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Research Article

SCREENING OF BIOACTIVE COMPOUNDS FROM MARINE SPONGES COLLECTED FROM KOVALAM, CHENNAI

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ABSTRACT

Objective: This study is designed to hunt for the presence of bioactive compounds from three marine sponges collected from Kovalam.

Methods: Zoochemical analysis is performed to screen for the presence of secondary metabolites. Based on those results, only two sponges which showed a significant presence of secondary metabolites has been subjected to gas chromatography-mass spectrometry (GC-MS) analysis to identify the unknown chemical compounds present in those sponges.

Results: On analyzing the results, two sponges, namely, *Dysidea herbacea* and *Sigmadocia pumila,* has shown a significant presence of secondary metabolites while the third sponge *Acanthella elongata* have shown moderate presence of secondary metabolites. Since the first two sponges results are remarkable, these two samples have been subjected to GC-MS analysis to separate and identify the unknown chemical compounds present in the sample.

Conclusion: Samples, namely, *D. herbacea* and *S. pumila*, indicated the presence of several components. From both the sponges, eleven different secondary metabolites were identified by GC-MS. Most of these compounds are widely used in cosmetic, pharmaceutical, and other industries and therefore a vital source for industrial biotechnology and related products in healthcare and skincare.

Keywords: *Dysidea herbacea, Sigmadocia pumila, Acanthella elongata,* Secondary metabolites, Gas chromatography-mass spectrometry analysis, Bioactive compounds.

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INTRODUCTION

Marine diversity is so high that majority of the living organisms are yet to be characterized morphologically and chemically [1]. Marine flora and fauna has kept scientists hooked up to look for new avenues for chemical research. Very less proportion of marine species has been studied [2]. Sponges (phylum porifera) are marine invertebrates, known to be multicellular and immobile but sessile [3]. These are a vital source for the researchers to look for novel bioactive compounds [4]. About 4851 compounds which are 30% of all marine natural products were discovered thus far; making them most prolific marine compound producers as nearly 200 new compounds were reported every year [5].

Infectious microorganisms evolve and develop resistance to existing pharmaceuticals; marine sponges provide novel leads against bacterial, fungal, and viral diseases [6]. Symbiotic relationship with various microorganisms makes them play vital roles such as photosynthetic carbon fixation, nitrification, and nitrogen fixation [7]. It has been reported that the metabolites synthesized by these symbionts could well become an ideal and vital source for the discovery of new drugs [8]. Some of the marine sponges are also known to have cosmetics activities such as anti-aging and anti-wrinkling effects, tyrosinase and metalloproteinase inhibition, and ultraviolet defense. Sponges are used to clean body, face as they soak up and hold more water. They are also used to clean insightful baby skin and whitening compounds [9]. Numerous studies have reported potential cytotoxic, antifungal, antibacterial, anti-inflammatory, and antioxidant activities from the sponges extracts [10-17].

Therefore, in this study, we have collected few sponges from the seashores on the outskirts of Chennai and have performed zoochemical characterization to reveal the presence of secondary metabolites, followed by gas chromatography-mass spectrometry (GC-MS) to identify major chemical compounds present in the samples.

METHODS

Sponge collection

Three species of marine sponges were collected from seashores of Kovalam, Kanchipuram, Tamil Nadu, with the help of Dr. Joe K. Kizhakudan, Principal Scientist from Central Marine Fisheries and Research Institute. Taxonomic identification of the samples was done and certified by Dr. Sivaleela, Scientist from Zoological Survey of India (ZSI). The samples were identified as *Dysidea herbacea*, *Sigmadocia pumila,* and *Acanthella elongata* (Fig. 1). Samples were preserved in ice boxes and maintained at −20°C until the experimental process. Voucher specimens preserved at 75% methanol was deposited at ZSI.

Extraction procedure

Five gram of the shade dried powdered sample was extracted with dichloromethane (100 ml) and methanol (100 ml) at room temperature overnight on a shaker. The extracts were filtered through filter paper and concentrated in vacuum and were stored at −20°C for further zoochemical analysis.

