Int. J. Adv. Res. Biol. Sci. 2(11): (2015): 280–288

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/ 1.15/ijarbs-2-11-35

The diversity of nudibranchs in Andaman Islands and screening of bioactive compounds from *Plakobranchus ocellatus*

Priyanka Devi[1] and Revathi. K[2]*

[1]Research Scholar, [2]Professor and Head, Department of Zoology, Ethiraj College for Women, Chennai-8, Tamilnadu, India
*Corresponding author: *reva63@rediffmail.com*

Abstract

The present study recorded a total number of 19 species of nudibranchs under 11 families from Andaman Islands were identified. The maximum nudibranches was found in Mayabunder (11 species) followed by Burmanallah (4 species) and Pongibalu (4 species). The seven species of Phyllidiidae family and three species of Chromodorididae, are the most species were found in three stations of Andaman Islands. The ethanolic extract of *P. ocellatus* showed the presence of maximum phytochemical compounds such as alkaloids, Anthraquinone, Phytosterols, saponin, steriods, phenolic compounds, Triterpenoids, Fixed oil and Fat, Aromatic acid Gum and Mucilage and Cardiac Glycosides. GC-MS analysis of the ethanolic extract of *P. ocellatus* showed the presence of twelve phytochemical constituents. The rich diversity in bioactive compounds from invertebrates has provided molecules that interfere with the prevention of a disease at many different points, which increase the chances of developing selective drugs against specific diseases.

Keywords: Andaman Islands, nudibranchs, *Plakobranchus ocellatus*, bioactive compounds.

Introduction

The isolation and extraction of novel bioactive secondary metabolites from marine organisms have a biomedical potential for future drug discovery as the oceans cover 70% of the planet's surface and life on earth originates from sea. In the last few decades, however, natural products chemists have started to discover the wealth of bioactive secondary metabolites that are produced by marine invertebrates such as sponges, soft corals, molluscs and others. Wide range of novel bioactive secondary metabolites exhibiting antibiotic, anti-parasitic, antiviral and anti-cancer properties etc., has been isolated from marine organisms and many to be discovered (Simmons *et al*., 2005). Among marine invertebrates, marine molluscs are the good source of bioactive metabolites. Many studies on bioactivity molecules with wide range of activities like antitumor, antiviral, antimicrobial, anti-inflammatory were reported from Molluscan groups (Kamiya *et al*., 1989; Anand *et al*., 1997). Marine invertebrates rely solely on innate immune mechanisms that include both humoral and cellular responses (Wright, 1981).

Nudibranchs are tiny organisms classified under the phylum Mollusca and are known to live in various habitats of the marine ecosystem. Many of the researchers noticed the antimicrobial compounds such as terpenoids, 9-thiocyanatopupukeanane sesquiterpene, deoxymanoalide and deoxy secomanoalide phospholipids, sterols and monoalkyl-diacylglycerol from nudibranchs (Yasman *et al*., 2003; Uddin *et al*., 2009; Sadhasivam *et al*., 2013; Zhukova, 2014). Also, nudibranch tissues and its associate actinomycetes have the potential antibacterial properties against human pathogens (Riyanti *et al*., 2009). Hence the present study was carried out to

prospect the bioactive potential of *Plakobranchus ocellatus* collected from intertidal regions of Andaman Islands, India.

Materials and Methods

Sampling and identification

Nudibranchs were collected during low tide by handpicking method from the three intertidal locations including Mayabunder (Lat: 12˚56.167”N and Long: 092˚58.113’E), in North Andaman and Pongibalu region (Lat: 11 30.956'N and Long: 92 39.206'E) and Burmanella (Lat:11˚30.998'N and Long: 92˚44.100'E) of South Andaman. The samples were kept in sterile seawater in containers and transported to the research laboratory. The specimens were identified based on the morphological characters and available literatures Jensen, 1990a; Gosliner, 1995, Gosliner *et al*., 2008, Rudman, 1982, 1983, 1984, 1986, 1995, and Brunckhorst, 1993. Two web-based portals, the Australian Museum’s Seaslug Forum (http://www.seaslugforum.net/) and Nudi Pixel (http://www.nudipixel.net) were also used for identification.

Preparation of ethanolic extracts

The specimens of *Plakobranchus ocellatus* were cut into small pieces using sterile scissors and homogenized in a mortar and pestle by following aseptic techniques. The homogenized samples were extracted with ethanol at room temperature for 48 hrs. The extracts were centrifuged at 27˚C at 10000 rpm for 10 min and the supernatant was collected and concentrated under vacuum in a rotary evaporator (Buchi) at 40˚C.

