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PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL ACTIVITY OF SELECTED SEAWEEDS FROM OKHA COAST, GUJARAT, INDIA

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ABSTRACT:

The study deals with the assessment of the chemical composition of carbohydrate, protein, phenol, flavanoid, chlorophyll, and carotenoid and antifungal activity from various marine seaweeds collected from Okha coast, Gujarat during September, 2013. The carbohydrate content was highest in *Cystoseira indica* Mairh; protein in *Gracillaria corticata* J.Agardh; phenol content in *Padina boergesenii*; flavanoid content found greater in *C. Indica*; chlorophyll content in *Monostroma latissimum* Wittrock; carotenoid content was more in *Dictyopteris acrostichoides* Bornet. Antifungal activity of these species belongs to red, green, and brown seaweeds was explored and extracted in acetone, ethanol and chloroform. The highest inhibiting effect was noted for *Sargassum tenerrimum* J.G. Agardh and *Turbinaria ornate* J. Agardh belong to brown algae, against *Aspergillus niger* and *Penicillium janthinellum* in chloroform extracts and ethanolic extract which are causing opportunistic infection of HIV-infected person, lung disease, aspergillosis, and otomycosis (fungal ear infections).

KEY WORDS: *Antifungal activity, Biochemical compounds, Marine seaweeds, Okha coast, Gujarat.*

INTRODUCTION:

Marine seaweeds comprise few thousand of species and they represent a

considerable part of the littoral biomass. According to their nutrient value and chemical composition, they are classified as red (Rhodophyta), brown (Phaeophyta), and green seaweeds (Chlorophyta) (Dawczynski *et al.*, 2007). Many seaweed species are used in the industry, principally for the extraction of phycocolloids (Jimenez-Escrig and Sanchez-Muniz 2000) and as a source of pharmaceutical substances. Also, they are used as herbal medicine, fertilizer, fungicides, and herbicides and for the direct use in human nutrition, too (Aguilera-Morales *et al.*, 2005; Cardozo et al. 2007). Seaweeds are known as a highly nutritive food containing vitamin, protein, mineral, fiber contents, and essential fatty acids (Ortiz *et al.*, 2006). Seaweeds are the only source of phytochemicals namely agar-agar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents.

Parekh *et al.*, (1977) studied the chemical composition seaweeds are the raw material for many industrial of 27 species of green seaweeds of Saurashtra coast. Dinesh *et al.*, (2007) studied the nutritive properties of 20 species of seaweeds from Gulf of Mannar. The seaweeds are also known to contain bioactive products that display antibacterial, antiviral and antifungal properties (Trono 1999). Seaweeds are exposed to seasonal variations of abiotic factors that influence their metabolic responses (photosynthesis and growth rates) and levels of proximate constituents (Ordu˜na-Rojas *et al.*, 2002).Seasonal variations in the chemical composition and nutritive value have been reported in common marine seaweeds from different parts of the world (Kaehler and Kennish (1996), Kumar (1993) and Mercer *et al.*, (1993).

A large number of algal extract products have been found to have antimicrobial activity. Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with antiviral, antibacterial, and antifungal activities (Caccamese *et al.*, 1980; Del Val *et al.*, 2001; Perry *et al.*, 1991). Zovko et al. (2012) studied antifungal activity against fungal strains of *C. albicans* with a high activity of algal extracts. Gao *et al.*, (2011) showed that a few extracts of marine algae have not only an antifungal activity but toxicity towards cancer cells. Several extractable compounds, such as cyclic polysulfides and halogenated compounds are toxic to microorganisms and, therefore, responsible for the antibiotic activity of some seaweeds (Fenical, 1975; Wrattens and Faulkner, 1976). Some commonly occurring marine algae *Caulerpa scalpelliformis, Ulva lactuca, Pandina tetrastromatica, Stoecchospermum marginatum* and *Acanthophora spicifera* have been collected from the coast of Tuticorin, Tamilnadu and evaluated for antifungal and antibacterial activity by using four solvents such as petroleum ether, chloroform, methanol and benzene by Jothibai margret *et al.*, (2008). Many marine algae were screened for their antimicrobial activity by Reichelt and Borowitzka (1984) and Salvador *et al.*, (2007) who studied antimicrobial activities of 82 marine algae. Bansemir *et al.*, (2006) have investigated the antibacterial activities of the extracts from 26 algal species prepared by dichloromethane,

methanol and water against five fish-pathogenic bacteria. Therefore, the present paper aims to analyze variations of the levels of proximate constituents like protein, carbohydrate, phenol, flavanoid, chlorophyll and carotenoid and antifungal activity of the seaweeds.