Zoochemical analysis

Individual tests were performed to identify the presence of common zoochemicals using conventional methods. To determine the presence of carbohydrate, 1 ml of Molisch's reagent and few drops of concentrated sulfuric acid were added to 2 ml of sponge extract [18]. The presence of tannin was confirmed by adding 2 ml of 5% ferric chloride to 1 ml of sponge extract [19]. To 2 ml of sponge extract, 2 ml of distilled

water was added and shaken in a graduated cylinder for 15 minutes lengthwise to determine the presence of saponins [20]. Addition of 1 ml of 2 N sodium hydroxide to 2 ml of sponge extract determined the presence of flavonoids. The presence of cardiac glycoside was confirmed with the addition of 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added to 0.5 ml of extract, and then, 1 ml of concentrated sulfuric acid. Addition of 2 ml of chloroform was added and concentrated sulfuric acid to 0.5 ml of extract determined the presence of terpenoids. To determine the presence of triterpenoids, 1 ml of Libermann-Burchard Reagent (acetic anhydride + conc.sulfuric acid) was added to 1.5 ml of extract. To determine the presence of phlobatannins and anthraquinones, few drops of 2% hydrochloric acid and few drops of 10% ammonia solution respectfully were added to each 1 ml of extract [21]. The presence of alkaloids was confirmed by adding 2 ml of concentrated hydrochloric acid and few drops of Mayer's reagent to 2 ml of sponge extract [22]. Test for quinones was performed by adding 1 ml of concentrated sulfuric acid to 1 ml of extract. Addition of 3 ml of chloroform and 10% ammonia solution to 2 ml of sponge extract determined the presence of glycosides. To test for the presence of phenol, 2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1 ml of sponge extract. Addition of 1 ml of 10% sodium hydroxide to 1 ml of extract determined the presence of coumarins [23]. To confirm the presence of steroids and phytosteroids, 1 ml of sponge extract equal volume of chloroform and few drops of concentrated sulfuric acid were added [24].

Gas chromatography-mass spectrometer analysis

Five gram freeze-dried sponge sample was extracted with chloroform and methanol in 2:1 ratio. The extract was evaporated under reduced pressure. The extract (500 mg) was chromatographed on a column (60 × 2.5 cm diameter) of silica gel using eluents of increasing polarity from hexane through ether to ethyl acetate. GC-MS analyses were carried out with a GC-MS QP-5000 (Shimadzu Corp.) fitted with a fused silica DB-5 capillary column (0.22 mm × 30 m, 0.25 µm film thickness) with helium gas at a flow rate of 1 ml/minute. The sample was injected in the splitless mode (sampling time, 5 minutes). The respective temperatures of the GC injection port and ionization chamber were 290°C and 280°C. Temperature programs for the column oven were as follows: program, 60°C for 1 minute, elevated to 130°C at 20°C/minute, then to 210°C at 10°C/minute, then to 260°C at 10°C/minute, then to 300°C at 10°C/minute; it was finally maintained at 300°C.

Fig. 1: Images of the collected samples from Kovalam

RESULTS AND DISCUSSION

The sponges which were initially subjected to zoochemical analysis showed their presence for secondary metabolites in different tests which was mentioned in Table 1 for the three sponges *D. herbacea, S. pumila,* and *A. elongata*. Tests for phenols (except dichloromethane extract of *S. pumila*) and steroids (except dichloromethane extract of *D. herbacea*) reported positive for these samples. The presence of alkaloids was confirmed in the methanolic extracts. Saponins were reported in all the extract types but dichloromethane extract of *S. pumila*. Flavonoids which are rich sources for various biological properties were able to be traced by the methanolic extracts of *D. herbacea* and *S. pumila*. On the other hand, both extracts of *D. herbacea* signaled the presence of carbohydrates. Glycosides, cardiac glycosides, terpenoids, coumarins, plobatannins, and anthraquinones were not present in our sponge samples at all. 16 tests for methanolic extracts and 10 for dichloromethane were positive and 58 were negative for 14 tests across three selected species. Overall, a remarkable presence is exhibited in methanol extract when compared to dichloromethane extract.

As the first two samples, namely, *D. herbacea* and *S. pumila,* has marked a significant presence of secondary metabolites, these two samples were further taken for GC-MS analysis to separate and identify various components present in the sample. From both the sponges, 11 components were separated at different retention time (RT).