Chemical screening of compounds (Harborne, 1973; Trease and Evans, 1996; Oloyede, 2005)

Detection of alkaloids: Dragendroff’s Test:

Filtrates were treated with dragendroff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.

Detection of anthraquinone: Borntrager Test:

Filtrate was mixed with ether, which was filtered, and to the filtrate caustic soda was added and aqueous ammonia. Red, pink or violet colour indicated the presence of anthraquinone glycoside.

Detection of glycosides: Baljets test:

The extract was treated with sodium picrate solution. Appearance of yellow to orange colour indicated the presence of glycoside with lactone ring.

Detection of cardiac glycosides: Keller–killiani test:

This test is performed only for the digitoxose sugar moiety. Drug was extracted with chloroform first. 0.4 ml acetic acid was added then along with FeCl3. After adding H2SO4 if purple colour is produced in the acid layer then presence of digitoxose sugar was confirmed.

Detection of saponins: Foam Test:

0.5 gm of extract was shaken with 2 ml of water. If foam produced persisted for ten minutes it indicated the presence of saponins.

Detection of phytosterols: Salkowski’s Test:

Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour indicated the presence of phytosterols.

Detection of Triterpenoids: Libermann Burchard’s test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicated the presence of phytosterols.

Detection of phenols: Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Detection of tannins: Gelatin Test:

The extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

Detection of flavonoids: Lead acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

Detection of amino acid: Ninhydrin Test:

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated the presence of amino acid.

Detection of Glycosides: Kellar Killani's test:

The extract was dissolved in water with glacial acetic acid and ferric chloride and concentrated sulphuric acid brown ring at the junction confirmed the presence of glycosides.

Detection of volatile oil:

To the extract, alcoholic solution of Sudan III was added. Red colour obtained by globules indicated the presence of volatile oil.

Detection of Fats and fixed oils:

To 5 drops of the sample were added 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide. The formation of a clear blue solution confirmed the test.

Detection of quinones:

To 1 ml of the extract, 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.

Detection of coumarins:

To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

Detection of Gums and Mucilages:

The aqueous solutions of extracts were mixed with absolute alcohol and dried in air and the residues were tested for swelling properties and for the presence of carbohydrates.

GC-MS-MS Analysis

The GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS with Fused silica 15m x 0.2 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the animal sample were identified.

Identification of compounds

Interpretation on mass-spectrum GC-MS-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and molecular formula of the test materials were ascertained.

Results and Discussion

The present study recorded a total number of 19 species of nudibranchs under 11 families from Andaman Islands were identified. The sampled area along with primary morphometric measurement, Gps coordinates along with the IUCN status of the species in Andaman Islands is given in table 1. The maximum nudibranches was found in Mayabunder (11 species) followed by Burmanallah (4 species) and Pongibalu (4 species). Opisthobranchiate taxonomy and ecology in Andaman and Nicobar Islands also recently gained attention with the works of Sreeraj et al., (2010). Taxonomic composition of the Nudibranchia is comparable to that reported for other sites in the Indian Ocean (Yonow, 1984, 2012, Yonow & Hayward 1991, Nithyanandan, 2012).

The seven species of Phyllidiidae family and three species of Chromodorididae, are the most species were found in three stations of Andaman Islands. The families, Tergipedidae, Dendrodorididae, Plakobranchidae, Aeolidiidae, Flabellinidae, Gymnodorididae, Doridiidae, Glaucidae and Hexabranchidae with only one species each, are the poorest families recorded in this area. The high diversity of Phyllidiidae and Chromodorididae recorded in Andaman oceans is in accordance with previous studies of Yonow, 1994, Yonow *et al*. 2001, Yonow 2008 and Yonow, 2012.

Consideration of abundance, seasonal variation and ecological relationships requires fundamental

scientific knowledge than does the recording of what species are present at specific localities. In many cases these relationships and detailed distribution are not well studied by scientists and necessary data is lacking. There is a great exigency to study these groups of organisms and to make those studies and their implications to conservation biology more widely and easily disseminated to non- specialists. Incorporation of these data is difficult operationally and creates complex challenges for developing conservation strategies and management plans. However, the inclusion of this information ultimately achieves more far reaching and continuing effective conservation of coral reef biodiversity.