STUDY AREA:

Okha Coast, situated at22°28'N and 69°05'E in the mouth of "Gulf of Kutch" on the north- westernmost part of Saurashtra in Gujarat (Fig. 1.) is one of the most important places of interest for algal growth in India. This coast being at the mouth of "Gulf of Kutch" experiences strong water currents round the year as compared to other parts of the country. The coast is characterized by rocks made up of tertiary formations alternating with patches of sand deposits making the area more hospitable for the growth of all types of marine algae throughout the year.

MATERIALS AND METHODS:

Sampling

The seaweeds samples were collected during September, 2013, picked with hand and immediately washed with seawater to remove the foreign particles, sand particles and epiphytes. Then it was kept in an ice box and immediately transported to the laboratory and washed thoroughly with tap water to remove the salt on the surface of the sample. After that, the species were identified by Bhavanath Jha *et al.*, (2009). They were spread on blotting paper to remove excess water. The dry air samples were placed in an oven at 50 °C and water content was calculated. Pulverized in the grinder and sieved through a screen with an aperture of 0.5 mm. Then, the powdered material was kept in airtight plastic bottles at room temperature until further analysis.

The total carbohydrate content was estimated by Anthrone method (Roe, 1955), protein was quantified by Biurette method (Raymont *et. Al.*, 1964). Total phenolic assay was determined by using Folin Ciocalteu assay (Sadasivam and manickam, 1992). Total flavanoid content was measured by the Aluminum chloride calorimetric assay (Zhishen *et. al.*, 1999).The amount of chlorophyll-a present in the alga was estimated by Arnon (1949). The amount of carotenoid was determined by Parsons and Strickland, (1963).

Extraction

The collected samples were air-dried and coarsely powdered. The powdered form of seaweeds was subjected to step wise extraction using acetone, chloroform and ethanol by soxhlation process. The three different extraction solvents were used according to the order of their polarity as different compounds get extracted in different solvents. The crude extracts were concentrated under reduced pressure to get their corresponding residues. The seaweed extracts were further subjected for antifungal activity by agar cup plate method (Sachin L Badole *et. al.*, 2011) Each seaweed extract were subjected to two fungal species

A. niger (NCBI accession number, KC545848) and *P. janthinellum(* NCBI No.KC545842) used for antifungal assay.

RESULTS:

Seaweeds were identified based on their morphological criteria mentioned in **Fig. 2.** Thirteen species of seaweeds belongs to Chlorophyceae , Phaeophyceae and Rhodophyceae collected from Okha coast ,Gujarat (**Table.1)** and their percent class wise distribution is represented in **Fig.3.**

Biochemical analysis

Biochemical analysis of carbohydrate, protein, total phenol, flavanoid, chlorophyll a and carotenoide content of seaweeds are presented in fig. (4a. to 4f). The carbohydrate content varied from 2.247±0.2 to 9.219±0.3 mg/g ; maximum carbohydrate was recorded in *C.indica* (9.21 ±0.4) followed by *C. sp* (7.30±0.4) and *D. acrostichoides* (6.99±0.2). Moreover, the minimum carbohydrate concentration was observed in *G.corticata* (2.247±0.2) followed by *C. trinedis* (2.96±0.2) and *G. micropterum* (2.98±0.4) (**fig. 4a).**

The protein concentration of seaweeds ranged from 0.429±0.02 to 1.8887±0.3mg/g; highest protein was registered in *G.corticata* (1.8887±0.4mg) followed by *P.boergesenii* (1.8392±0.4) and *M. latissimum* (1.384±0.2), *C. indica* (1.367±0.1), *S.cinctum* (1.2507±0.2). Whereas the lowest protein content was recorded from *S. tenerrimum* (0.429±0.02) followed by *D.acrostichoides* (0.4235±0.02), *T.ornate* (0.4939±0.01) and *D.dichotoma* (0.7777±0.02) (**fig. 4b).**

The phenol content of seaweeds fluctuated from 0.658±0.02 to 3.808±0.5 mg/g; maximum phenol was encountered in *P. boergesenii* (3.808±0.5) followed by *S. tenerrimum* (3.598±0.4), *S. cinereum* (2.765±0.4) and *S.cinctum* (2.576±0.2). However , the minimum phenol was noticed in *Cladophora.sp* (0.658±0.06) followed by *M. latissimum* (0.707±0.08), *D. dichotoma* (0.882±0.03) and *G. micropterum* (0.959±0.01) (**fig. 4c).**