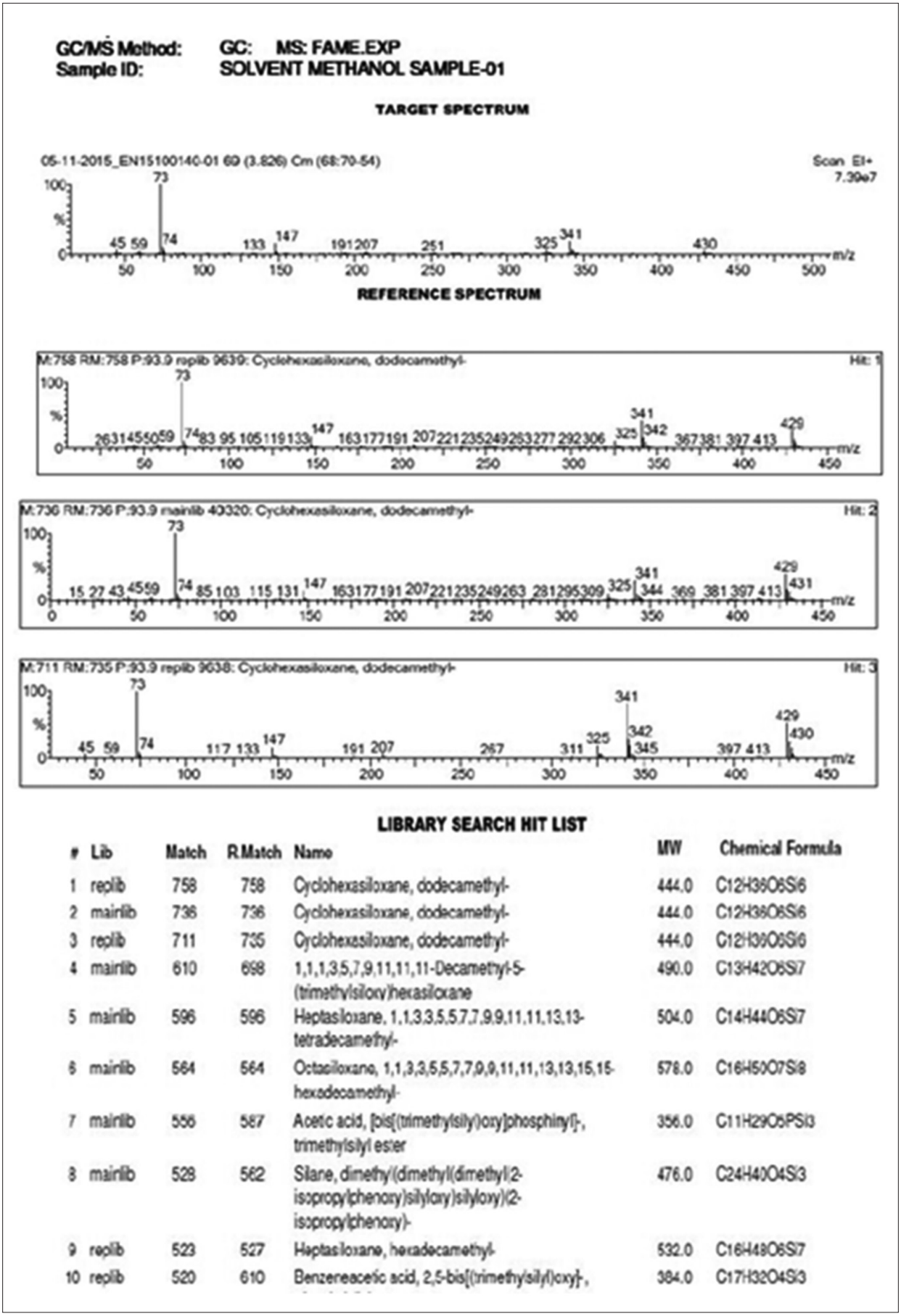
D. herbacea (Fig. 2) revealed the occurrence of components such as cyclohexasiloxane, dodecamethyl at 3.826 RT, 3 isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris (trimethylsiloxy) tetrasiloxane at 4.685 RT, 3-Tosyl sedoheptulose at 5.061 RT, cyclooctasiloxane hexadecamethyl at 6.080 RT, 2-(2,4,4,6,6,8,8-heptamethyltetrasiloxan 2 yloxy)-2,4,4,6,6,8,8,10,10-nonmethylcyclopentasiloxane at 8.668 RT, 1,1,1,3,5,5,7,7,7-nanomethyl-3-(trimethylsiloxy)-tetrasiloxane at 11.962 RT, ethanedioic acid bis(trimethylsilyl)ester at 15.414 RT, trisiloxane 1,1,1,5,5,5 hexamethyl-3.3-bis(trimethylsilyl)oxy at 21.504 RT, octasiloxane 1, 1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl at 26.827 RT, 1,1,1,3,5,5,5-heptamethyltrisiloxane at 28.829 RT, 3,6-dioxy 2,4,5,7 tetrasiloctane 2,2,4,4,5,5,7,7 octamethyl at 29.224 RT. Two compounds, namely, 3 isopropoxy-1,1,1,7,7,7hexamethyl 3,5,5-tris(trimethylsiloxy) tetrasiloxane at 4.685 RT and 3-tosyl sedoheptulose at 5.061 RT are predominant in this sponge sample.

