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Greensynthesis and Characterisation of Silver Nanoparticles Using *Phallusia nigra*

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ABSTRACT

Biosynthetic technique is used for the production, characterization and application of silver nanoparticles (AgNPs), as reducing agent, which can be utilized in biomedical research and environmental cleaning applications. The reducing agents used to produce the nanoparticles were from the ethanolic extracts made from the simple ascidian Phallusia *nigra*. Prepared AgNP was observed by UV-Visible and IR spectroscopy. UV-Visible spectrum showed a peak as plasmon absorption in the range of 417 and 425 nm. AgNPs characterise their size and shape by Atom Force Microscopy (AFM). XRD pattern showed the characteristic Bragg peaks of (111), (200), (220) and (311) facets of the face center cubic (fcc). Antioxidants present in the ascidian reduce the Ag metal ions, for formation of silver nanoparticles and AgNPs presented in the ethanolic medium were quite stable, even up to 4 months of incubation. This work proved the capability of using biomaterial towards the synthesis of silver nanoparticle, by adopting the principles of green chemistry.

Keywords
Green synthesis, phallusia nigra, AFM.

INTRODUCTION

Marine sedentary organisms of ascidians belong to biofouling community. They are found in piers, pilings, harbour installations, materials used in aquaculture operations etc. *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae. Ascidians are consumed as food in many parts of the world and there are coastal aqua farms in Japan as well as Thailand for the culture of ascidians. *Microcosmus sulcatus*, *Styela plicata* and *Polycarpa pomaria* are taken as food in the Mediterranean.[1] *Halocynthia roretzi* in Japan, is even cultured in the North of Honsyu[2] for human consumption and *Pyura chilensis* is popular in South America[3] as a food source. Margalino and Destefano found that the flesh of *Microcosmus sulcatus* is almost as digestible as whole egg and the protein content higher.[4] Literature survey show that the animal possesses antipyretic[5], analgesic[6], anaesthetic[7] wound healing[8] and antimicrobial activities.[9-13] and Chemical investigation and antioxidant, antitumour effect to DLA, EAC cells using colonial ascidian has been done [14- 24]. The objective of this work is to investigate the silver nanoparticles in *Phallusia nigra*. In the present report, we report the synthesis and Characterization of silver nanoparticles of simple ascidian *Phallusia nigra* using for the first time. The morphology structure and stability of the synthesized silver nanoparticles were studied using scanning electron microscope (SEM and AFM studies).

Materials and methods

Collection and identification

Green Gate area (8˚48’N and 78˚11’E) of Thoothukudi Port, Tamil Nadu, *Phallusia nigra* (Plate:1) was collected by SCUBA diving and identified using Key to identification of Indian ascidians.[26] A voucher specimen (AS 2083) was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002 Tamilnadu, India.

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Plate 1: *Phallusia nigra* Sav.

Preparation of extract

The whole animal was dried, homogenized to get a coarse powder. The powder was successively extracted with various solvents such as petroleum ether (400-600 °C), benzene, chloroform, ethanol, methanol and water. The ethanolic extract of *Phallusia nigra* was used to prepare nanoparticles like silver.

Synthesis of silver nanoparticles

A. Preparation of Silver nitrate

Silver nitrate (analytical grade) was prepared by dissolving the solid $AgNO_3$ in DI water.

B. Preparation of *Phallusia nigra* extract

Phallusia nigra simple ascidian, was collected and washed thoroughly to remove the adhering soil and dust and heated 100 g/L fine piece at 80◦C for 30 min followed by filtering through filter paper to separate out the broth. The pH of the extract was 4.10. The extract was stored at 4◦C for further experiments

C. Silver nano particles

Phallusia nigra extract is used to produce silver nanoparticles, when *nigra* extract is mixed with AgNO3 solution in 1:8 ratio reduction is followed by on immediate change in yellowish to brown color in the aqueous solution of the plant extract due to excitation of surface Plasmon vibration in silver nanoparticle. Further formation of AgNPs in aqueous extract can be monitored by color change.

