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Bioactive compounds from marine yeast inhibits lung cancer

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ABSTRACT

Anticancer agents derived from natural products such as various sources like plants, animals and microorganisms. Marine-derived microorganism has become important sources of new bioactive molecules. The products isolated from marine sources possess cytotoxic activities against many cancers. Secondary metabolites as a source of *Candida albicans* natural drugs many of the medicines prescribed today are natural products obtained from terrestrial plants and microorganisms. The major components (80-90%) of cell wall of are carbohydrates, mannose or polymers of mannose covalently associated with proteins to form glycoproteins. The American Cancer Society has estimated 159,260 deaths due to lung cancer from diagnosis of 224,210 new cases in the United States in 2014. The International Agency for Research on Cancer estimated over 1 million new cancers cases in India in 2012 (10, 14, 934). In this study, we aimed to find new bioactive compounds from crude culture extracts produced by *C. albicans* as isolated from coastal mangrove ecosystem and docking studies were carried out.

INTRODUCTION

Natural products play a relevant role in cancer therapy today with substantial numbers of anticancer agents used in the clinic being either natural or derived from natural products from various sources such as plants, animals and microorganisms (also of marine origin). Large-scale anticancer drug discovery and screening programs such as those promoted by the National Cancer Institute (NCI) have played an important role in the development of anticancer natural compounds (Nobili, 2009). Thereafter, marine-derived microorganism has become important sources of new bioactive molecules (Lam, 2007). The first marine derived bioactive natural product reported from *Acremonium chrysogenum* which became the source of the parent compound of modern cephalosporin antibiotics now indispensible for the treatment of numerous bacterial infections (Proksch *et al.*, 2008). There is a large chemical diversity in marine plants. The products isolated from marine sources possess cytotoxic activities against many cancers. The marine environment contains a great diversity of organisms exhibiting a variety of molecules with unique structural features not found in terrestrial natural products (Schumacher *et al.*, 2011; Folmer *et al.*, 2008). Because of the great interest in marine bioactive compounds, researchers isolated hundreds of compounds each year and evaluated them for their anticancer properties. Didemnin B was the first marine compound isolated from the Caribbean tunicate *Tridemnum solidum* in 1981 (Ahuja *et al.*, 2000). Secondary metabolites as a source of natural drugs many of the medicines prescribed today are natural products obtained from terrestrial plants and microorganisms. The use of plant-derived secondary metabolites as drugs has come to us as a legacy of folk medicine based on herbal remedies (Nagle *et al.*, 2004, Newman *et al.*, 2004). High throughput screening of natural products is novel technologies in the recent discovery of new natural products from plants, animals, marine organisms, and microorganisms. Actinomycin D, mitomycin C, bleomycin, doxorubicin, and Lasparaginase are drugs derived from microorganisms (Orlikova *et al.*, 2014). Bryostatin 1 is best known as an anticancer agent.

This compound is able to induce ubiquitination and proteasome degradation of Bcl-2 in lymphoblastic leukemia and permits the growth of progenitor cells from bone marrow. Bryostatins were isolated from a species of bryozoan Bugulaneritina. This species lives in symbiosis with a bacterium that secretes an active biomolecule: bryostatin.

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Bryostatins are potent activators of protein kinase C (PKC) and regulate the activation, growth, and differentiation of cells (Wall *et al.,* 2000). Cancer is ongoing major health problem in developed as well as undeveloped countries. The great cancer incidence worldwide increases the search for new, safer and efficient anticancer agents, aiming the prevention or the cure of this illness. Although many classes of drugs are being used for the treatment of cancer, the need for more potent selective antitumor agents is still not precluded. Lung cancer is a prominent cause of cancer-associated mortality worldwide. The main reason for high mortality due to lung cancer is attributable to the fact that the diagnosis is generally made when it has spread beyond a curable stage and cannot be treated surgically or with radiation therapy. Therefore, new approaches like dietary modifications could be extremely useful in reducing lung cancer incidences (Naghma Khan, 2015). It is the second most common cancer in both men and women, and is by far the leading cause of cancer death among both men and women. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined. Most lung cancers could be prevented, because they are related to smoking (or secondhand smoke), or less often to exposure to radon or other environmental factors. But some lung cancers occur in people without any known risk factors for the disease. It is not yet clear if these cancers can be prevented. Most lung cancers have already spread widely and are at an advanced stage when they are first found. These cancers are very hard to cure (American cancer foundation). The American Cancer Society has estimated 159,260 deaths due to lung cancer from diagnosis of 224,210 new cases in the United States in 2014 (American Cancer Society, 2015). There was 14.1 million new cases of cancer were estimated globally in 2012, and more than half of these new cancers (56.8%) and cancer deaths (64.9%) occurred in developing countries (Naghma Khan, 2015). The International Agency for Research on Cancer estimated over 1 million new cancers cases in India in 2012 (10, 14, 934), with one third of these occurring in the breast, cervix, lip and oral cavity. Cancer is the third most common cause of death among non-communicable diseases in India and accounted for 6.6% (n = 663,032) of all deaths in 2010 (Mallath *et al.,* 2014).In accordance with worldwide trends, cancer rates will continue to rise in India due to the rising burden of chronic disease risk factors such as tobacco alcohol, physical activity, diet and body fatness (Mathur *et al.,* 2014). The majority of lung cancer patients have disease diagnosed at later stages which is not curable by current treatment options. Therefore, effective chemo preventive agents against lung cancer are greatly desired. Indications from these research studies on lung cancer suggest that the dietary agents modulate several signaling pathways and inhibit inflammatory processes. In Western countries, dietary factors account for about 30% of cancers, suggesting that diet plays an important role in the prevention of cancer (Doll and Peto1981). Therefore, the concept of chemoprevention would be very beneficial for persons who are in the high risk category for developing lung cancer, such as heavy smokers, ex-smokers and patients with resected primary lung cancer. The inducible transcription factor Nuclear Factor-kappaB (NF-kB) plays an important role in the regulation of immune, inflammatory and carcinogenic responses. While normal NF-kB activation is necessary for cell survival and immunity, deregulated NF-kB expression is a characteristic phenomenon in cancer development, as well as in several inflammatory diseases. Hence, NF-kB has become a major target in drug discovery, and several natural and synthetic compounds have been investigated for their potential to inhibit NF-kB. Natural products isolated from marine organisms have also been shown to have a great potential in drug discovery (Newman *et al.,* 2004; Senthilraja *et al.,* 2015). Already a comparative docking study was carried out to prove the bioactive compounds from marine products against liver cancer and diabetic mellitus (Senthilraja *et al.,* 2013*)*With the continuous emergence of new diseases and the development of drug resistance in harmful cancer cells, bacteria, virus and there is a continuous need for the development of new drugs with novel mechanisms of action. *Candida albicans* is a dimorphic opportunistic pathogen that grows as yeast or as a mycelial fungus depending on the environmental conditions. The major components (80-90%) of cell wall of *C. albicans* are carbohydrates, mannose or polymers of mannose covalently associated with proteins to form glycoproteins, also referred to as manno-proteins. These proteins contain β-glucans that are branched polymers of glucose containing β-1, 3 and β-1, 6 linkages and chitin, which is an unbranchedhomopolymer of N-acetyl-D-glucoseamine containing β-1, 4 bonds. Proteins (6-25%) and lipids (1-7%) are also present as minor wall components. 2, 3 1 β-Glucans and chitin (0.6-9%) are also present as structural components of the wall (47-60% by weight) (Chaffin *et al.,* 1998).