S. pumila (Fig. 3) revealed the presence of cyclopentasiloxane decamethyl at 3.156 RT, cyclohexasiloxane dodecamethyl at 3.827 RT, 3 isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris(trimethylsiloxy)

Table 1: Results of the zoo chemical tests for the presence of classes of secondary metabolites in two extracts

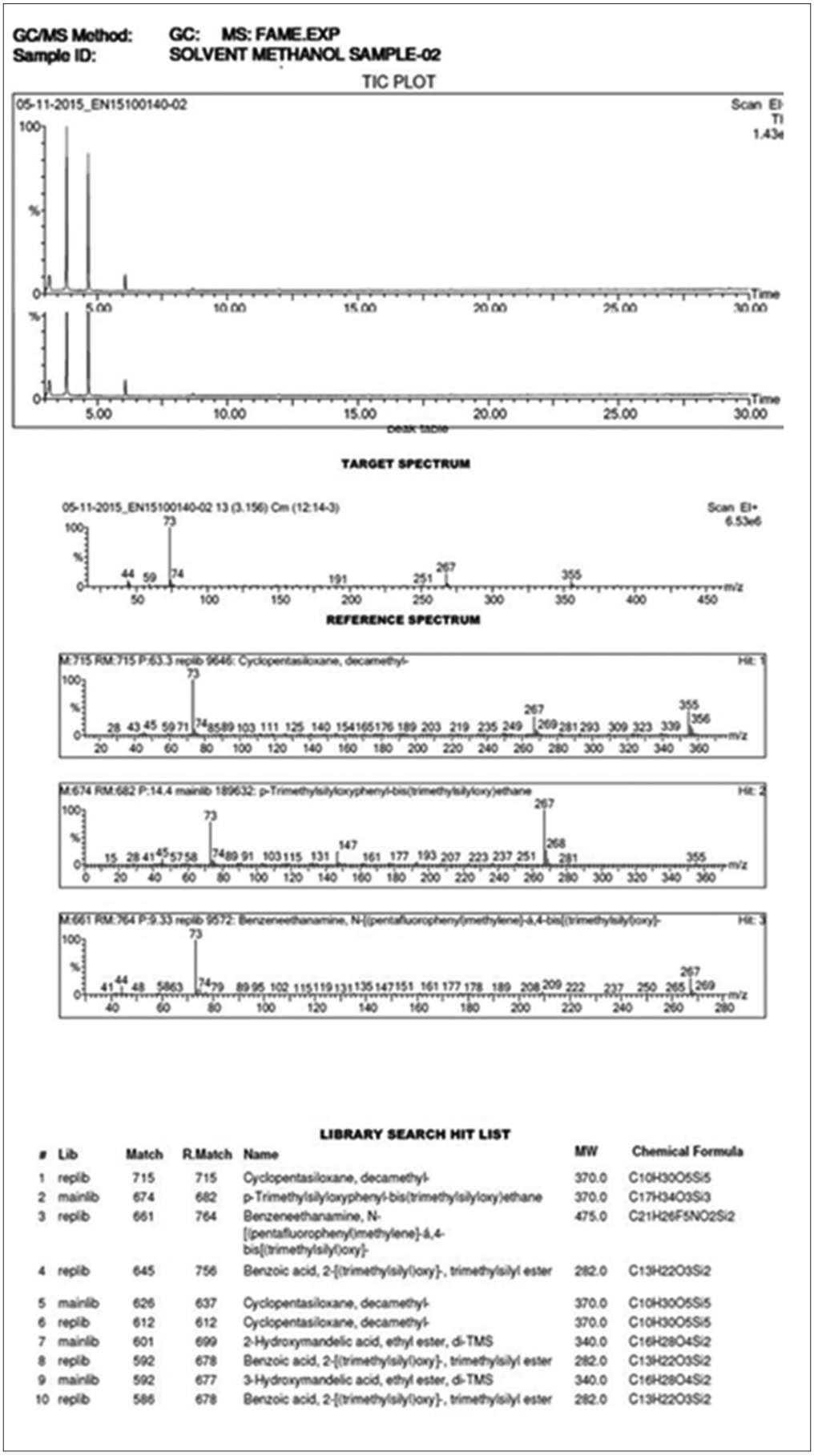
S.No.	Zoo chemical tests	DHM	DHDM	SPM	SPDM	AEM	AEDM
1	Carbohydrate test						
2	Tannins test						
3	Saponins test						
4	Flavonoids test						
5	Alkaloid test						
6	Quinones test						
7	Glycosides test						
8	Cardiac glycosides test						
9	Terpenoids test						
10	Phenols test						
11	Coumarins test						
12	Steroids						
13	Phlobatannins test						
14	Anthraquinones test						