AgNPs exhibit this yellowish-brown color in aqueous solution due to excitation of surface plasmon resonance in the AgNPs.

Characterisation:

FTIR

The FT-IR spectra of the samples were determined using a fourier transform Infrared spectrometer.

UV-Visible Spectroscopy

The surface Plasmon resonances (SPR) of synthesized nanoparticles have been studied by UV-

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Vis double-beam bio-spectrophotometer.
After the addition the *Phallusia nigra* extract into the aqueous solution of silver nitrate, the solution was filled in glass cuvette of path length 10mm and UVVis spectral analysis has been done in the range of 300 to 700 nm. DI water was used as blank.

XRD Analysis

Synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy (Philips PAN analytical) with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. The particle size of the prepared samples were determined by using Scherrer′s equation as follows $D\approx 0.9\boldsymbol{\lambda}\beta\cos\theta$ Where D is the crystal size, λ is the wavelength of X-ray, Ɵ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

SEM Analysis

Silver nanoparticles from *Phallusia nigra* extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs, of the addition of zinc nitrate solutions, the SEM slides were prepared by making a smear of the solutions on slides by an accelerating voltage 20 KV.

AFM studies

A thin film of the prepared sample of silver NPs were deposited on a silica glass plate by dropping few drops of the AgNPs on the plate and allowed to dry at room temperature in the dark (to avoid nanoparticles diameter growth due to temperature and/or light).

Antioxidant Studies

Cyclic voltammograms trace the transfer of electrons during an oxidation-reduction (redox) reaction. The system starts off with an initial potential at which no redox can takes place.
The shape of the voltammogram depends on the mechanism of the electrode process. The number of electrons transferred in each peak can be determined by cyclic voltammetry.

Results and Discussion

IR Analysis

The IR spectrum of Ag nanoparticles shown band at 3452.32 cm-1, 2384.41 cm-1, 1629 cm-1, 1383.53 cm-1corresponds to O-H stretching, H bonded alcohols and phenols, Carbonyl stretching, N-H bond 1°amines correspons to C-N stretching of the aromatic amino group and C-O stretching alcohols, ethers respectively in figure 1. FTIR spectrum of Ag Nano particles suggested that Ag nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acids. According to Niraimathi *et al.*, (2013) the FT-IR spectra suggest that the proteins present in the plant extract acting as a capping agent. 624.34 cm-1 shows any metal in the extract.

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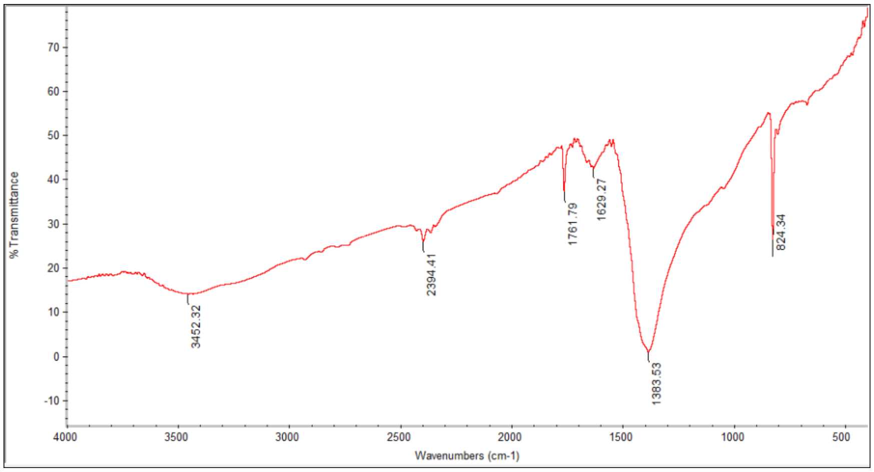