Table 1: Morphometric account of specimens:

Sl. no	Species Name	Dia./Len. (cm)	Sampled area	Coordinates		IUCN Status
				Lat.	Long.	
1	*Phestilla lugubris* (Bergh, 1870)	3-4	Burmanallah	011°34’252’’N	092°44’184’’E	DD
2.	*Dendrodoris fumata* (Ruppel&Leuckart, 1831)	4-10				
3	*Plakobranchus ocellatus* (van Hasselt, 1824)	4-6				
4	*Cerberilla annulata* (Quoy&Gaimard, 1832)	5-7				
5	*Flabellina exoptata* (Gosliner and Willan,1991)	2-3	Mayabunder	12°56.167’N	092°58.113’E	DD
6	*Phyllidia varicosa* (Lamarck, 1801)	8-11				
7	*Phyllidia alyta* (Yonow, 1996)	4-5				
8	*Phyllidia ocellata* (Cuvier, 1804)	5-7				
9	*Phyllidiella zeylanica* (Kelaart, 1859)	6-8				
10	*Phyllidiopsis phiphiensis* (Brunckhorst, 1993)	2-3				
11	*Glossodoris atromarginata* (Cuvier, 1804)	9-10				
12	*Gymnodoris striata* (Bergh, 1905)	5-6				
13	*Jorunna funebris* (Kelaart, 1858)	6-8				
14	*Phyllidiella pustulosa* (Cuvier, 1804)	5-7				
15	*Pteraeolidia ianthina* (Angas,1864)	9-11				
16	*Chromodoris gleniei* (Kelaart, 1858)	4-5	Pongibalu	11°30.573’N	92°39.123’E	DD
17	*Goniobranchus gemina* (Rudman, 1987)	4-5				
18	*Hexabranchuss anguineus* (Ruppell and Leuckart, 1828)	30-40				
19	*Phyllidia varicosa* (Lamarck, 1801)	8-11				

D=diameter, L=length, DD= data deficient

Chemical Screening

In the phytochemical analysis, the chemical compounds such as alkaloids, Anthraquinone, Glycosides, Aminoacids, Flavonoids, Phytosterols, Saponin, Steroids, Tannins, Phenolic compounds, Triterpenoids, Fixed oil and Fat, Aromatic acid, Coumarin, Quinones, Gum and Mucilage and Cardiac

Glycosides were tested in ethanol extracts of *Plakobranchus ocellatus*. The ethanolic extract of *P. ocellatus* showed the presence of maximum phytochemical compounds such as alkaloids, Anthraquinone, Phytosterols, saponin, steriods, Phenolic compounds, Triterpenoids, Fixed oil and Fat, Aromatic acid Gum and Mucilage and Cardiac Glycosides and the results were tabulated in Table 2.

Table 2: Chemical Analysis:

Name of the Test	Sample
Alkaloids	++
Anthraquinone	++
Glycosides	--
Aminoacids	--
Flavonoids	--
Phytosterols	++
Saponin	++
Steroids	++
Tannins	--
Phenolic compounds	++
Triterpenoids	++
Fixed oil and Fat	++
Aromatic acid	++
Coumarin	--
Quinones	--
Gum and Mucilage	++
Cardiac Glycosides	++