DHM: *Dysidea herbacea* methanol extract, DHDM: *Dysidea herbacea* dichloromethane extract, SPM: *Sigmadocia pumila* methanol extract, SPDM: *Sigmadocia pumila* dichloromethane extract, AEM: *Acanthella elongata* methanol extract, AEDM: *Acanthella elongata* dichloromethane extract, , red color - negative result, green color - positive result



#	Lib	Match	R.Match	Name	MW	Chemical Formula
1	replib	758	758	Cyclohexasiloxane, dodecamethyl-	444.0	C12H36O6Si6
2	mainlib	736	736	Cyclohexasiloxane, dodecamethyl-	444.0	C12H36O6Si6
3	replib	711	735	Cyclohexasiloxane, dodecamethyl-	444.0	C12H36O6Si6
4	mainlib	610	698	1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(trimethylsiloxy)hexasiloxane	490.0	C13H42O6Si7
5	mainlib	596	596	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	504.0	C14H44O6Si7
6	mainlib	564	564	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	576.0	C16H50O7Si8
7	mainlib	556	587	Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester	356.0	C11H29O5PSi3
8	mainlib	528	562	Silane, dimethyl(dimethyl(dimethyl(2-isopropyl(phenoxy)silyloxy)silyloxy)(2-isopropyl(phenoxy)-	476.0	C24H40O4Si3
9	replib	523	527	Heptasiloxane, hexadecamethyl-	532.0	C16H48O6Si7
10	replib	520	610	Benzeneacetic acid, 2,5-bis[(trimethylsilyl)oxy]-,	384.0	C17H32O4Si3

Fig. 2: Results of gas chromatography-mass spectrometry analysis for the sample *Dysidea herbacea*



#	Lib	Match	R.Match	Name	MW	Chemical Formula
1	replib	715	715	Cyclopentasiloxane, decamethyl-	370.0	C10H30O5Si5
2	mainlib	674	682	p-Trimethylsilyloxyphenyl-bis(trimethylsilyloxy)ethane	370.0	C17H34O3Si3
3	replib	661	764	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-á,4-bis[(trimethylsilyl)oxy]-	475.0	C21H26F5NO2Si2
4	replib	645	756	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	282.0	C13H22O3Si2
5	mainlib	626	637	Cyclopentasiloxane, decamethyl-	370.0	C10H30O5Si5
6	replib	612	612	Cyclopentasiloxane, decamethyl-	370.0	C10H30O5Si5
7	mainlib	601	699	2-Hydroxymandelic acid, ethyl ester, di-TMS	340.0	C16H28O4Si2
8	replib	592	678	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	282.0	C13H22O3Si2
9	mainlib	592	677	3-Hydroxymandelic acid, ethyl ester, di-TMS	340.0	C16H28O4Si2
10	replib	586	678	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	282.0	C13H22O3Si2