Figure 1: IR spectrum for Ag nano particle

UV Spectrophotometric analysis:

Silver nanoparticles (AgNPs) appear yellowish brown in colour in aqueous medium as a result of surface Plasmon vibrations. As extracts were added to aqueous silver nitrate solution, the colour of the solution changed from faint light to yellowish brown to reddish brown and finally to colloidal brown indicating AgNP formation. Similar changes in colour have also been observed in previous studies and hence confirmed the completion of reaction between extract and AgNO3. This was confirmed by the UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of AgNPs formed in the reaction media has absorption peak at 451 nm due to surface plasmon resonance of AgNPs. The UV-vis spectra recorded, implied that most rapid bioreduction was achieved using nigra extract as reducing agent. This was denoted by broadening of the peak which indicated the formation of polydispersed large nanoparticles due to slow reduction rates. The UV-vis spectra also revealed that formation of AgNPs occurred rapidly within the first 15 mins only and the AgNPs in solution remained stable even after 24 h of completion of reaction.

Table 1 UV Analysis of Ag nano particles

FREQUENCY	**OD**
300	0.2636
310	0.2715
320	0.2805
330	0.31
340	0.333
350	0.2476
360	0.2193
370	0.1846
380	0.1696
390	0.1535
400	0.142
410	0.1385
420	0.1156

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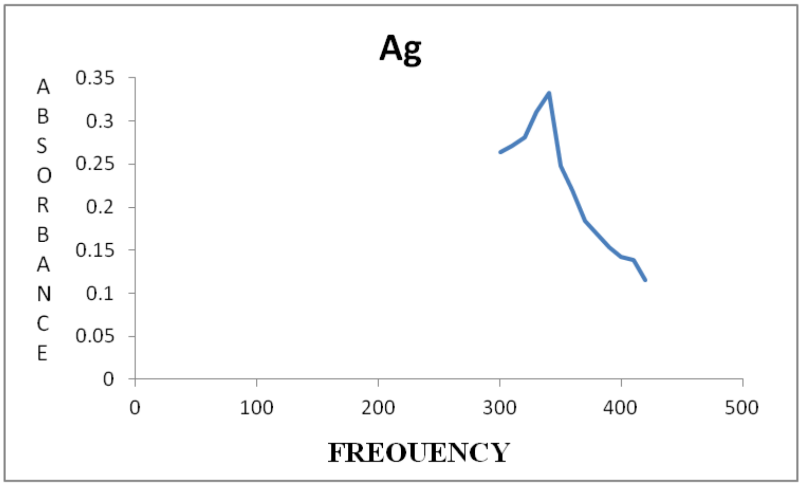

Figure 2: UV spectra for Ag nano particles

XRD Analysis

Table 2: XRD Analysis

2−*THETA*	**FWHM**	**PARTICLE SIZE**	**PLANE**
34.4549	0.1673	51.9467	111
35.5059	0.1338	65.1437	111
54.4249	0.2676	32.1474	22-1

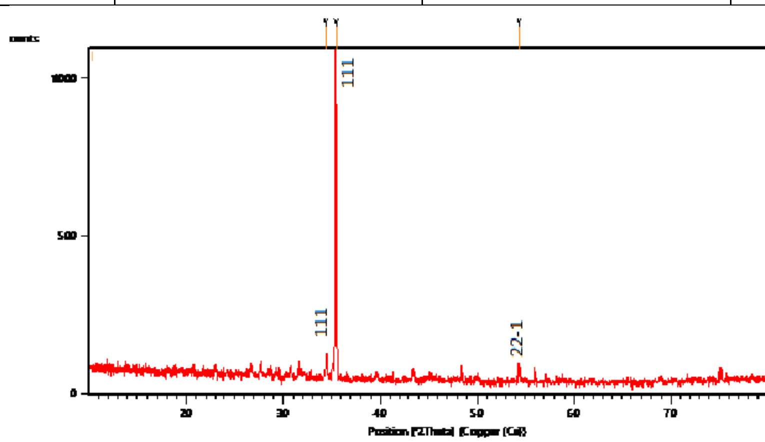

Figure 3: XRD Analysis of Ag nano particles

SEM Analysis

The SEM images of the AgNPs are shown in Figure 4. It is seen that AgNPs of different shapes

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were obtained in case of nigra extract being used as reducing and capping agents. Extract formed approximately spherical and cuboidal AgNPs ,respectively.

