PREDICATION OF BIOLOGICAL ACTIVITY OF ALGAL ANTITUMOR DRUGS USING PASS

R. Azhaguraj[1*], E. Arokia Lenin[2], C. Viswanathan[3,] B. Sangeetha[1] and M. Selvanayagam[1†]

[1*†]Department of Advanced Zoology and Biotechnology, Loyola Institute of Frontier Energy, Loyola College (Autonomous), Chennai-600 034 Tamil Nadu, India. Tel-04428178200, Cell-09176019044, Fax-044-28175566 ,azhaguloyolascholar@gmail.com, drmssel@yahoo.co.in

[2]Department of Advanced Zoology and Biotechnology, St. Xavier's College, (Autonomous), Palayamkottai, Tamil Nadu, India.627 002.

[3]Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Parangipettai, Tamil Nadu, India 608 502.

Summary

The computer system PASS provides simultaneous predication of several hundreds of biological activity types of any drug like compounds. Over the past decade seaweeds have become an interesting source of new classes of pharmacologically active natural products. The majority of biologically active compounds have both pharmacotherapeutic and side/toxic actions. To estimate general efficacy and safety of the molecules under study, their biological potential should be thoroughly evaluated. In this study the PASS (Predication of activity spectra for substances)computer program, which is able to simultaneously predict more than one thousand biological and toxicological activities from only the structural formulas of the chemicals, was used to predict the biological activity profile of 19 algal secondary metabolites. PASS predications were successfully compared to the available information on the pharmacological and toxicological activity of these compounds.

Key words: PASS software; antitumor activity, marine drugs, Cancer.

Introduction

Bioinformatics is progressing from the mere analysis of nucleic acid and amino acid sequences to the search for new targets and ligands as leads for new drugs. There are cases where a ligand, capable of binding to one protein binds to a homologus or similar protein. The similarity of targets can be transferred to the similarity of drug like ligands, and some new ligands can be found on the basis of structure activity relationship **[1]**. Experimental determination of drug efficacy and safety is a time and cost consuming procedure. There exist standard tests for drug safety assessment and different strategies of search for new lead compound. Biological testing is organized taking into account similarity/dissimilarity of new compounds to the known biologically active substances several similarity/dissimilarity suggestions are used both in drug design and screening to determine if particular tests are necessary and sufficient for comprehensive estimation of new compound activity **[2].**

Biological activity is a result of a chemical compounds interaction with a biological entity. In clinical study, a biological entity is represented by a human organism. In preclinical testing it is the experimental animals (in vivo) and experimental models (in vitro). Biological activity depends on the peculiarities of a compound (structure and physic- chemicals properties), biological entity (species, sex, age, *etc.*), mode of treatment (dose, route, *etc*). Any biologically active compound reveals a wide spectrum of different effects. Some of them are useful in the treatment of definite diseases, but the others cause various side and toxic effects. Total complex of activities caused by the compound in biological entities is called the biological activity spectrum of the substance.Such evaluation can be done using internet with the software PASS, which estimates the probabilities of 900 types of biological activity on the basis of structural formulae of compounds with the accuracy of 85 %. PASS predications are based on the analysis of structure-activity relationship (SAR)for the training set of about 46000 biologically active compounds**.[2-3]**.Therefore, pass once trained is able to predict simultaneously all biological activities which are included in the training set . To provide the best quality of predication new information about biologically active compounds is collected permanently from papers and electronic sources and after the experts evaluation, is regularly added to the training set**.[4-6].**

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. Algae are heterogeneous group of plants with a long fossil history. Two major types of algae can be identified, the macro algae(seaweeds)and microalgae(phytoplankton).Hopkins marine station pointed to over 5000 species of green algae known,1500 species off brown algae almost exclusively found in marine habitats and with over 4000 species of red algae. Man has used the sea for many years as a productive source for several economically useful materials, especially to supplement his diet **[7]**. Algal chemistry has interested many researchers in order to develop new drugs. The knowledge about the chemical composition of marine organisms is an essential element for assessing chemotaxonomic, chemical ecology, and natural products studies, including that directed towards evaluating the pharmacological roles. In recent years an increasing number of marine natural products have been reported to display antimicrobial activities and anti tumor compounds, have been isolated from sponges, tunicates, algae and other organisms **[8][9]**.In most cases, the evaluation of anticancer potential of crude extracts from different sea organisms has been carried out by in vivo cytotoxicity tests in malignant cell cultures **[10]**[**11**] Jolles(1963), were the first report the influences of degraded sulfated laminarine (an algal extract) on tumor growth**[12].**Isolation of cytotoxic antitumor substances from marine organisms has been reported in several references during the last 40 years **[13-16]**, while in recent years, hundreds of potential anti tumour agents have been isolated from marine origin especially from marine algae**[17]** and **[15].**Marine algae derived compounds have played an important role in the development of several clinically useful anticancer agents.