The maximum flavanoid concentration fluctuated from 0.024±0.002 to 0.186±0.03 mg/g; higher content was recorded in *C. indica* (0.186±0.03) followed by *P.boergesenii (*0.168±0.02) and *S. cinereum* (0.165±0.02). However the lower content was observed in *G.corticata* (0.024±0.002) followed by *C. sp* (0.033± 0.002) and *C. trinedis* (0.063±0.001) (**fig. 4d).**

The chlorophyll a., concentration ranged from 0.019±0.001 to 0.1258±0.02 mg/10ml; maximum found in *M. latissimum* (0.1258±0.02), *D. acrostichoides* (0.1078±0.02) and *C.sp* (0.0845±0.001). The minimum content was observed in *G.corticata(* 0.019±0.001), *D.dichotoma* (0.0294±0.002) and *T.ornate* (0.039±0.001) (**fig. 4e).**

The carotenoid concentration fluctuated from 0.042±0.001 to 0.161±0.01; the content was greater in *D.acrostichoides(* 0.161±0.01) followed by *P. boergesenii* (0.138±0.01) and *G. micropterum*

(0.124±0.01). The lower concentration was found in *D.dichotoma* (0.042±0.001) followed by *T.ornate* (0.057±0.002) and *G.corticata* (0.074±0.002) (**fig. 4f).**

Antifungal assay

Different extracts of eight seaweed species were tested for their antifungal activity against two strains *Aspergillus niger* and *Penicillium jenthinellum*, by cup plate method. The chloroform extracts of *S. tenerrimum* and *T.ornate* brown algae, showed considerable antifungal activity. *Sargassum cinereum* , and *Cladophora sp* also shown the resistance against tested organism. The results of antifungal activity against tested pathogens were tabulated in the **Table 2** for the crude extractions of *S. tenerrimum* and *T.ornate* for antifungal activity. **Table 3 represent** zone diameter of other species shown the moderate and low activity against tested pathogen. The zone of inhibition of high activity seaweeds extract for two different pathogen depicted in **Fig. 5.**

DISCUSSION:

The results of the phytochemical analysis and antifungal screening revealed the presence of high amount of biochemical compounds and antifungal substances in seaweeds studied. From the study, maximum carbohydrate was recorded in *C.indica* belongs to phaeophycean species and some of the Chlorophycean members. Similarly, Chakraborthy and Santra (2008) recorded higher carbohydrate in the green seaweeds like *Ulva lactuca* (35.27%) and *E. intestinalis* (30.58%). Kaliaperumal *et al.,* (1987) also reported similar kind of results that the green seaweeds have high carbohydrate than the red and brown seaweeds. Pise N. M and Sabale A. B., (2010) investigations revealed the maximum carbohydrate being recorded in Sargassum a brown alga and minimum was found in *Gracillaria*, a member of Rhodophyceae which is corroborated with the present investigations

In the present study the highest protein content was encountered in the brown alga *P.boergesenii* and red algae *G.corticata* than the green alga. Similarly Dinesh *et al.,* (2007) recorded highest protein content in brown alga Tubinaria ornata from Gulf of Mannar region and Anitha *et al.,* (2008) recorded maximum protein in the brown alga *Turbinaria conoides* and minimum in *Gracilaria corticata* from the same Mandapam coast. Besides, Selvi *et al.,* (1999) reported more protein content in red alga *Hypnea valentiae where as* Mairh *et al.,* (1983) reported 22.22% of crude protein *in Ulva fasciata*.

The amount of total phenol and flavanoid was higher in the brown seaweeds *P. boergesenii* and *C. indica*, respectively. Marry and Vimalabai (2003) screened four brown seaweeds from Tuticorin coast for their phenol content and reported highest value in *Padina tetrastromatica*. Pedersen (1964) reported that the phenol content increased with the increasing age of the tissue and with increasing salinity. The highest total chlorophyll was recorded in the green alga *M. latissimum* and minimum in the red alga *G.corticata*. Similarly Muthuraman and Ranganathan (2004) reported maximum chlorophyll in the green alga Caulerpa scalpelliformis among the 12 seaweeds tested which include Phaeophycean and

Rhodophycean member also. The highest carotenoid content was recorded in the brown seaweed *D.acrostichoides*, similarly Muthuraman and Ranganathan (2004) reported maximum carotenoid content in the brown seaweed *S. wightii*.