Among the various sources of anticancer drugs microorganisms have more advantages regarding to the potentials in producing diverse compounds and in the manipulation of the production. In this study, we aimed to find a new source of low and non-toxic, natural anticancer agent produced by yeasts, isolated from coastal mangrove ecosystem and used their culture extracts to screen for bioactive compounds and docking studies were carried out.

MATERIALS AND METHOD

Yeast culture

The samples were be collected from Pichavaram is located near Chidambaram in Cuddalore District, Tamil Nadu, in South India. (Lat.11º43'N and Long. 79º77'E), mangrove soil. They were purified by repeated sub culture and maintained in slants. The sub culture separately in Yeast Malt agar medium (YM) broth medium (Wickerham, 1951) containing yeast extract (1.0%) and malt extract (1.5%) prepared in 50 % seawater. The purified Culture 18srRNA identified cells as *Candida albicans* the sequence was submitted in Genbank Accession No. KP760068.

Crude extraction of Yeast

Candida albicans were grown in Yeast Malt Agar medium. After 48 hours of incubation at 24 ºC, the cell was

harvested by centrifugation and washed twice with 50 mm-TrisHCl buffer (pH 7.5). Cells were shaken vigorously for 2 hrs at 4°C and then pelleted was collected.

Characterization of Secondary Metabolites Using GC–MS Analysis

GC–MS analysis was done to identify and confirm the various bioactive compounds present in the crude extract. The culture free supernatant which was extracted in ethyl acetate was concentrated and analyzed in a GC–MS equipment (PerkinElmer Clarus 500). Experimental conditions of GC–MS system were as follows: Capillary Column Elite-5MS, Column ID 250µm. Flow rate of mobile phase (carrier gas: Helium) was set at 1.0 mL/min. The oven temperature was 50 °C at8 °C/min raised to 220 °C (5 min) at 7 °C/min to 280 °C (15 min). Injections of 2µL were effected at 250 °C in the splitless mode (0.8 min) into a split-splitless injector. Transfer line was heated to 280 °C and the ion trap temperature was 220 °C. The ion source was operated in the electron impact mode with 70 eV electron energy, scan range 40–600 amu.

Docking analysis

The structural information of the macromolecules determined by x-ray crystallographic and NMR methods are available in the PDB. The Lung cancer responsible protein (PDB I.D: 2XP2) was downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/). Bioactive compound retrieved from crude extract of *Candida albicans* by GC-MS analysis were used as ligand the properties was retrieved from Pubchem. The ligand molecules were generated and the three dimensional optimizations were done and then saved MOL file format. The docking analysis is performed by Discover Studio Version 4.5 (Biovia Dassault System, USA) for the lung cancer protein interacts with GC-MS of crude extract compounds. The protein ligand docking energy values performance of the 5 compounds was based on the Scoring functions which are implemented in docking program.

The docking result values are defined in Libdock value, when the higher docking energies values show the ligand bind to target protein with strong binding.

Result

The structural elucidation of the compound was based on the results of GC-MS analysis Retention Time (RT) peaks (12.23, 14.02, 15.63, 17.12, 18.43, and 19.72) are shown Fig: 1 and Fig: 2 3,4,5,6,7. The spectrum was comparable to the standard report data. The Properties and 2D structure of the compound obtained from the GC-MS analysis were given in the Table: 1. The compound retrived from GC-MS are used as ligand molecules for docking.