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. Most anticancer drugs act inhibiting DNA synthesis or some other process in the cell growth cycle. Because anti cancer drugs generally affect rapidly dividing cells, other non-cancerous cells will also be affected. the way in Which the other cells are affected determines the side effects of the individual drugs .other cells affected include blood cells, which fight infection, help the blood to clot ,and carry oxygen to all parts of the body.

Many of these side effects can now be controlled, thanks to new or improved drugs. With the known ethno botanical knowledge and phytochemical interpretations there are a number of drugs that could be effective against the cancer. The outcome of Insilco knowledge on the designing of drugs is yet another boom for the designers to minimize the work, the PASS(predication of activity spectra for substances) is one such server that predicts the possibility of a drug to be active against a target based on the physico-chemical methods using comparisons and various algorithms **[4], [18-19]**.

Material and Methods

The structures of 8α11-dihydroxypachydictyol A from *Dictyota* sp **[20],[21]** Bis –prenylated quinons from *Perithalia capillaris* **[22]** Cyckozonarone and Meroditerpens atomarianones A from *Taonia atomaria* **[23], [24],** Terpeniod C and Styoplactone from *Styolpopdium zonale* **[25],[26]**12-hydroxygeranylgeraniol from *Bifurcaria bifurcate* **[27**] Dictyone acetate from *Cystoseria myrica* **[28**], 24-Ethyl cholesta-4,24(28)-diene 3-one and 24-ethylcholesta-4,28(29)-diene 3-one, from *Turbinaria conoids***[29]** Diterpene - xenicane from *Dityota dichotoma* and Sargol II from S*argassum tortile* **[30]** are drawn using the Chem sketch package 11.0 belonging to the ACD Chem laboratory.

The structure of the phytochemicals present in the marine algae are (Zonaral, Zonarone , Isozonaral from *Dictyopteris zonarioides***[31]** leptosin from S*argassum tortile* [**32]**,Turbinaric acid from *Turbinaria ornate***[33]**, dolabelane diterpene from *Dictyota* sp**[20]**and Ribofuranosides *Hizikia fusiforme*[**34]** are obtained from the pubchem compound repository. The biological activity spectrum was drawn using the activity predication server PASS-(Predication Of Activity Spectra of Substances). PASS 4.20 predications search includes 9314 biologically active substances PASS 4.20 predicts the probabilities of presence/absence for114 biological actions simultaneously main and side pharmacological effects, mechanisms, specific toxicity).the biological activity spectrum of pass was designed and prediction was made with the comparison from the source data available in http://195.178.207.233/PASS/predict.php. biological activity spectrum of a compound presents exhibit its activity despite the difference in essential conditions of its experimental determination.

Results and Discussion

Antitumor compounds like (i) 8α11-dihydroxypachydictyol A, (ii) Bis-prenylated quinons, (iii) Cyckozonarone, (iv)Meroditerpens atomarianones A, (**Table** 1),(v) Terpeniod C, (vi) Styoplactone; (vii)12-hydroxygeranylgeraniol, (viii) Dictyone acetate, (**Table** 2),(ix)24-Ethyl cholesta-4,24(28)-diene 3-one, (x) 24-ethylcholesta-4,28(29)-diene 3-one, (xi) Diterpene – xenicane, (xii)Sargol II (**Table 3**) (xiii) Zonaral, (xiv) Zonarone, (xv) Isozonaral, (xvi) leptosin, (**Table 4**) (xvii) Turbinaric acid,(xviii) dolabelane diterpene, (xix) Ribofuranosides (**Table 5)** were predicted. PASS predicated search results shows that the available information on the pharmacological and toxicological activity of these compounds and they are corroborative with the previous reports **[4-6][18-19].**