The study was evaluated the activity of different species of seaweeds from the Okha coast against pathogenic fungus. As for the tests with pathogenic fungus, the extracts showed differences in their activity, depending on the solvent used in the extraction. The brown seaweeds shows high antifungal activity as compare to red and green algae. The chloroform and ethanol extract of *Sargassum tenerrimum* and *Turbinaria ornate* showed highest antifungal activity against tested pathogenic organism than other seaweeds where as *S. cinereum* , and *Cladophora sp*. shown moderate activity against tested pathogen. The acetone extract of seaweeds showed minimum activity against both organisms. In the present study, the species of Phaeophyta showed the strongest activities against fungi, which was in agreement with the findings of Padmakumar and Ayyakkannu (1997). The brown seaweeds contain high amount of flavanoid and phenolic compound could be the reason for antifungal activity, Cowan *et al.*, (1999) which further confirms the greater amounts of phenolic compound in brown algae in the present investigation.

CONCLUSION

The study reveals that the seaweeds contain high amount of biochemical constituents besides the crude extracts of the seaweeds showed promising activity against the test fungal pathogens, henceforth, seaweeds collected from Okha coast, Gujarat region is potential capacity for biochemical compounds which makes them for screening of natural products for pharmaceutical industry.

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Table.1 List of the Seaweeds belong to different classes, collected from Okha coast, Gujarat

Chlorophyta	**Phaeophyta**	**Rhodophyta**
Sp1 –*Monostroma latissimum* Wittrock	Sp3- *Padina boergesenii* Allender &Kraft	Sp12- *Gracillaria corticata* J.Agardh
Sp2- *Cladophora sp*	Sp4-*Dictyopteris acrostichoides Bornet*	Sp13- *Geidium micropterum* Kutzing
	Sp5- *Sargassum tenerrimum J.G. Agardh*	
	Sp6- *Sargassum cinctum J.Agardh*	
	Sp7- *Sargassum cinereum* J.Agardh	
	Sp8- *Cystoseira indica* Mairh	
	Sp9- *Cystoseira trinedis* C.Agardh	
	Sp10- *Dictyota dichotoma* Lamouroax	
	Sp11- *Turbinaria ornate* J.Agardh	

Table 2. Antifungal activity of two species of high MIC

Test Organisms	Inhibition zone diameter (mm)						
	Sargassum tenerrimum				Standard		
	Chloroform extract		Ethanol extract				
Concentration	50 µl	100 µl	50 µl	100 µl	Fluconazole 10 mcg	Ketoconazole 10 mcg	Amphotericin B 20mcg
A. niger	19	20	9	12	10	17	18
P. jenthinellum	12	14	13	15	12	20	19
	Turbinaria ornate						
	50 µl	100 µl	50 µl	100 µl	Fluconazole 10 mcg	Ketoconazole 10 mcg	Amphotericin B 20mcg
A. niger	11	13	10	14	10	17	18
P. jenthinellum	13	16	10	11	12	20	19
Control	Chloroform 100µl				Ethanol 100µl		
A. niger	8				9		
P. jenthinellum	9				10		

Table 3. Antifungal activity of seaweeds of moderate and low activity

		Inhibition zone diameter (mm)			
Seaweed species		*A. niger*		*P. janthinellum*	
		50 µl	100 µl	50 µl	100 µl
C. sp	**CE**	12	12	11	13
	AE	7	9	NA	7
	EE	6	9	7	7
D. acrostichoides	**CE**	19	20	8	9
	AE	NA	NA	NA	NA
	EE	8	12	13	15
S. cinereum	**CE**	NA	6	9	11
	AE	NA	8	NA	NA
	EE	7	7	4	5
C. indica	**CE**	NA	NA	12	12
	AE	7	9	NA	7
	EE	5	9	6	5
C. trinedis	**CE**	7	7	13	16
	AE	7	8	7	8
	EE	10	11	5	6
G. micropterum	**CE**	11	13	9	10
	AE	NA	NA	NA	7
	EE	8	7	5	6

Fig 1. Map of Gujarat showing the study site of Okha Coast, Gujarat, India

Fig 2. Identified Seaweeds collected from the site.