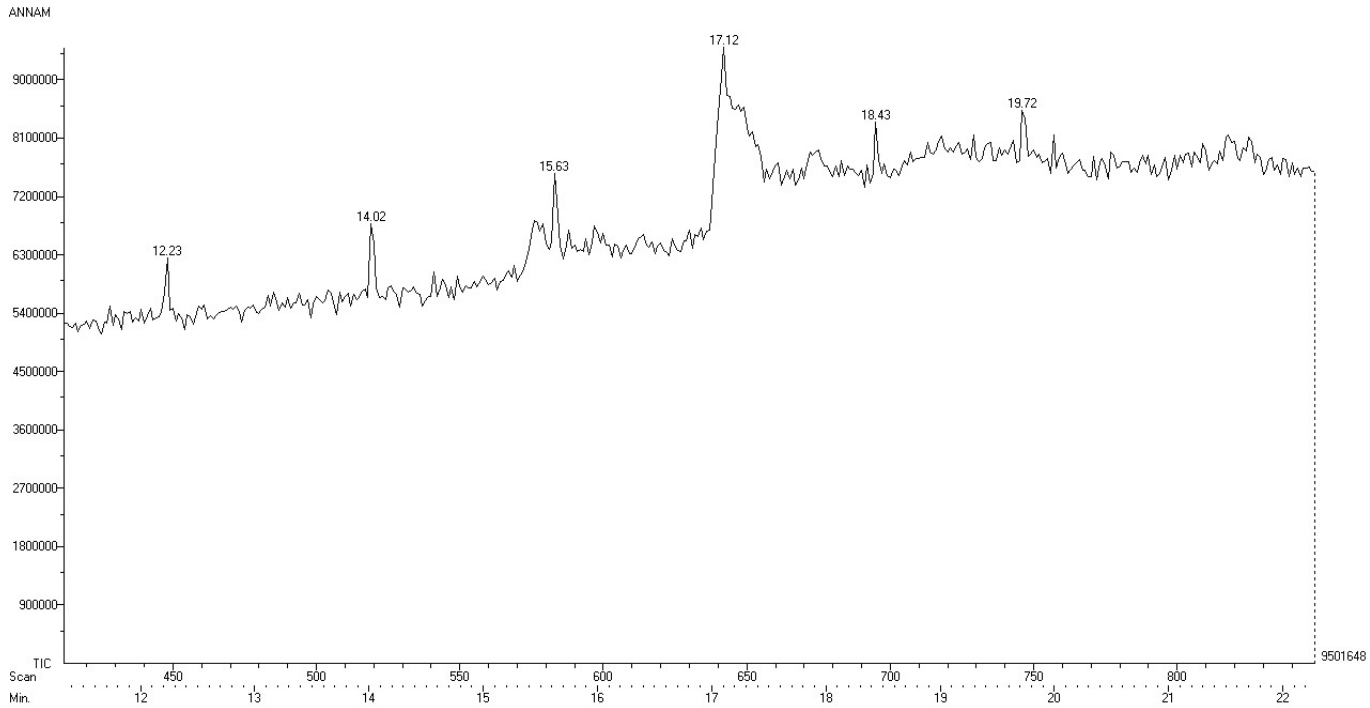

Fig: 1: GC-MS analysis of extracts of Candida albicans.