Table 1 : List of Biological Activities Predicated by PASS version 4.2.(8α11-dihydroxypachydictyol A, Bis-prenylated quinons, Cyckozonarone,and Meroditerpens atomarianones A,

S. No	Pa	Pi	Activities
8- Alpha Dihydroxypachydictyol A			
1	0,830	0,014	Antimetastatic
2	0,816	0,005	TERT expression inhibitor
3	0,743	0,019	CDP-glycerol glycerophosphotransferase inhibitor
Bis - Prenylated quinons			
4	0,830	0,014	Antimetastatic
5	0,816,	0,005	TERT expression inhibitor
6	0,743	0,019	CDP-glycerol glycerophosphotransferase inhibitor
	0,727	0,008	Insulin and insulin analogs
Cyckozonarone			
7	0,925	0,018	Transferase stimulant
8	0,916	0,012	CYP2C12 substrate
9	0,910	0.007	Testosterone 17 beta-dehydrogenase (NADP+)Inhibitor
10	0,876	0,004	Oxidoreductase inhibitor
11	0,850	0,005	Mucomembranous protector
12	0,849	0,004	CYP2B5 substrate
13	0,821	0,004	Antiviral (Influenza)
14	0,810	0,005	Hypercholesterolemic
15	0,819	0,017	Phosphatase inhibitor
16	0,822	0,022	Antiiseborrheic
Meroditerpens Atomarianones A			
17	0,910	0,007	Testosterone 17 beta-dehydrogenase (NADP+) Inhibitor
18	0,875	0,024	CYP2C12 Substrate
19	0,840	0,017	Reproductive dysfunction
20	0,829	0,007	Oxidoreductase inhibitor
21	0,815	0,005	CYP2B5 Substrate
22	0,815	0,005	27-hydroxy cholesterol 7 alpha-monooxygenase inhibitor
23	0,812	0,016	CYP2Jsubstrate
24	0,812	0,016	CYP2J2 substrate
25	0,800	0,016	Steroid 21-monoxygenase inhibitor
26	0,784	0,024	Phosphatase inhibitor

TABLE 2. List of Biological Activities Predicated by PASS version 4.2. (Terpeniod C, Styoplactone; 12-hydroxygeranylgeraniol, and Dictyone acetate,

S.No	Pa	Pi	Activities
Terpenoid c			
1	0,848	0,006	Antiinflammatory
2	0,820	0,003	Cholertic
3	0,799	0,021	Phosphatase inhibitor
4	0,740	0,005	Antioxidant
5	0,748	0,015	Oxidoreductase inhibitor
6	0,702	0,015	Antipruritic
7	0,712	0,032	Mucomembranous protector
8	0,729	0,141	Transferase stimulant
Styoplactone			
9	0,981	0,001	Phosphatase inhibitor
10	0,882	0,008	Acylcarnitine hydrolase inhibitor
11	0,845	0,011	Antimetastatic
12	0,832	0,018	Alkenylglycerophosphocholine hydrolase inhibitor
13	0,817	0,004	Cycloartenol synthase inhibitor
14	0,835	0,023	Testosterone 17 beta –dehydrogenase(NADP+) inhibitor
15	0,815	0,011	Alkylacetylglycerophosphatase inhibitor
16	0,767	0,008	Cholesterol synthesis inhibitor
17	0,831	0,083	Transferase stimulant
18	0,749	0,008	TERT expression inhibitor
12-hydroxygeranylgeraniol			
19	0,926	0,002	Prenyl-diphosphatase inhibitor
20	0,920	0,002	Undecaprenyl-phosphatemannosyltranferase inhibitor
21	0,906	0,003	(-)-(4S)- limonene synthase inhibitor

22	0,899	0,001	Retional dehydrogenase inhibitor
23	0,862	0,021	Retional oxidase inhibitor
24	0,839	0,004	CYP2E1 inhibitor
25	0,836	0,005	Hyperglycemic
26	0,794	0,003	Dolich- phosphatase inhibitor
27	0,794	0,006	Protoporphyrinogenvoxidase inhibitor
28	0,792	0,006	TERT expression inhibitor
Dictone acetate			
30	0,909	0,004	Antimetastatic
31	0,799	0,004	Proliferative disease treatment
32	0,795	0,022	Phosphatase inhibitor
33	0,769	0,004	Adenomatous polyposis treatment
34	0,744	0,001	CYP24A1 Substrte
35	0,745	0,023	Prostaglandin –E2 9-reductase inhibitor