The EDX spectrum (Fig. 5) contains intense peaks of Na and Mg in addition to Fe and O. The atomic percentages obtained from EDX quantification were 75.85% of O, 3.35% of Na, 1.68% of Mg and 18.48 % of Ag. These values could be helpful in reflecting the atomic content the surface and near surface regions of the NPs.

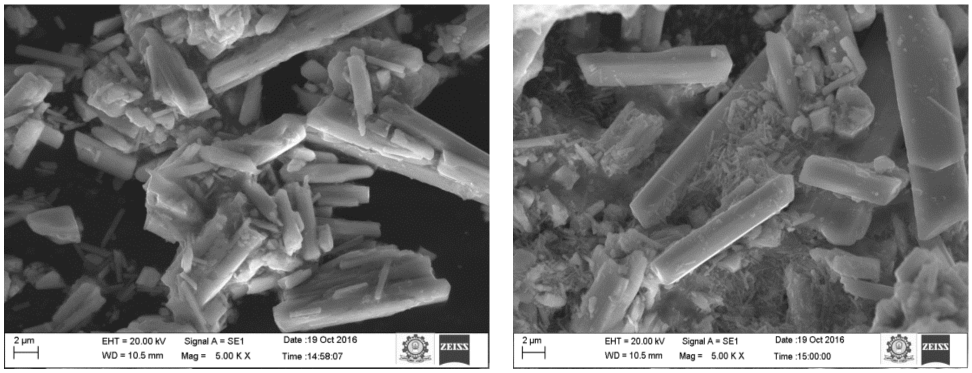

Figure 4: SEM Image of silver nano particles

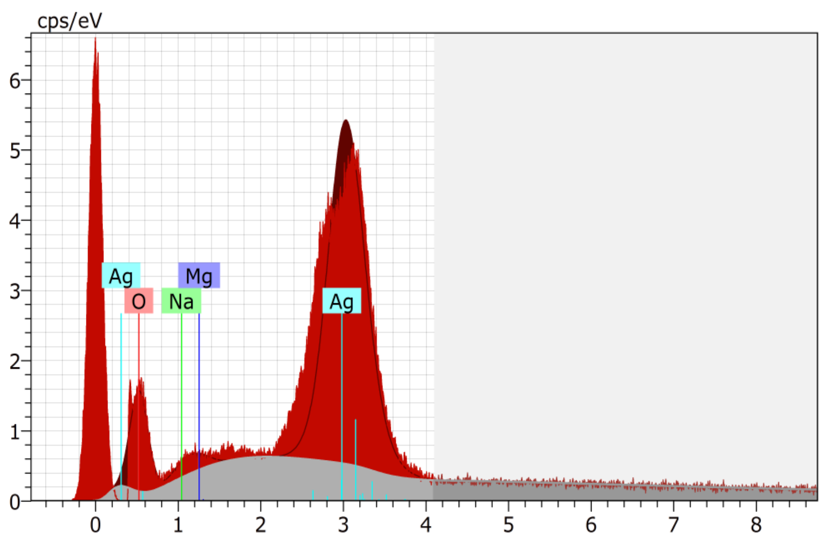

Figure 5 EDX Analysis of silver nano particles

AFM Analysis

The AgNPs was characterized by Atomic Force Microscopy (AFM) (Figures 6). AFM results showed particles with spherical shape surrounded by biological molecules, which prevent AgNPs from aggregation. As shown in Figure 4 the average size of AgNPs is within the nm range.