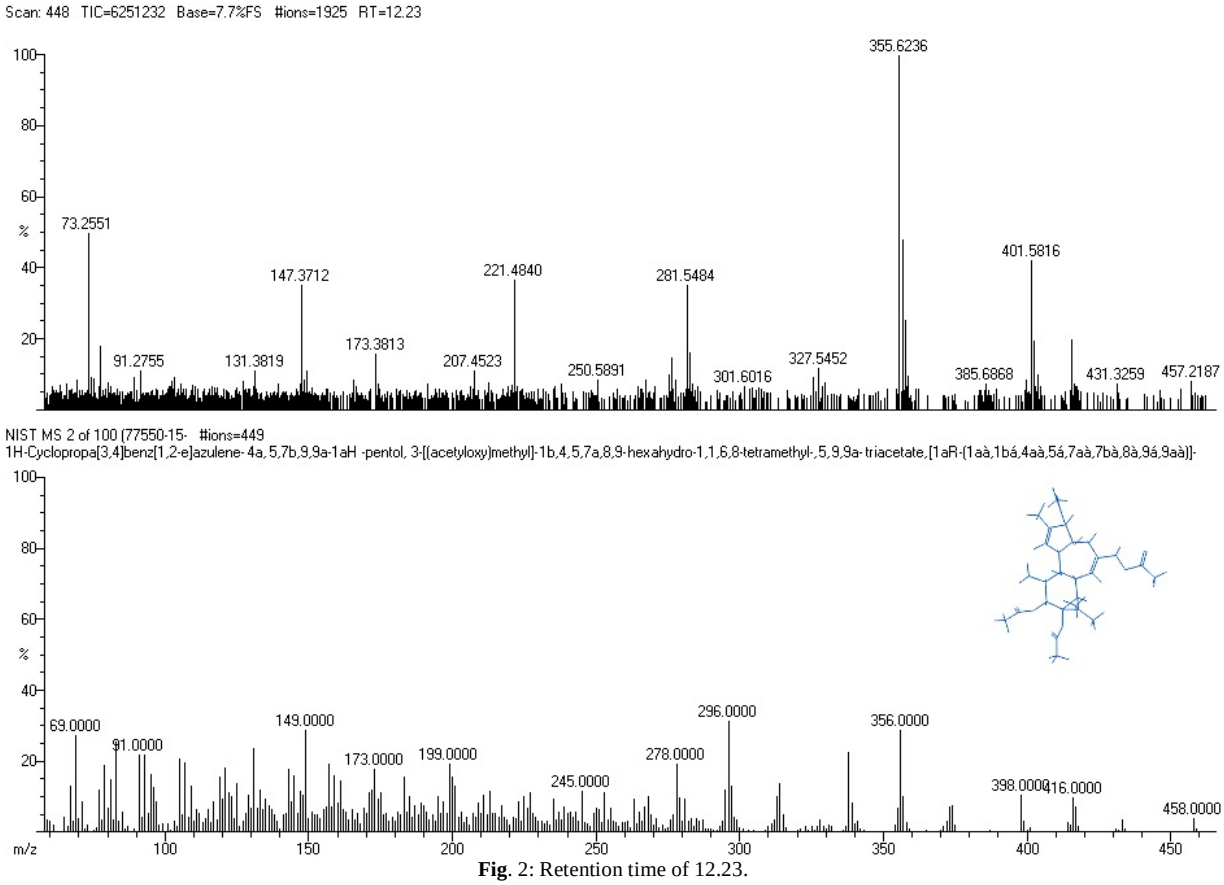

Fig. 2: Retention time of 12.23.