Table 3: List of Biological Activities Predicated by PASS version 4.2. (24-Ethyl cholesta-4,24(28)-diene 3-one, 24-ethylcholesta-4,28(29)-diene 3-one, Diterpene – xenicane, and Sargol II

S.No	Pa	Pi	Activities
24- ethyl cholesta -4,24(28)-diene 3-one			
1	0,803	0,002	Mitochondrial electron transport inhibitor
2	0,823	0,023	Acrocylindropepsin inhibitor
3	0,823	0,023	Chymosin inhibibitor
4	0,823	0,023	Saccharopepsin inhibitor
5	0,859	0,064	Transferase stimulant
6	0,784	0,032	Thermopsin inhibitor

7	0,739	0,003	Plastoquinol-plastocyanin reductase inhibitor
8	0,754	0,031	Pro –opiomelanocortin converting enzyme inhibitior
9	0,755	0,041	Polyporopepsin inhibitor
10	0,743	0,034	Sugar –phosphatase inhibitor
24-ethylcholestra-4,28(29)-diene 3-one			
11	0,839	0,022	Testosterone 17 beta-dehydrogenase (NADP+)Inhibitor
12	0,796	0,004	Proliferative diseases treatment
13	0,803	0,020	Phosphatase inhibitor
14	0,805	0,027	GST A substrate
15	0,789	0,012	Mucomembranous protector
16	0,759	0,009	Fatty-acyl-CoA synthhase inhibitor
17	0,765	0,017	Membrane permeability inhibitor
18	0,780	0,035	Acrocylindropepsin inhibitor
19	0,780	0,035	Chymosin inhibitor
20	0,780	0,035	Hypocholesterolemic
Diterpene xenicane			
21	0,925	0,003	Cardiovascular analeptic
22	0,915	0,004	Phosphatase inhibitor
23	0,835	0,013	Antimetastatic
24	0,809	0,011	H+-exporting ATPase inhibitor
25	0,783	0,007	NOS2 expresssion inhibitor
26	0,807	0,039	Membrane integrity agonist
27	0,783	0,034	Neurotoxic
28	0,786	0,046	Ubiquional-cytochrome –c reductase inhibitor
29	0,769	0,029	Hyperthermic
30	0,776	0,044	Emetic
Sargol II			
31	0,941	0,002	Antioxidant

32	0,926	0,009	CYP2C12 Substrate
33	0,896	0,003	TERT expression inhibitor
34	0,868	0,003	NOS2 expression inhibitor
35	0,827	0,004	Apoptosis agonist
36	0,824	0,008	Antineoplastic (lung cancer)
37	0,816	0,006	Kinase inhibitor
38	0,835	0,030	Retinal oxidase inhibitor
39	0,833	0,032	Membrane integrity agonist
40	0,797	0,001	Anticarcinogenic

Table 4. : List of Biological Activities Predicated by PASS version 4.2. (Zonaral, Zonarone, Isozonaral, and leptosin)

S.No	Pa	Pi	Activitites
Zonaral			
1	0,880	0,006	CYP2B6 inhibitor
2	0,862	0,009	Phosphatase inhibitor
3	0,811	0,018	Antimmmetastic
4	0,773	0,005	Proliferative diseases treatment
5	0,776	0,007	NOS2 expression inhibitor
6	0,761	0,003	Cholertic
7	0,762	0,011	hypercholesterolemic
8	0,761	0,021	Prostaglandin-E2 9-reductase inhibitor
9	0,740	0,008	CYP2C8 Inhibitor
10	0,717	0,004	Nitric oxide antagonist
Zonorone			
11	0,864	0,009	Phosphatase inhibitor
12	0,808	0,018	Antimetastatic