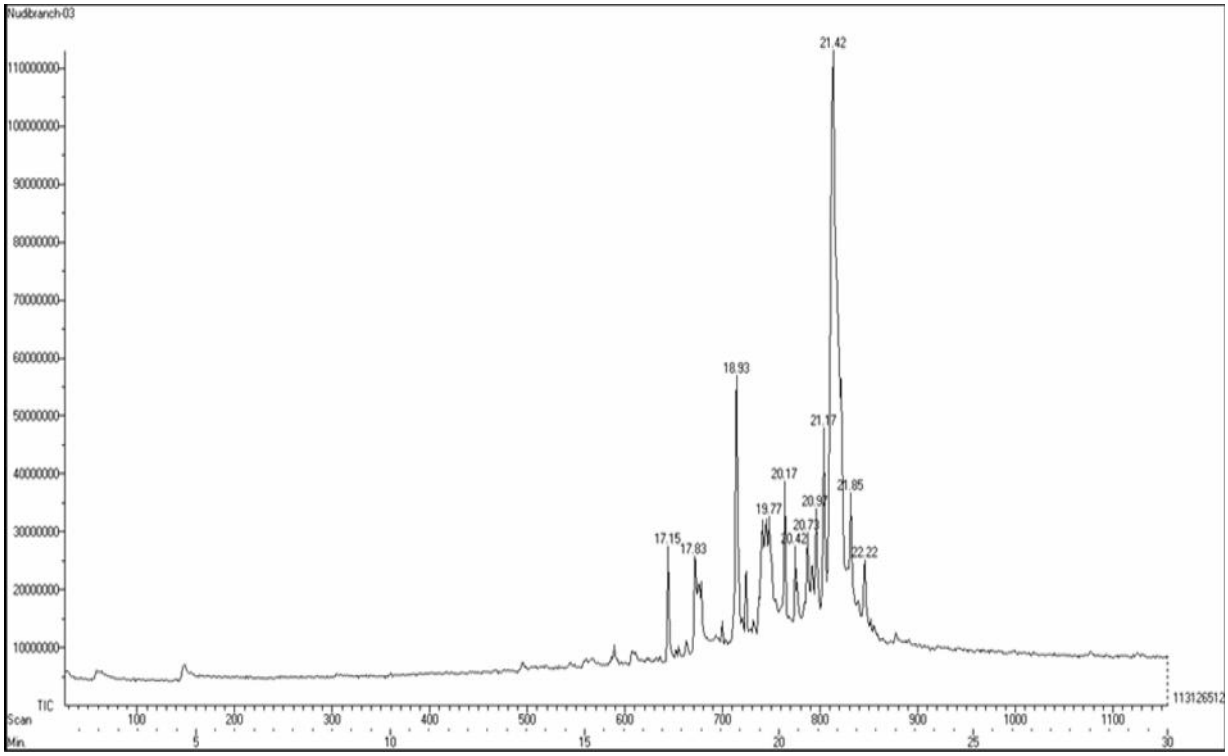
GC-MS analysis of the ethanolic extract of *P. ocellatus* showed the presence of twelve phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library, the twelve phytocompounds were characterized and identified [Table 3]. The individual mass spectra of all the phytochemicals identified in the whole animal ethanolic extract of *P. ocellatus* are presented in Figure 1**.**

Table 3: GC-MS-MS Analysis:

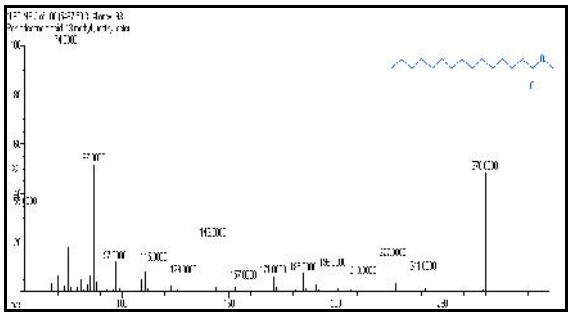
Nudibrancian: *Plakobranchus ocellatus*

S.No	Retention Time [Min]	Compound Name
1	17.15	Pentadecanoic acid-14-methyl ester
2	17.83	Hexadecanoic acid-ethyl ester
3	18.83	Octadecanoic acid-Methyl ester
4	19.77	10-Hydroxy,5,7-Dimethoxy-2,3-Dimethyl-1,4-Anthracene Dione
5	20.17	Pregnanetrione
6	20.42	6H-D, Benzopyran-9-methanol, tetrahydro-hydroxy, dimethyl-3-pentyl
7	20.73	10,13-Dimethyl 3-oxa Doceca hydro Cyclopentaphenantharanyl ester
8	20.97	Androsten-Ethynyl-3,17-Diol
9	21.17	Oxandrosta-11,5-Diene-3-One
10	21.42	Methyl,2,3-Hydroxy-5,7,9-estratiene-17-yl-propionate
11	21.85	Retinoic acid methyl ester
12	22.22	Carbamic acid-methylene-Di 4,1- PhenyleneBis-Di methyl ester

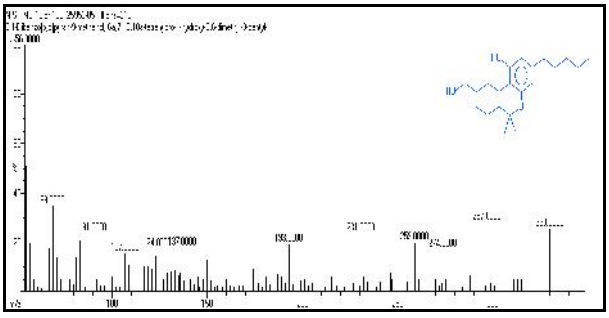
Figure 1: GC MS Analysis of ethanolic extract of *P.ocellatus*