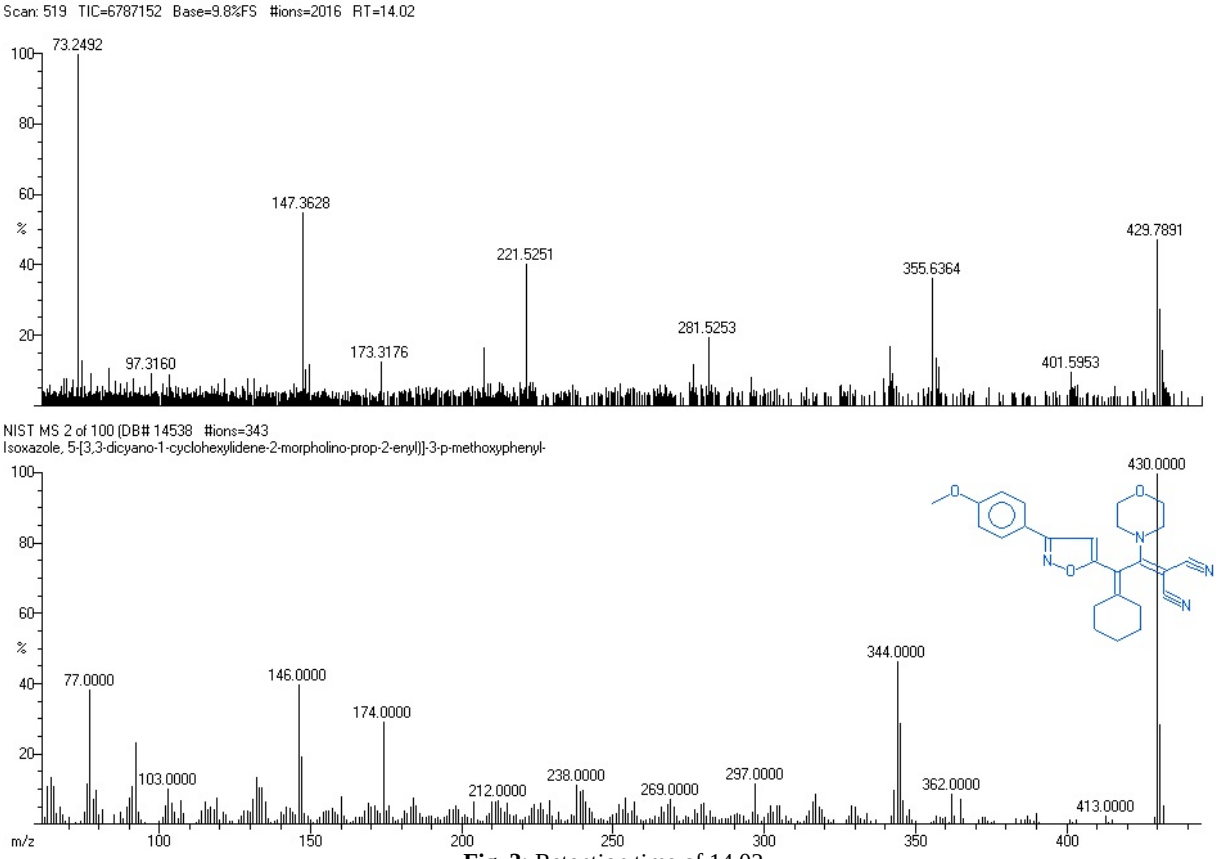

Fig. 3: Retention time of 14.02

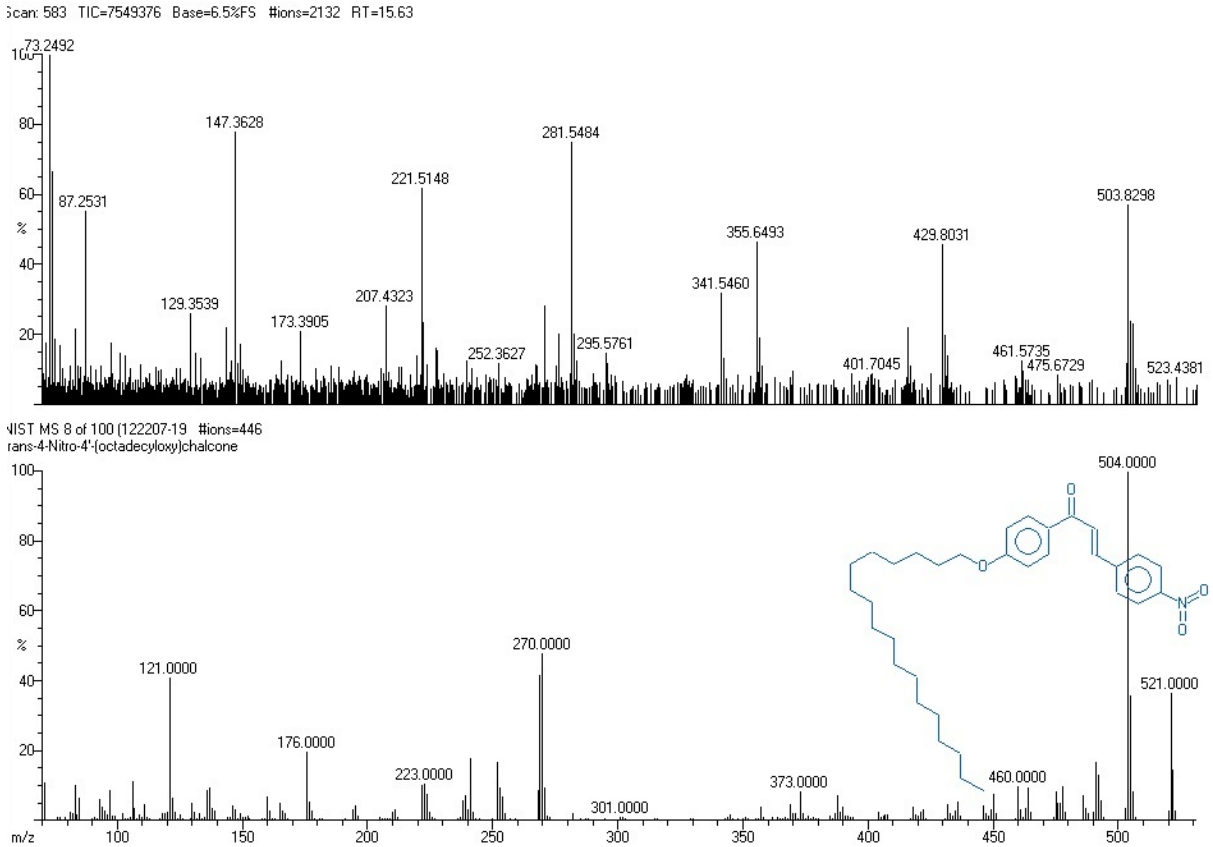

Fig. 4: Retention time of 15.63.

