 150 school students participated in various competitions such as Essay Writing, Drawing and Quiz.

Mr. Sivakumar, Headmaster, Government High School, Mudasalodai, gave the welcome address and delivered a special lecture about the need of children's day in India.

Prof. Dr. M. Srinivasan, Director and Dean, ENVIS Co-ordinator, gave inaugural address and emphasised on the importance of the 'children's Day. He mentioned that the children should be given integrated education that would build a better society in the future.

Prof. Dr. A. Annatharaman, CAS in Marine Biology delivered felicitation address. Dr.T Lenin, Programme Officer, ENVIS RP proposed the vote of thanks and the programme was successfully organized by the CAS in Marine Biology, ENVISRP team.



Brochure



Inauguration of the Children's day programme



School students, participating in competitions



Group photo with school children

National Pollution Control Day – 02.12.2021

ENVISRP, CAS in Marine Biology (CASMB) observed the 'National Pollution Control Day-2021 and conducted a Webinar on 'Soil Waste Management'. Dr. S. Kumerasan, Assistant Professor CAS in Marine Biology, delivered the welcome address and explained about the significance of pollution control day besides the socio-economic importance of waste management.

Prof. Dr. M. Srinivasan, Dean and Director of the Centre and the ENVIS Co-ordinator who inaugurated the programme recalled the Bhopal Gas tragedy and highlighted the significance of 'National Pollution Control Day'.

The Chief Guest, Dr. Kathirvelu Sambabdan, Head, Department of Botany, Arignar Anna Government Arts College, Karaikkal presided over the function and highlighted various aspects such as types of pollution, hazardous wastes, global warming, solid waste management, sustainable development goals and the mantra namely Reduce, Reuse and Recycle (RRR).

Seventy seven (77) persons attended the Webinar.



Brochure



Participants of National Pollution Control Day function



Chief Guest, explaining about sustainable development

Florida Manatee in a Fresh Water Spring

Florida Manatee (*Trichechus manatus latirostris*) swimming within a fresh water spring on Crystal River in Florida. Note the tree roots on the right of the frame which make up a portion of this unique ecosystem. Fish aggregate around the manatee and eat the algae on the manatee's body.



Copyright © Brian Skerry

Source: <https://ocean.si.edu/ocean-life/marine-mammals/florida-manatee-fresh-water-spring>

Webinar on Biodiversity

“Ek Bharat Shreshtha Bharat (EBSB) Scheme” promotes a sustained and structured cultural connect between citizens of different regions. In a fitting manner, ENVISRP of both CASMB (Tamil Nadu) and DzUM (Tamil Nadu) along with JK ENVIS HUB (Jammu and Kashmir) jointly organized a webinar on biodiversity. Experts of both the states delivered lectures to participants who gained knowledge on the biodiversity of both the states.

Prof. Dr. M. Srinivasan, Dean and Director of the Centre and the ENVIS Co-ordinator welcomed the gathering and inaugurated the programme.

Dr. Majid Farooq, ENVIS, Co-ordinator, Department of Ecology, Environment & Remote Sensing, Kashmir, explained about their ENVIS activities before introducing the Speaker.

The Chief Guest, Dr. Anzar A. Khuroo, Member of J&K Biodiversity Council, Centre for Biodiversity and Taxonomy, University of Kashmir, briefly explained about the “Biodiversity of Jammu and Kashmir”, covering the aspects of major forest types and current threats to them.

Dr. K. Sivakumar, Associate Professor, CAS in Marine Biology highlighted the “Indian Ocean Atoll Mangroves: Diversity and Conservation Perspectives”

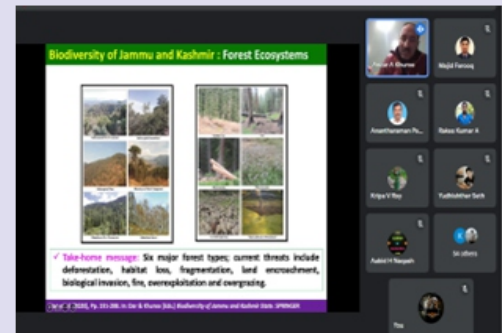
Finally, Dr. C. Arulvasu, Associate Professor, ENVIS Coordinator, Department of Zoology, University of Madras, proposed the vote of thanks.