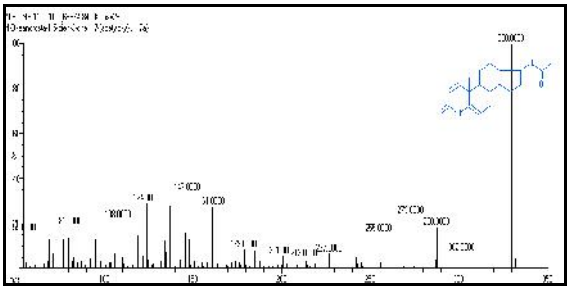
1.Pentadecanoic acid-14-methyl ester



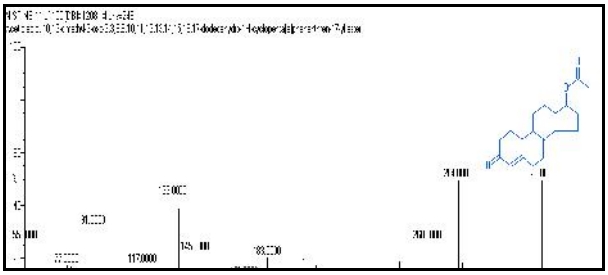
2.6H-D, Benzopyran-9-methanol, tetrahydro-hydroxy, dimethyl-3-pentyl



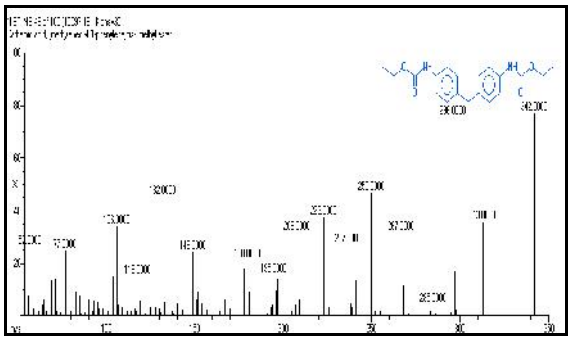
3. Oxandrosta-11, 5-Diene-3-One



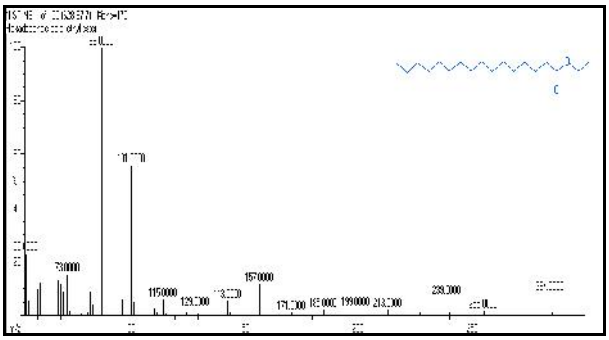
4. 10,13-Dimethyl 3-oxa Doceca hydro Cyclo penta phenantharanyl ester



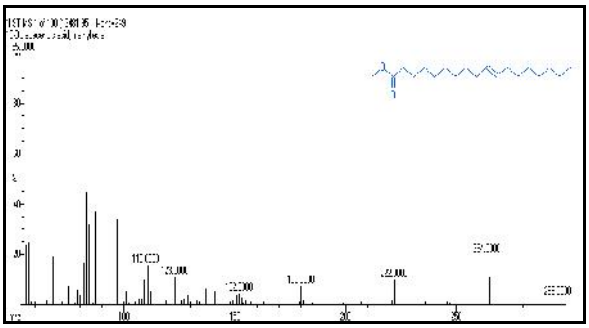
5. Carbamic acid-methylene-Di 4,1-PhenyleneBis-Di methyl ester