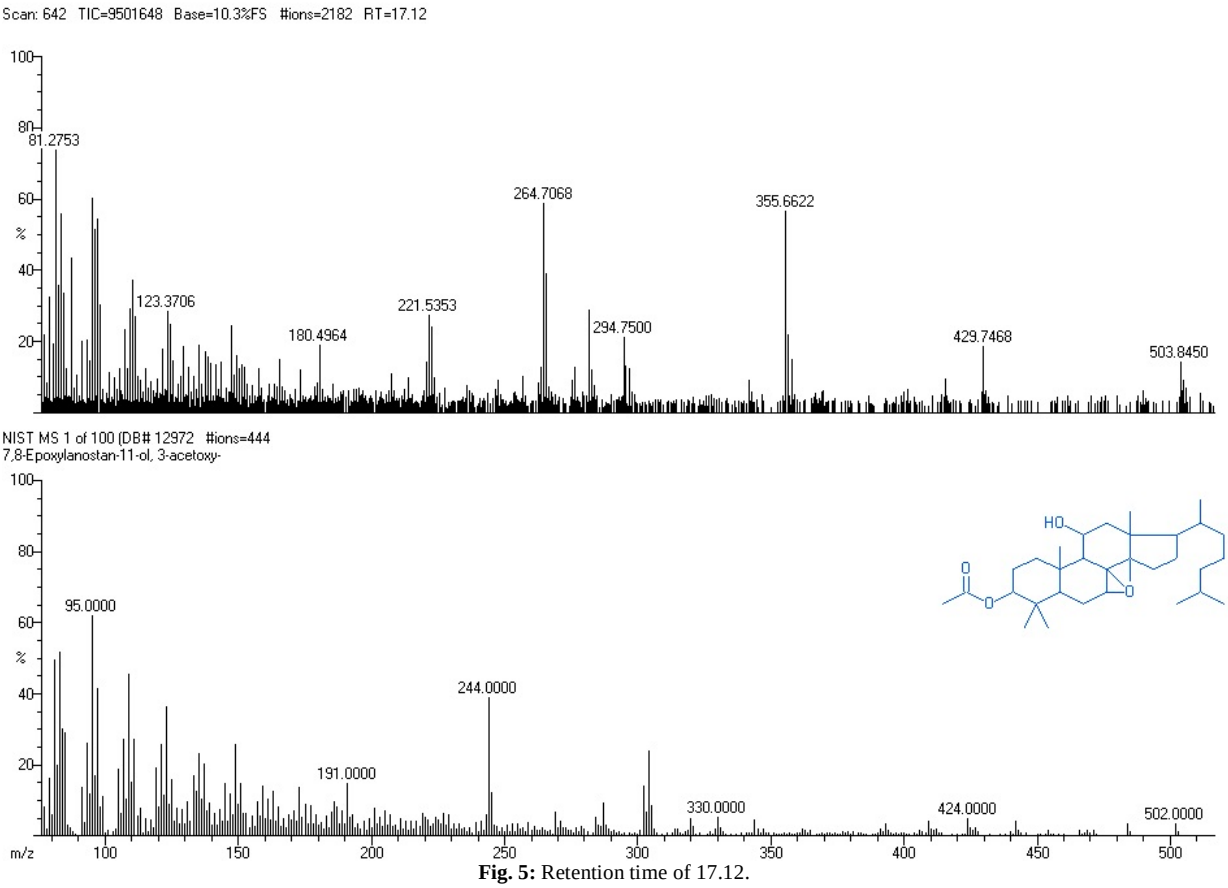

Fig. 5: Retention time of 17.12.

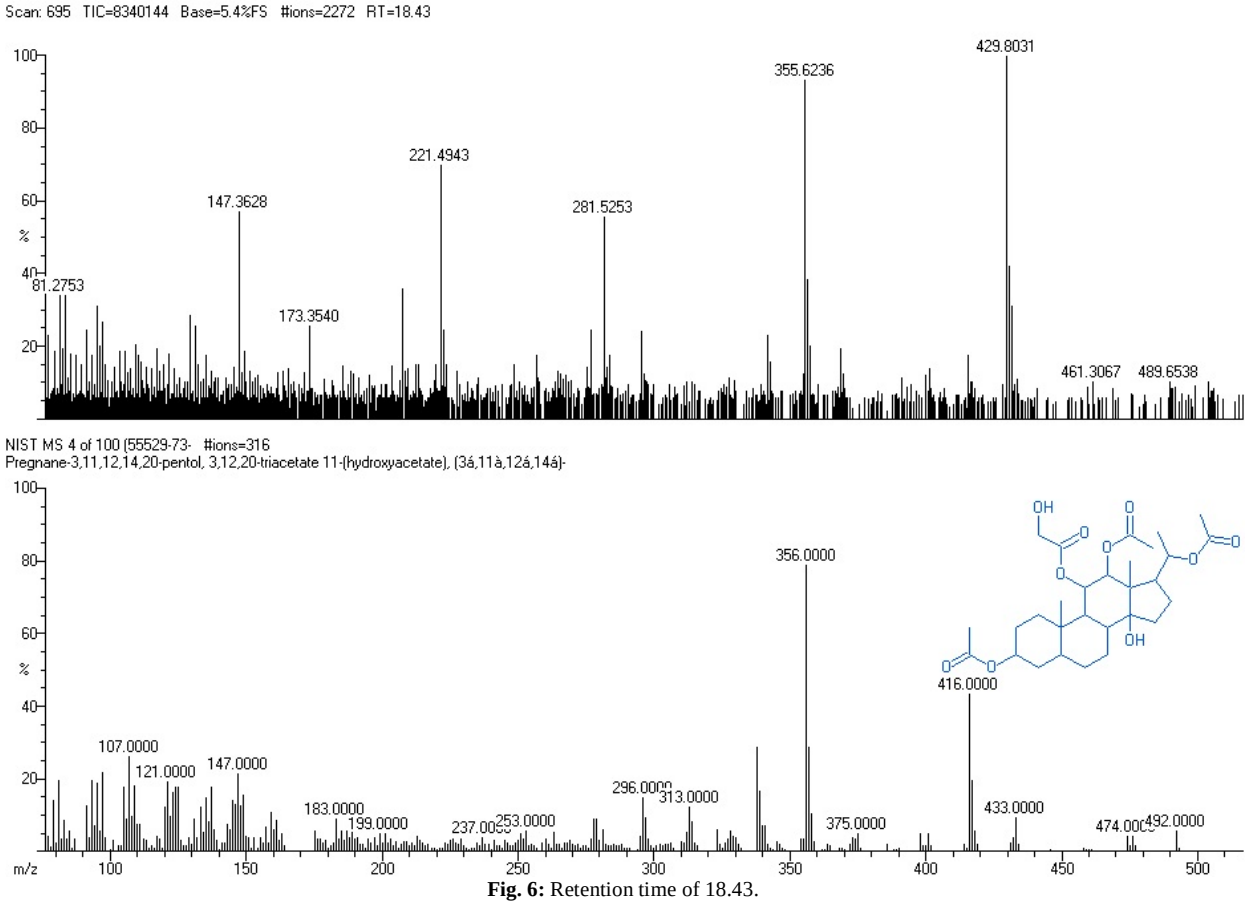

Fig. 6: Retention time of 18.43.