13	0,765	0,008	NOS2 expression inhibitor
14	0,750	0,006	Proliferative diseases management
15	0,737	0,015	Anti-inflammatory
16	0,726	0,018	CYP2B6 Inhibitor
17	0,732	0,024	Prostaglandin E2 9- reductase inhibitor
18	0,708	0,027	Hypercholesterolemic
19	0,762	0,124	Transferase stimulant
Isozonaral			
20	0,788	0,007	Hypercholesterolemic
21	0,793	0,022	Phosphatase inhibitor
22	0,744	0,005	Antioxidant
23	0,823	0,088	Transferase stimulant
24	0,723	0,012	NOS2 expression inhibitor
25	0,728	0,018	Oxidoreductase inhibitor
Leptosin			
26	0,956	0,003	Membrane integrity agonist
27	0,935	0,002	Antioxidant
28	0,894	0,003	Chemopreventive
29	0,878	0,003	Monophenol monooxygenase inhibitor
30	0,861	0,003	Vasoprotector
31	0,864	0,014	Transferase inhibitor
32	0,850	0,002	Free radical scavenger
33	0,851	0,015	Benzoate co A ligase inhibitor
34	0,837	0,008	CDP-glycerol glycerophophotransferase inhibitor
35	0,825	0,004	TERT expression inhibitor

Table 5: List of Biological Activities Predicated by PASS version 4.2 ,(Turbinaric acid, dolabelane diterpene, and Ribofuranosides.)

S.No	Pa	Pi	Activitites
Turbinaric acid			
1	0,957	0,002	CYPP2E1 Inhibitor
2	0,945	0.001	Prenyl –diphosphate inhibitor
3	0,941	0,002	(-)-(4S)-Limonene synthase inhibitor
4	0,929	0,003	VCAM1expression inhibitor
5	0,925	0,003	CYP2J2 Substrate
6	0,922	0,002	Undecarprenyl- phosphate mannosy lytransferase inhibitor
Dolabllane			
7	0,846	0,004	TERT expression inhibitor
8	0,805	0,020	Phosphatase inhibitor
9	0,790	0,016	CYP3A4 Substrate
10	0,765	0,011	Antineoplastic
11	0,768	0,017	CYP3A substrate
12	0,752	0,005	Apoptosis agonist
13	0,748	0,008	CYP3A4 Inhibitor
14	0,727	0,012	CYP3A5 substate
Ribofuranosides			
15	0,954	0,003	Benzoate-Co A ligase inhibitor
16	0,946	0,003	D-arabinose 1- dehydrogenase inhibitor
17	0,938	0,004	Alkenylglycerophosphocholine hydrolase inhibitor
18	0,935	0,005	Signal peptidase II inhibitor
19	0,919	0,002	Managanese peroxidase inhibitor
20	0,917	0,003	Fucosterol-epoxide lyase inhibitor
21	0,915	0,003	Phosphatidate phosphatase inhibitor
22	0,913	0,005	Sugar- phosphatase inhibitor
23	0,908	0,002	Beta-mannosidase inhibitor

24	0,902	0,003	Mucinaminylsserine mucinaminidase inhibitor

PASS inlet predicates biological activity spectrum on the basis of structural formula of the compound. Establishing quantitative relationship between molecular structure and broad biological effects has been long standing challenge in science. Currently, no method exists for forecasting broad biological activity profiles of medicinal agents even narrow boundaries of structurally similar molecules. **[4-6][18]**.Pa and Pi are the estimates of probability for the compound to be active and inactive respectively for each type of activity from the biological activity spectrum their values vary from 0.000 to 1.000. it is reasonably that only those types of activities may be revealed by the compound, which Pa >Pi and so they are put into the biological activity spectrum. if Pa> 0.7 the compound is very likely to reveal this activity in experiments, but in this case the chance of being the analogue of the known pharmaceutical agents for this compound is also high, if 0.5 <Pa<0.7 the compound is likely to reveal this activity in experiments, but this probality is less, and the compounds is not so similar to the known pharmaceutical agents **[19][5]**. While planning experiments and choosing the activities on which the compound has to be tested, it is necessary to keep in mind the balance between the novelty of pharmacological action and the risk to obtain negative result in experimental testing. Certainly, in this present study, such a vital approach has been made to take into account the particular interest in some kinds of activities other than principal anti-tumor activity of the drugs from this algal extract. The accuracy of biological activity predication through PASS with reference to 19 algal drugs constituents is about 90%. Now a band width of drugs for the cancer treatment is analyzed, it must be improved and lot of studies have to be continued to ensure its activity invitro and in vivo.