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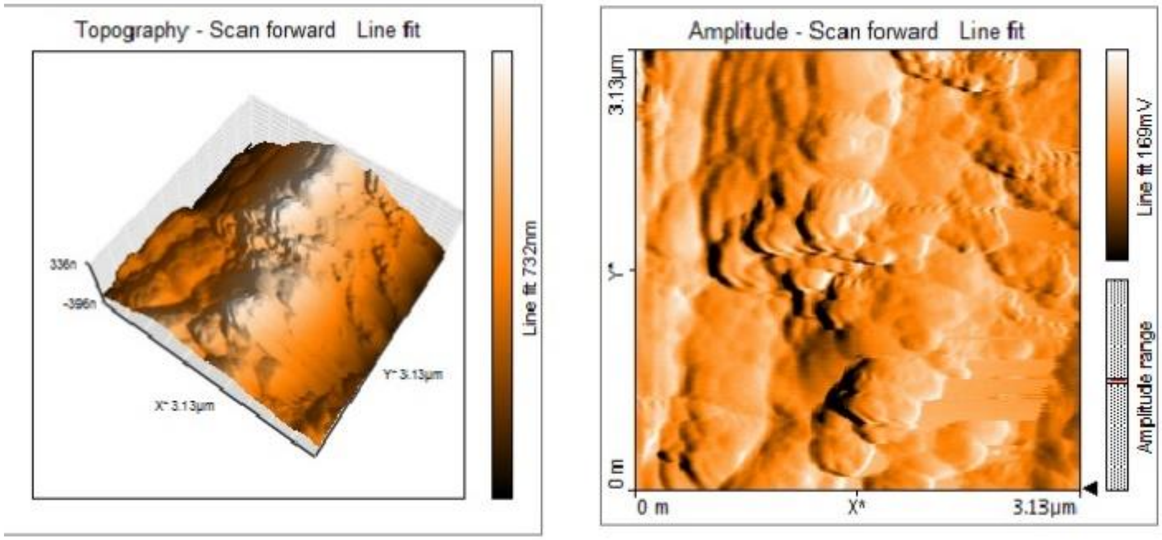