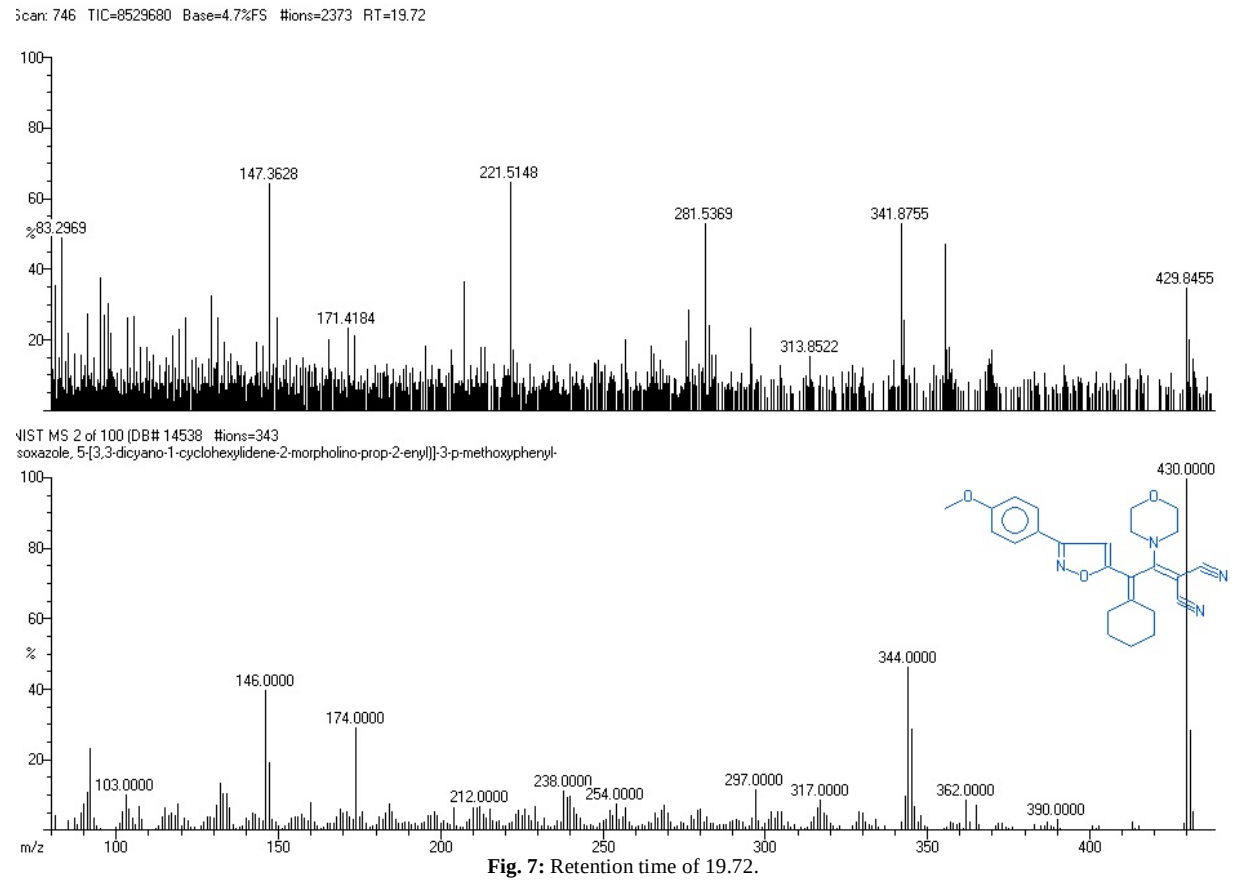

Fig. 7: Retention time of 19.72.

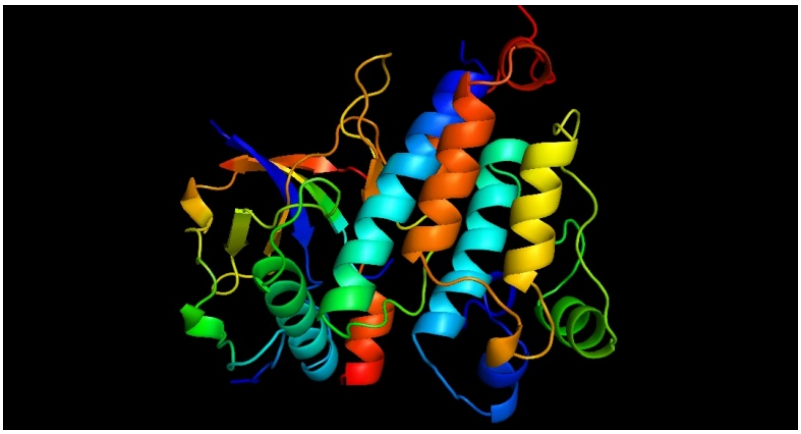

Fig. 8: Structure of the lung cancer protein PDB: ID: 2XP2.

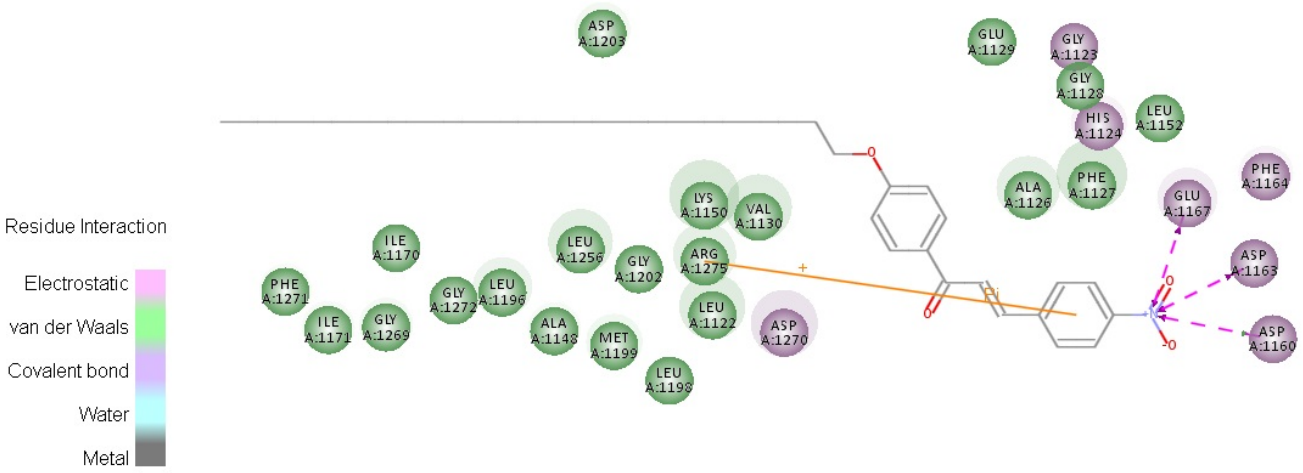

Fig. 9: 2D interaction of trans-4-Nitro-4'-(octadecyloxy)chalcone and 2XP2.