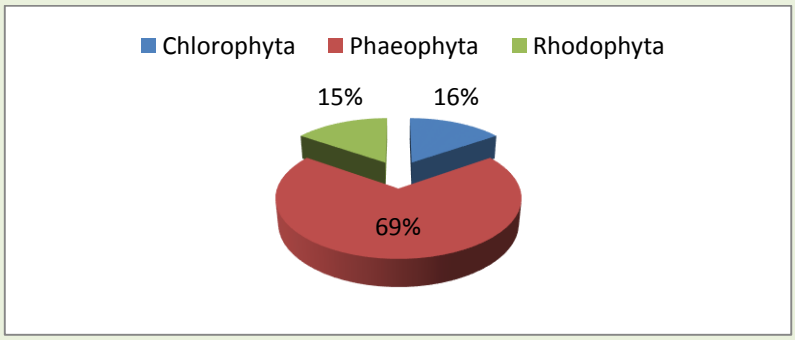

Fig. 3 Percent distribution of Seaweeds at selected site

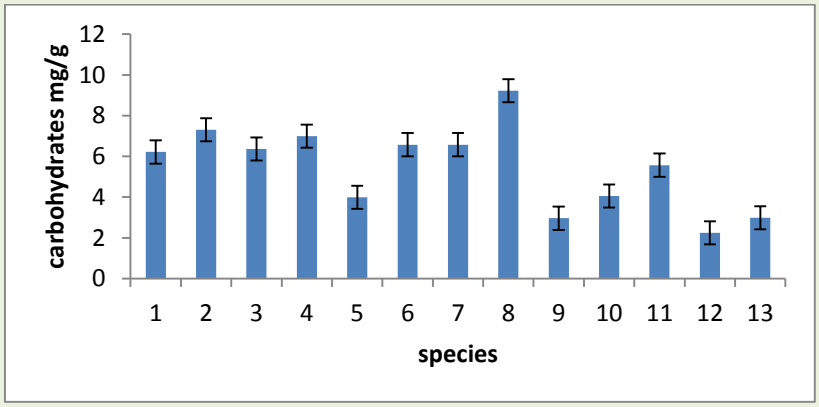

Fig: 4a. Carbohydrate content of different seaweeds collected from Okha coast

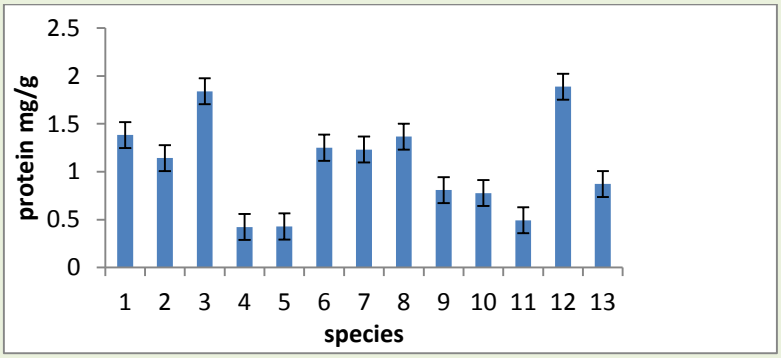

Fig: 4b. Protein content of different seaweeds collected from Okha coast