Conclusion

The application of computerized system PASS in the process of new drugs R&D in many cases provide the possibilities to select compounds with desirable spectra of therapeutic effects and minimum side actions prior to experimental testing or even synthesis. The results show that antitumor drugs could possess several pharmacological activities such as Anti inflammatory,Antineoplastic (nonsmall cell lung cancer),Antineoplastic (lung cancer), Antica rcinogenic ,Choleretic ,Antioxidant ,Antipruritic ,Cardiovascular analeptic ,Hypercholesterol emic,Antiseborrheic, Antimetastatic, Phosphatase inhibitor, Neurotoxic ,Hyperthermic ,Antiv iral (Arbovirus), Apoptosis agonist, TERT expression inhibitor, ,Chemopreventive, Hypokale mia, Antithrombotic. The PASS software is useful for the study of biological activity of anti tumor compounds isolated from the brown algae.

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References

1. Alexy Lagunin, Alla Stepanchikova, Dmitrii Filimonov and Vladimir Poroikov . PASS: Prediction of activity spectra for biologically active substances. Bioinformatics 2000;16(8): 747-748.

2. Sadym A, Lagunin A, Filimov D and Poroikov V.Predication of biological activity spectra via the internet. SAR and QASR in Environmental Research 2003; 14(5-6): 339-347.

3.Anzali S, Anikal G, Cexanne B, Krug M, Fillimonov D and Poroikov V. Discriminating between drugs and non drugs by predication of activity spectra for substances (PASS). J.Med.Chem.2001; 44(15):2432-2437.

4. John de Britto, Leon Stephan Raj T, and Abiya Chelliah D. Prediction of biological activity spectra for few anticancer drugs derived from plant sources. Ethnobotanical leaflets 2001; 12: 801-810.

5. Maridass M, Raju G, Thangavel K and Ghanthikumar S. Predication of anti-HIV activity of flavanoid constituents through PASS . Ethnobotanical leaflets 2008; 12: 954-94.

6.Maridass M. PASS: Prediction of activity spectra for biologically active constituent of Polygodial (A Drimane type of Dialdehyde Sesquiterpene).2008; 3:191-197.

7. Prescott G.W. Economics of algae "The Algae:A Review" 1984. Otto Koeltz Science Publishers d-629. Koenig-Stein. W. Germany.

8. Chapman DJ, and Gellenbeck KW. An historical perspective of algal biotechnology in: "Algae and cyanobacterial biotechnology"(eds Cresswell, R, C, Ress T.A.V and Shav V) Longman group, U.K. 1983; 1-27pp.

9..Gamal AA, Biological importance of marine algae. Saudi Pharmaceutical Journal.2009; doi:10.1016/j.jsps.2009.12.001.

10.Russell FE. Advances of Marine Biology. Academic Press, New York.1963; 3: 255-256.

11. Jolles B, Remington M and Andrews PS. Effects of sulphated degraded laminarin on experimental tumour growth. Brit.J.Cancer. 1963;17: 109-115.

12. Ahmed M M, Ibrahim Mostafa H. Mostafa. M. Hisham El-Masry, Manal M. A. El-Naggar. Active biological materials inhibiting tumor initiation extracted from marine algae. Egyptian Journal of Aquatic Research. 2005;31(1):146-155.

13. Hansen, G.I. A revised check list and preliminary assessment of the macrobenthic marine algae of Oregon. 1997. pp.1-26.In T. Kaye (eds),conservation and management of native Flora and Fungi. Oregon State University Press, Corvallis.

14.Burrows E.M. Seaweeds of the British Isles.Vol.2.Chlorophyta Natural History Museum Publications, London, 1991; ix:238.

15. Fadli M, Aracil J.M, Jeanty G. Banaigs B. and Francisco, C. Novel meteroterpnoids from *Cystoseria mediterranea* use of crown gall bioassay as a primary screen for lipophilic anti-neoplastic agents. J. Nat. Prod. 1991:54(1):261-264.