Figure 6 AFM Image of silver nano particle

Antioxidant studies
Cyclic Voltammetric studies

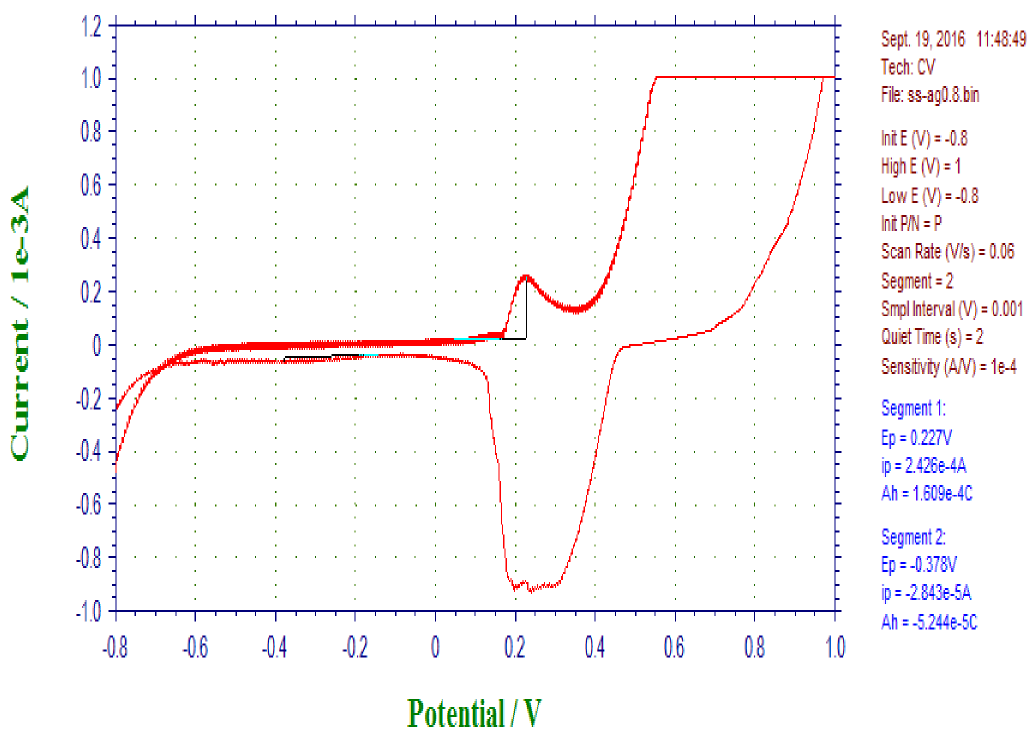

Figure 7Cyclic voltammetic studies using silver Nanoparticle

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Cyclic voltammograms were recorded in the scan rate 0.1 for 1.0 ml of silver nanoparticles synthesised using *Phallusia nigra* extract. The voltammogram of nanoparticles exhibited one sharp anodic peak and cathodic peak. This behaviour is associated with the electroactive nature of silver nanoparticles.

Conclusion

To conclude we have used unreported, inexpensive, nontoxic, ecofriendly and abundantly available *Phallusia nigra* for the rapid synthesis of silver NPs in the acceptable range. This green synthesis approach shows that the environmentally benign and renewable *Phallusia nigra* extract can be used as an effective stabilizing as well as reducing agent for the synthesis of silver nanoparticles.

References

[1] Harant H, Les Tuniciers comestibies. Atti del 11 congresso Inter. Nazionvale d' Igiene di Medicina Mediterranca Palermo, 1951; 1-3.

[2] Tokioka, T., Ascidians of Sagami Bay, "Iwanami Bhoten, Tokyo, 1953.

[3] Van Name, W.G., The North and South American ascidians. Bull. Am. Mus. Nat. Hist., 1945; 84: 1-476.

[4] Margalino, G.A. and Destefano. M., Contributo alla conoscenza della digeribilita delle Ascidie Eduli. Thalassia jonica, 1960; 3: 69-82.

[5] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Antipyretic and Analgesic activity of Phallusia nigra Savigny, 1816. Annals of Biological Research, 2011; 20, 2(4): 192-196.

[6] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D, Anaesthetic activity of Phallusia nigra Savigny. Annals of Biological Research, 2012; 3(4): 1863-1865.

[7] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D, Anti-Inflammatory activity of Simple Ascidian, Phallusia nigra Savigny. International Journal of Pharmaceutical sciences Review and Research, 2013; 22(2): 162-167.

[8] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D.Wound healing activity of the methanolic extract of Phallusia nigra Savigny. International Journal of Chemical and Pharmaceutical Sciences, 2013; 45-51.

[9] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D, Antimicrobial activity of the methanolic extract of Phallusia nigra Savigny. Journal of Natural Product and Plant Resources, 2012; 2(5): 579-583.

[10] Shanmuga priya D, Kohila Subathra Christy H, S. Sankaravadivu. Antimicrobial activity of a simple ascidian, Phallusia nigra. World journal of pharmacy and Pharmaceutical sciences, 2015; 4(7): 822-827.

[11] Shanmuga priya D, Kohila Subathra Christy H, S.Sankaravadivu and C.Stella Packiam Antioxidant activity of the simple ascidian, Phallusia nigra of Tuticorin cost. International journal of Pharmaceutical chemistry, 2015; 410-412.

[12] Shanmuga priya D, Kohila Subathra Christy H, S.Sankaravadivu and C. Stella Packaim Antidiabetic activity of the ethanolic extracts of a simple ascidian, Phallusia nigra. World journal of pharmacy and Pharmaceutical sciences, 2015; 4(11): 1557-1563.