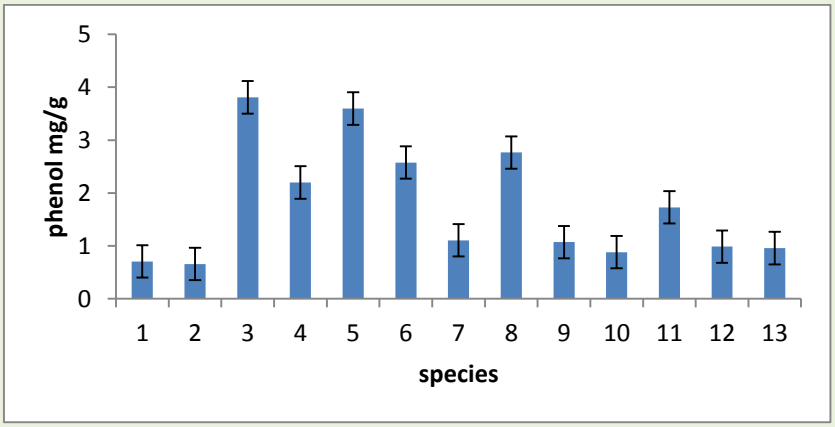

Fig: 4c. Phenol content of different seaweeds collected from Okha coast

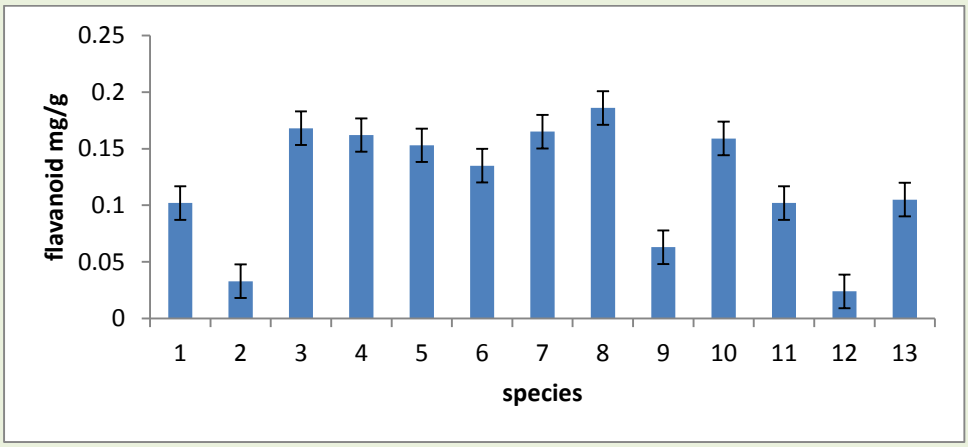

Fig: 4d. Flavanoid content of different seaweeds collected from Okha coast

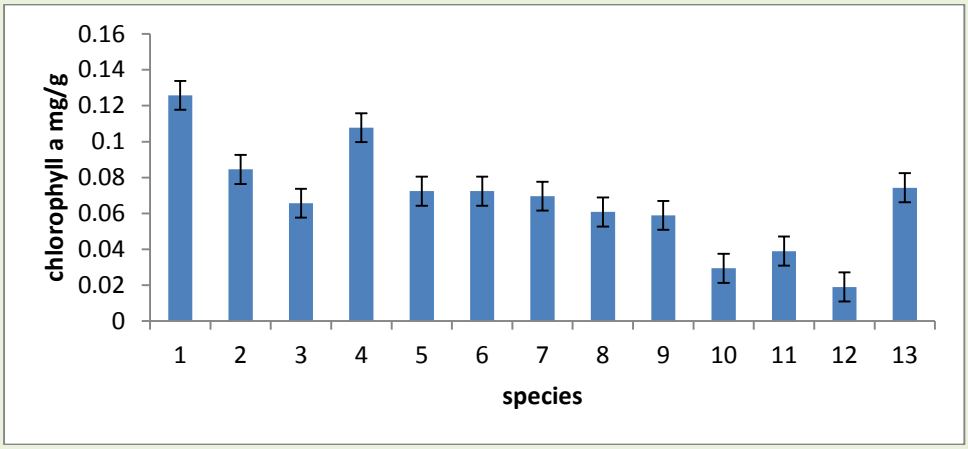