16.Gonzalez AG, Darias Vand Estevez E. Chemo-therapeutic activity of polyhalogenated terpens from Spanish algae. J. Planta Medica, 1982;44:4-46.

17. Adams NM. 1994.Seaweeds of New Zealand : An illustrated guide. Canterbury University Press, Christchruch, New Zealand.

18.John de Britto, Prediction of biological activity of anti-malarial drugs using PASS. Indian Journal of Multidisciplinary Research.2008; 4(2): 271-274.

19. Abiya Chelliah D. Biological activity predication of an ethno medicinal plant *Cinnamomum camphora* through Bio-informatics. Ethnobotanical leaflets, 2008; 12: 181-190.

20. Tringali C, Prattelia M and Nicols G. Structure and conformation of new diterpens based on the dolabellane skeleton from *Dictyota* species. Tetrahedron,1984;40:799-803.

21. Jongaramruong J and Kongkam N . Novel diterpens with cytotoxic, anti-malarial and anti-tuberculosis activities from activities from a brown alga *Dictyota* sp. J. Asian Nat.Prod.Res. 2007; 9:743-51.

22.Blackman AJ, Dragar C and Wells RJ. A new Phenol from the brown alga *Perithalia caudata* contain a reverse isoprene unit at the 4th position. J. Aust.J.Chem.1979; 32: 2783-86.

23.WU CX, LI ZG and LI HW. Effect of berbamine on action potential in isolated human atrial tissues.Asia.Pac.J.Pharmacol,1990;5:191-193.

24. Abates D, Vigias C, Galanakis, D, Norris JN Moreau D, Roussakis C, and Rousis V. Atomariaanones A and B two cytotoxic meroditerpene from the brown alga *Taonia atomaria* .Tetrahedron let. 2005; 46: 8525-29.

25. Dorta E Cueto M Diaz-Marreo AR and Daris J . Stypolactone ,an interesting diterpenoid from the brown alga *Stypodium zonale*. Tetrahedron let, 2002; 65: 9043-46.

26. .Dorta E, Cueto M, Bito I and Darias J. New terpeniods from the brown alga *Stypopodium zonale* J. Nat. Prod.2002; 65:727-1730.

27. Gulioli G, Oratalo-Magne A, Daoudi M, Thomas Guyon H, Vallis R and Piovetti l. trihydroxylated liner diterpens from the brown alga *Bifurcaria bifurcate*. Phytochemistry. 2004; 65: 2063-69.

28. Ayyad S-E-N, Abdal-Halim OB, Shier WT and Hoye TR. Cytotoxic hydroazulene diterpens from the brown algae *Cystoseria myrica* Z. Biosci. 2003; 58: 33-38.

29. Sheu JH, Wang GH, Sung PJ and Duh CY. New cytotoxic oxygenated fucosterols from the brown algae *Turbinaria conoides*.J.Nat.Prod.1999;62: 224-227.

30. Numata A, Kambara S, Takaashi C, Fujiki R Yoneda M Fujita E and Nabeshima Y. Cytotoxic activity of marine algae and a cytotoxic principle of the brown algae *Sargassum tortile*. Chem.Pharm.Bull.1991; 39: 2129-31.

31. Finical W, Sims JJ, Squartritod, Wing RM, and Radick P.Zonarol and Isozonaral, fungitoxic hydroquinones from the brown seaweed *Dictyopteris zonariodes*. J. Org. Chem.1973; 38: 2383-86.

32. Yamada T, Iwamto C, Yamagaki N, Yamanouchi T, Minoura K, Yamouri T, Udehara Y, and OH Tumemura K, and Numata A. Leptosins M-N cytotoxic metabolites from a *Leptosphaeria* species separated from a marine algae, structure determination and biological activities Tetrahedron .2002;58 :479-487.

33. Asari F, Kusumi T, and Kakisawa H. Turbinaric acid A cytotoxic secosqualene carboxylic acid from the brown algae *Turbinaria orante*. J. Nat. Prod.1989; 52:1167-69.

34. Edmonds SI , Mortia M and Shibata Y. Isolation and identification of Arsenic containing ribfurnaoside and inorganic arsenic from japans edible seaweed *Hizikia fusiforme* . J. Chem. Soc. Perkin Trans I. 1987; 577-580.