[13] Shanmuga priya D, Kohila Subathra Christy H, S. Sankaravadivu. Hepatprotective activity of the ethanolic extracts a simple ascidian, Phallusia nigra against ccl4 induced hepatotoxicity in rats. World journal of pharmaceutical research, 2015; 5(1): 648-655.

[14] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2013. Infrared andgas chromatogram-mass spectral studies of the ethanolic extract of Ecteinascidia venui

[15] Meenakshi, 2000. International Journal of Chemical and Pharmaceutical Sciences, 4(2):84-89, ISSN: 0976-9390, Impact factor: 0.684

[16] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2013. Spectrophotometric studies of a colonial ascidian Ecteinascidia venui Meenakshi, 2000. International journal of pharmacy and biological sciences, 3(4): 159-163, ISSN: 2321-3272, Impact factor: 0.885, UGC Journal number 46322.

[17] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2015. Preliminary Screening and IR Spectral studies of a colonial ascidian Ecteinascidia venui Meenakshi, 2000. Journal of Chemical, biological and physical sciences, 5(4): 4205-4210, Impact factor: 1.310.

[18] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2016. AntitumourActivity of Ecteinascidia venui Meenakshi, 2000 against Dalton's Lymphoma Ascites. International Journal of Pharma Research and Health sciences, 2016; 4(3): 1214-1222, ISSN: 2348-6465, Impact factor: 0.039.

[19] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2016. Assessment of Acute and Subchronic Oral Toxicity of Ecteinascidia venui Meenakshi, 2000. World Journal of Pharmacy and Pharmaceutical Sciences, 5(7): 1225-1234, ISSN: 2278-4357, Impact factor: 7.421.

[20] Sankaravadivu, S, R. Jothibai Margret and V.K Meenakshi. 2016. In vitro antioxidant studies of a colonial ascidian Ecteinascidia venui Meenakshi, 2000. International Journal of Pharmaceutical Chemistry, 6(6): 169-177, Impact factor: 0.498.

[21] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2016. Ecteinascidia venui Meenakshi, 2000 induces immunomodulations against Dalton's lymphoma ascites. International Journal of Medicinal Chemistry and Analysis, 6(2): 79-86, ISSN: 2279-7595, Impact factor: 1.34.

[22] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2016. Ecteinascidia venui Meenakshi 2000 against Ehrlich Ascites carcinoma - Anticancer Potential. European Journal of Pharmaceutical and Medical Research, 3(9): 329-334, ISSN:2394-3211, Impact factor: 4.8971.

[23] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2016. Immunonature of Ecteinascidia venui Meenakshi, 2000 against Ehlrich Ascites Carcinoma. International Journal of Current Research, 8(11):41678-41684, ISSN: 0975-833X, Impact factor: 7.749.

[24] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2016. Evaluation of anticancer activity of Ecteinascidia venui Meenakssi, 2000 against S-180. Scholars Journal of Applied Sciences, 4(12A):4231-4238, ISSN: 2347-954X, Impact factor: 0.671.

[25] Sankaravadivu, S, R. Jothibai Margret and V.K. Meenakshi. 2018. Synthesis and

characterization of iron oxide nanoparticles using colonial tunicate Ecteinascidia venui. International Journal of Emerging Technology and Innovative Research, 5(7):393 -397, ISSN: 2349-5162, Impact factor: 3.24. UGC journal number 63975.

[26] Meenakshi, V.K. Biology of a few chosen ascidians. Ph. D Thesis, Manonmaniam Sundaranar University, Tirunelveli, 1997; 157-173.

[27] Naheed Ahmad, Seema Sharma, Green Synthesis of Silver Nanoparticles Using Extracts of Ananas comosus, Green and Sustainable Chemistry 02(04):141-147