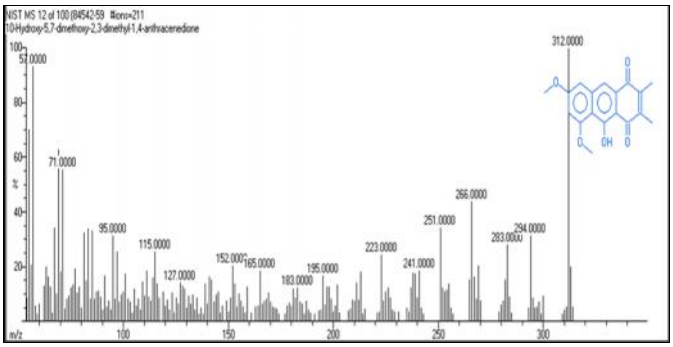
6. Hexadecanoic acid-ethyl ester



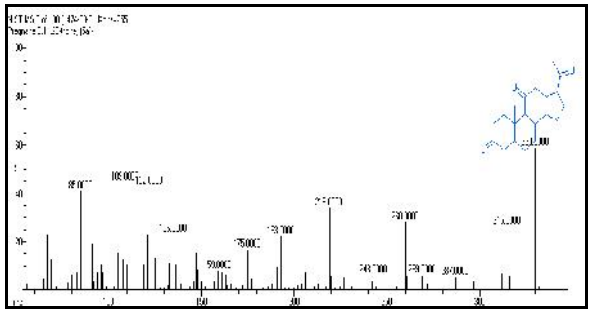
7. Octadecanoic acid-Methyl ester



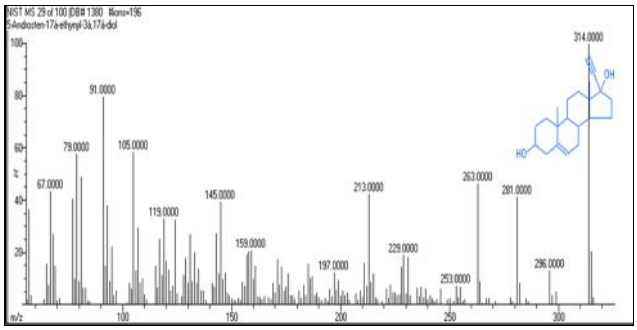
8. 10-Hydroxy,5,7-Dimethoxy-2,3-Dimethyl-1,4-Anthracene Dione



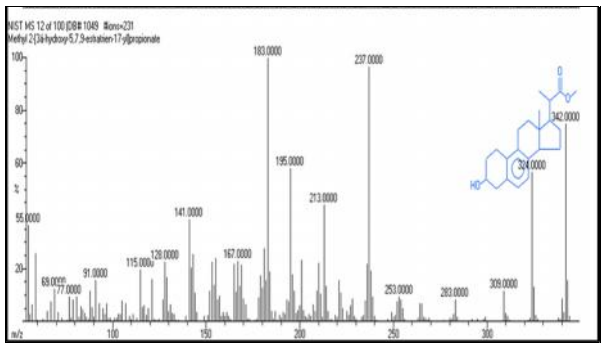
9. Pregnanetrione



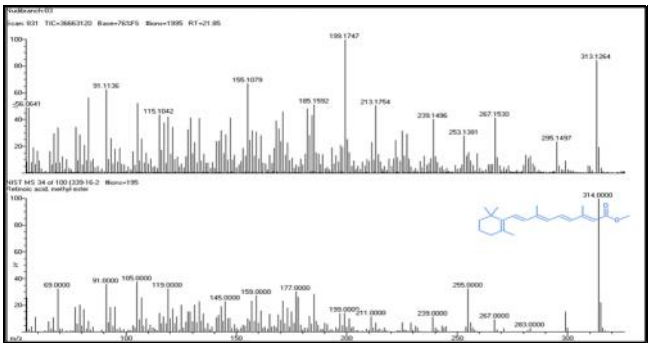
10. Androsten-Ethynyl-3,17-Diol



11. Methyl,2,3-Hydroxy-5,7,9-estratiene-17-yl-propionate



12. Retinoic acid methyl ester



The results revealed the presence of Pentadecanoic acid-14-methyl ester, Hexadecanoic acid-ethyl ester, Octadecanoic acid-Methyl ester, 10-Hydroxy,5,7-Dimethoxy-2,3-Dimethyl-1,4-Anthracene Dione, Pregnane trione, 6H-D, Benzopyran-9-methanol, tetrahydro-hydroxy, dimethyl-3-pentyl, 10,13-Dimethyl 3-oxa Doceca hydro Cyclopenta phenantharanyl ester, Androsten-Ethynyl-3,17-Diol Oxandrosta-11,5-Diene-3-One , Methyl,2,3-Hydroxy-5,7,9-estratiene-17-yl-propionate, Retinoic acid methyl ester and Carbamic acid-methylene-Di 4,1-Phenylene Bis-Di methyl ester. The spectrum profile of GC-MS confirmed the presence of twelve major components with the retention time 17.15, 17.83, 18.83, 19.77, 20.17, 20.42, 20.73, 20.97, 21.17, 21.42, 21.85 and 22.22 respectively (Table 3). The major compounds identified are all shown to have cancer preventive, antioxidant, hypochloesterolemic, nematicide, pesticide, lubricant, anti- androgenic and haemolytic activity.