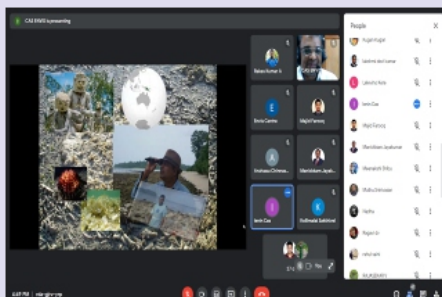
Brochure



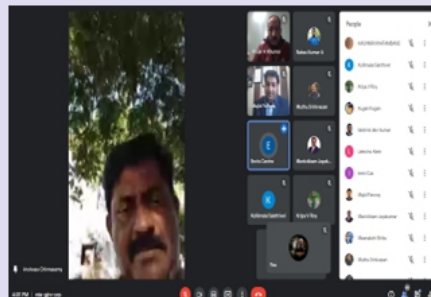
Dr. Majid Farooq, ENVIS Co-ordinator, EE&RS, Department of Ecology, Jammu & Kashmir introducing the speaker



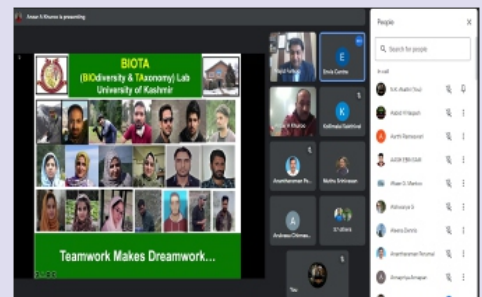
Chief Guest, Dr. Anzar A. Khuroo, explaining about J & K biodiversity



Dr. K. Sivakumar, Associate Professor explaining about mangrove diversity



Dr. C. Arulvasu, Associate Professor and ENVIS Coordinator-DzUM proposing the vote of thanks



Biodiversity webinar participants

Artificial Intelligence

A Basic Approach



Dr. Shweta KR
Dr. Krishnaveni A
Nayantara KR

AN Publications

About the Authors



Dr. Shweta K.R is an accomplished expert in Computer Science, with seven years of experience in teaching and five years of experience in Research & Development. She hold a PhD in Computer Science from Bharathiar University Coimbatore from 2017- 2022, and have conducted extensive research on "Optimized cost estimation using Machine Learning model for software project development in earlier stage".



Dr.A.Krishnaveni is currently working as an Assistant Professor in the Department of Computer Science at Thassim Beevi Abdul Kader College for Women, Kilakarai, Ramanathapuram, Tamilnadu. She was a Doctoral Candidate in Computer Science at the Chikkanna Government Arts College, Tirupur in 2019-2021.



Nayantara Kalathil Radhakrishnan received her master's degree in Information Security from Royal Holloway, University of London in 2023. She graduated from Cochin University of Science and Technology in 2020 with a BTech in Electronics and Communication Engineering. Her research interests include embedded security cryptography, cyber-crime, and human aspects of security

AN Publications

#29, Moorthy street, Balavinayagar
nagar, Tiruvallur-602001, Tamilnadu, India
9087236988



www.anpublications.in



Basics of Computer Networks and OSI Model

Dr.A.Shanthisona
Ms.G.Sharmila
Dr.A.Krishnaveni



AN Publications

About the Authors



Dr.A.Shanthisona is currently working as an Assistant Professor in the PG & Research Department of Computer Science & Placement Coordinator at Tiruppur Kumaran College for Women. She was a Doctoral Candidate in Computer Science at the Vidyasagar College of arts and science, Udumalpet ,Bharathiar University,Coimbatore during 2016-2022.



Sharmila G, MCA, M.Phil, [PhD] is currently working as Associate Professor, in St. Anne's First Grade College for Women, Bengaluru, Karnataka, India. She has more than a decade of teaching experience. Her areas of interest are networks, computer architecture and assembly language.



Dr.A.Krishnaveni is currently working as an Assistant Professor in the Department of Computer Science at Thassim Beevi Abdul Kader College for Women, Kilakarai, Ramanathapuram, Tamilnadu. She was a Doctoral Candidate in Computer Science at the Chikkanna Government Arts College, Tirupur in 2019-2021.



978-81-963342-0-8

AN Publications

#29,Moorthy street,Balavinayagar
nagar,Tiruvallur-602001,Tamilnadu,India
9087236988

www.anpublications.in