Fig: 4e. Chlorophyll a content of different seaweeds collected from Okha coast

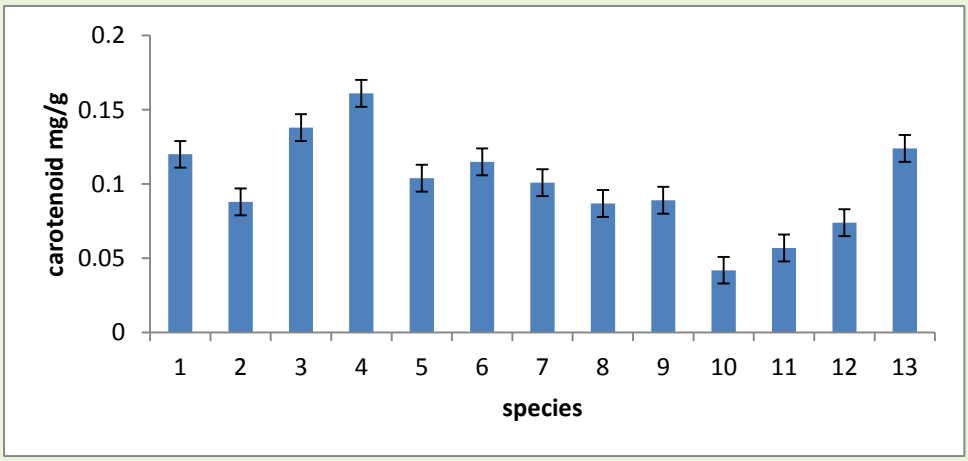

Fig: 4f. Carotenoid content of different seaweeds collected from Okha coast

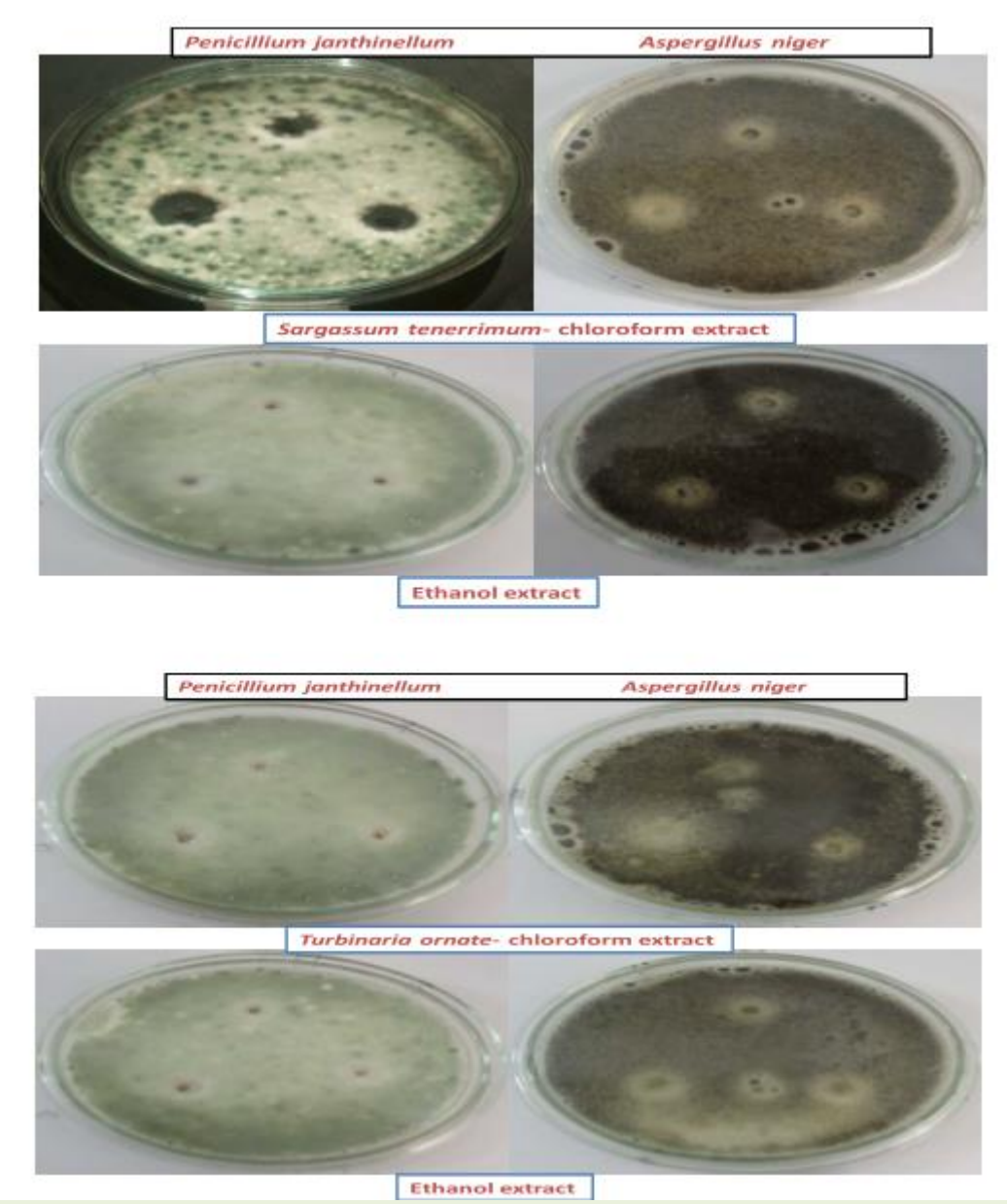

Fig: 5. Antifungal assay

Note: (CE-chloroform extract, AE-acetone extract, EE-ethanol extract, NA- no activity)