Fig. 3: Results of gas chromatography-mass spectrometry analysis for the sample *Sigmadocia pumila*

tetrasiloxane at 4.655 RT, acetic acid ([aminocarbonyl]amino)oxo at 4.835 RT, hexasiloxanetetradecamethyl at 6.082 RT, malonic acid bis(2 trimethylsilyethyl) ester at 8.672 RT, 1,3 dioxolane-2-methanol at 11.958 RT, 1,1,1,3,5,5,7,7,7-nanomethyl-3-(trimethylsiloxy)-tetrasiloxane at 15.388 RT, trisiloxane 1,1,1,5,5,5 hexamethyl-3.3-bis(trimethylsilyl)oxy at 18.578, cyclotrisiloxanehexamethyl at 28.772 RT, and 1,1,1,3,5,5,5-heptamethyltrisiloxane at 29.228 RT. Two compounds, namely, cyclohexasiloxanedodecamethyl at 3.827 RT, 3 isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris(trimethylsiloxy) tetrasiloxane at 4.655 RT are predominant in *S. pumila.*

DISCUSSION

Marine supplies are a potent source for unexplored chemical moieties that may have vital biological and economic properties. Marine diaspora over the years has proven its importance as several of compounds of economic significance are isolated and mass produced across the globe. Very little of these living organisms was studied and therefore provides ample opportunities to hunt for vital substances. Three species chosen here in this study such as *D. herbacea, S. pumila,* and *A. elongata* were used to assess zoochemical properties with the help of methanolic and dichloromethane solvents. Both these extracts were able to track many vital secondary metabolites of different importance. Although glycosides, cardiac glycosides, terpenoids, coumarins, phlobatannins, and anthraquinones were not present in any of these extracts, other class of secondary metabolites such as saponins, phenols, steroids, alkaloids, and flavonoids was traced in these samples. Saponins are class of glycosides with soap-like foaming properties produced in abundance in various plant species. This particular property of saponins has led to its utilization as natural emulsifiers in cosmetic industry [25]. Alkaloids are nitrogenous organic compounds produced by variety of organisms such as bacteria, fungi plants, and animals. Alkaloids have wide range of applications in pharmacology including antimalarial, antiasthma, and anticancer. Alkaloids are commonly used in cosmetic industry as one of the ingredient in skin care mixtures. Alkaloids are used for skin protective purposes, against wrinkles, skin tightening extracts, etc [26]. These flavanoids are class of plant pigments commonly used in cosmetics as antioxidant and for soothing actions [27].

Cyclohexasiloxanedodecamethyl (dodecamethylcyclohexasiloxane [D6]) isolated from *S. pumila* is found to have many industrial applications. This compound is used in the production of silicone polymers, organosilicon substances, and a wide application in personal and household care products. Personal care products such as antiperspirants, skin creams and lotions, skin cleansing product, and hair care products [28].

It is also reported that sponge-associated bacterial extracts have cytotoxicity effect on MOLT4 cell lines by the result of cell cycle analysis hence claimed anticancer activity [29] which suggests sponges could play an active role in anticancer activity. The extract of marine sponge *A. globostellata* found to possess bioactive properties such as flavonoids, terpenoids, phenolic compounds, and aromatic acids and exhibited a good antioxidant activity [30] also claims that secondary metabolites from sponges could serve as an antioxidant.

3 isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris (trimethylsiloxy) tetrasiloxane has been reported in many species which include brown rice [31], leaves of *Commelina benghalensis L*, and root and rhizome of *Smilax zeylanica*. In our study, 3 isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris (trimethylsiloxy) tetrasiloxane was known to be predominant in two sponges *D. herbacea* and *S. pumila*. Cyclooctasiloxane hexadecamethyl was reported to have antimicrobial property and it was also reported in *Oscillatoria* species [32] and *Solanum nigram* [33].

CONCLUSION

Three different sponges collected from different locations were extracted and various zoochemical tests were performed to identify some of the secondary metabolites produced by them. Samples, namely, *D. herbacea* and *S. pumila* indicated the presence of several components. From both the sponges, 11 different secondary metabolites were identified by GC-MS. Most of these compounds are widely used in cosmetic, pharmaceutical, and other industries, and therefore, a vital source for industrial biotechnology and related products in healthcare and skincare.

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