Opisthobranch molluscs are more putative and interesting marine animals to extract bioactive compounds (Williams *et al*., 1986; Sadhasivam *et al*., 2013; Ramya *etal*., 2014). They are all almost shell-less benthic animals with inability to escape from predators, competitors fighting for escape with slow moving without any physical defenses but they have some effective chemical answers. *Elysia*, a genus included in family *Plakobranchidae* are reported that it accumulates bioactive compounds (Ashour *et al*., 2006) which sequestered from green algae with strong antifungal effects (Shilabin *et al*., 2007). The bioactive compounds found in *P. ocellatus* are able to produce their own defensive compounds (Cimino and Gavagnin, 2006).

Drug discoveries from marine non-chordates have an important research field since decades. Currently, interest in evaluating marine invertebrate products, with the aim of obtaining new potential disease preventive drugs with few side effects, is still growing. In many cases, the bioactive compounds from marine organisms are difficult to obtain in sufficient amounts and researchers, therefore, have to start mimicry Mother Nature for preparation of synthetic compounds or drugs. The rich diversity in bioactive compounds from invertebrates has provided molecules that interfere with the prevention of a disease at many different points, which increase the chances of developing selective drugs against specific disease(s). Marine invertebrates have provided many examples of novel secondary metabolites that possess varied chemical status and potent antimalarial, antiinflamatory, anticarcinogenic, antibacterial, antifungal activities etc.

References

Anand, T., Rajaganapathi, J. and Patterson Edward, J.K. 1997. Antibacterial activity of marine mollusks from Portonovo region. *Ind J Mar Sci*., 26: 206-208.

Ashour, M., Edrada, R., Ebel, R., Wray, V. and Watjen, W. *et al*., 2006. Kahalalide derivatives from the Indian sacoglossan mollusk *Elysia grandifolia*. *J. Nat. Prod*., 69: 1547-1553.

Australian Museum's Seaslug Forum (http://www.seaslugforum.net/).

Brunckhorst, D.J. 1993. The systematics and phylogeny of phyllidiid nudibranchs (Doridoidea). *Records of the Australian Museum* 16(Supplement)*:*1–107.

Cimino, G. and Gavagnin, M. 2006. Molluscs: From Chemo-ecological Study to Biotechnological Application (Progress in Molecular and Subcellular Biology/Marine Molecular Biotechnology). Springer, USA., ISBN-13: 978-3540308799, Pages: 387.

Gosliner, T.M., 1995. The genus Thuridilla (*Opisthobranchia*: Elysiidae) from the tropical Indo-Pacific, with a revision of the phylogeny and systematics of the Elysiidae. Proc. California Acad. Scie., 49: 1-54.

Gosliner, T.M., Behrens, D.W. and Valdes, A. 2008. Indo Pacific Nudibranchs and Sea Slugs. Sea challengers, pp: 425.

Harborne, J. B. 1976. Phytochemical methods, First Edition, Chapman and Hall Ltd, London. 52 -55.

Jensen, K.R., 1990. Three new species of Ascoglossa (*Mollusca*, Opisthobranchia) from Hong Kong and a description of the internal anatomy of *Costasiella pallida.* Jensen, 1985. Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and southern China, Hong Kong, April 2-24, 1986, Hong Kong Univeristy Press, Hing Kong, pp: 419-432.

Kamiya, H., Muromoto, K., Salai, G.K., Endo, Y.M. and Yamamzaki, M. 1989. Purification and Characterization of an antibacterial and antineoplastic protein serum of a sea hare *Aplysia juliena*. *Toxicon*., 27(2): 1269-1277.

Nithyanandan M. 2012. New and rare nudibranch records from Kuwait, Persian Gulf (Mollusca: Opisthobranchia). Marine Biodiversity Records 5, 1-7.

NudiPixel http://www.nudipixel.net/.

Oloyede , O.I. 2005. Chemical profile of unripe pulp of *Carica papaya*. *Pakistan Journal of Nutrition*, 4: 379-381.

Ramya, M.S., Ravichandran, S. and Anbuchezhian, R. 2014. *In vitro* antioxidant properties of marine Sea Slug *Armina babai* from Pazhayaar, Southeast coast of India. *Int. J. Nat. Prod. Res*., 4: 105-109

Riyanti, Widada, J. and Radjasa, O.K. 2009. Isolation and screening of antimicrobial producing-actinomycetes symbionts in nudibranch. *Indones J Biotechnol*; 14(1): 1132-8.

Rudman, W.B. 1982. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris quadricolor*, *C. lineolata* and *Hypselodoris nigrolineata* colour groups. *Zoological Journal of the Linnaean Society* 76: 183–241.

Rudman, W.B. 1983. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris splendida*, *C. aspersa* and*Hypselodoris placida* colour groups. *Zoological Journal of the Linnaean Society* 78: 105–173.

Rudman, W.B. 1984. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: a review of the genera. *Zoological Journal of the Linnaean Society* 81(2&3): 115–273.

Rudman, W.B. 1986. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Noumea purpurea* and *Chromodoris decora* colour groups. *Zoological Journal of the Linnaean Society* 86: 309–353.

Rudman, W.B. 1995. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: further species from New Caledonia and the*Noumea romeri* group. *Molluscan Research* 16: 1–43.

Sadhasivam, G., A. Muthuvel, W.M. Vitthal, A. Pachaiyappan, M. Kumar and B. Thangavel, 2013. *In vitro* antibacterial, alpha-amylase inhibition potential of three nudibranchs extracts from South East coast of India. J. Coastal Life Med., 1: 186-192.

Shilabin, A.G., Kasanah, N., Wedge, D.E. and Hamann, M.T. 2007. Lysosome and HER3 (ErbB3) selective anticancer agent kahalalide F: Semisynthetic modifications and antifungal lead-exploration studies. *J. Med. Chem*., 50: 4340-4350.

Simmons, T.L., Andrianasolo, E., McPhail, K., Flatt, P. and Gerwick, W.H. 2005. Marine natural products as anticancer drugs. *Mol Cancer Ther.* 3: 333-342.

Sreeraj, C.R., Rajan. P.T., Raghuraman. R., Raghunathan., R C., Rajkumar, Titus Immanuel and Ramakrishna. 2010. On some new records of sea slugs (Class: Gastropoda, Subclass: Opisthobranchia) from Andaman and Nicobar Islands, pp. 289–298. In: *Recent Trends in Biodiversity of Andaman and Nicobar Islands.* Zoological Survey of India, Kolkata.

Trease. G.E. and Evans, W.C. 1989. Pharmacognosy 2nd Edn. Braille Tiridel and Macmillan Publishers.

Uddin, H.M., Otsuka, M., Muroi, T., Ono, A., Hanif, N. and Matsuda, S., *et al*. 2009 Deoxymanoalides from the nudibranch *Chromodoris willani*. *ChemPharm Bull (Tokyo)* 57(8): 885-7.

Williams, D.E., Ayer, S.W. and Andersen, R.J. 1986. Diaulusterols A and B from the skin extracts of the dorid nudibranch *Diaulula sandiegensis*. Canadian J. Chem., 64: 1527-1529.

Wright, R.K. 1981. Urochordates In: Ratcliff NA, Rowley AF (eds), Invertebrate blood cells, Academic Press, New York, vol 2, pp 565-626, 1981.

Yasman, Y., Edrada, R.A., Wray, V. and Proksch, P. 2003. New 9-thiocyanatopupukeanane sesquiterpenes from the nudibranch *Phyllidia varicosa* and its sponge-prey *Axinyssa aculeate*. *J Nat Prod.,* 66: 1512-4.

Yonow, N. 2001. Results of the Rumphius Biohistorical Expedition to Ambon (1990).Part 11.Doridacea of the families Chromodorididae and Hexabranchidae (Mollusca, Gastropoda, Opisthobranchia, Nudibranchia), including additional Molukkan material. Zoologische Mededelingen, Leiden 75 (15), 1–50.

Yonow, N. 2008. Sea Slugs of the Red Sea. Pensoft Publishers, Sofia-Moscow, 304 pp.

Yonow, N. 2012. Opisthobranchs from the western Indian Ocean, with descriptions of two new species and ten new records (Mollusca, Gastropoda). ZooKeys 197, 1–129.

Yonow, N. and Hayward, P.J. 1991. Opisthobranches de l'Ile Maurice, avec la description de deuxespèces nouvelles (Mollusca: Opisthobranchia).— Revue française d'aquariologie herpétologie 18 (1): 1-30.

Yonow, N. 1984. Doridacean nudibranchs from Sri Lanka, with descriptions of four new species.— The Veliger 26 (3): 214-228.

Yonow, N. 1994. Opisthobranchs from the Maldive Islands, including descriptions of seven new species (Mollusca: Gastropoda).— Revue française d'aquariologie herpétologie. 20 (4): 97-130.

Yonow, N. 2012. Opisthobranchs from the western Indian Ocean, with descriptions of two new species and ten new records (Mollusca, Gastropoda). ZooKeys **197**, 1–129.

Zhukova, N.V. 2014. Lipids and fatty acids of nudibranch mollusks: potential sources of bioactive compounds. *Mar Drugs.,* 12: 4578-92.