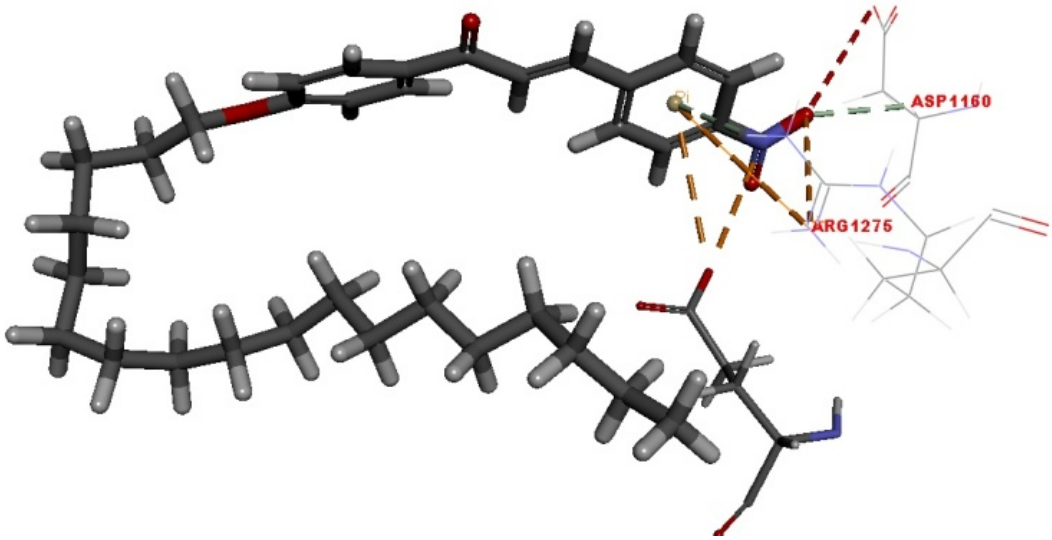

Fig. 10: 3D Interaction of trans-4-nitro-4'-(octadecyloxy)chalcone and 2XP2

Protein structure

The structure of the target protein (PDB ID: 2XP2) (Fig: 8), were retrieved from the RCSB Protein Data Bank, http://www.rcsb.org/ pdb

Protein and ligand docking analysis

The docking analysis is performed by Discover Studio Version 4.5 (Biovia Dassault system, USA) for the EGFR Non-Small cell lung cancer protein interacts with GCMS of crude protein extract of *Candida albicans*, fig: 9 and fig 10 represents the 2D and 3D interaction of trans-4-Nitro-4'-(octadecyloxy) chalcone and 2XP2.

CONCLUSION

Nature has provided the largest library of medicinal tools and has become a foundation for sophisticated traditional pharmacopeia all over the world. Nowadays, natural products maintain their importance in modern cancer drug discovery. The marine environment has proven to be a rich source of chemical and biological diversity and marine organisms are highly potential sources for commercializing interesting compounds for various industrial applications. Secondary metabolites produced by organisms often include active compounds serving predominantly for defense, predation, or communication. Hence, it is not surprising that those biologically highly active compounds have become an inherent acquisition to modern medicine. Regarding the latest advancements in drug discovery research from marine sources, more than ten marine-derived compounds are actually approved anticancer drugs or in clinical trials (phase I–III) as (Mayer *et al.*, 2010) and highlighted by the recent drug approval of the marine-derived product eribulinmesylate (Halaven®; Eisai Inc.) by the FDA in mid-November 2010 (FDA) (Gradishar, 2011). The activity of these compounds against molecular mechanisms involved in inflammation and cancer is nowadays an active field of research. Compounds from natural sources can therefore be considered as valuable agents in both prevention and therapy. Although advances in the field of chemo-preventive and therapeutic medicine have been made regularly over the last ten years, the search for novel anticancer treatments continues. The marine environment, with its rich variety of organisms, is a largely untapped source of novel compounds with potent antitumor activity (Senthilraja and Kathiresan, 2015). Although many reviews of marine anticancer compounds have been published, we focus here on selected compounds from marine yeast *Candida albicans* that act as an anticancer. GC-MS analysis secondary metabolites ofcrude extracts marine yeast *Candida albicans* that shows different compounds (Table: 1).

The compounds were used to structure-based drug design forNon-Small Cell lung cancer. *In silico* technique strongly supports and helps to identify the novel and more potent inhibitors through the mechanism of Ligand-Receptor interaction. From the 5 compound "Trans-4-Nitro-4'-(octadecyloxy)chalcone" has showed strong interaction with the lung cancer protein the Libdock score value is 138.499 which is higher than all other compounds obtained from GC-MS analysis. This study has presented marine products and underlines the importance of marine derived compounds as promising in the drug discovery research area. The data presented here undoubtedly indicate the great value of marine products as well as marine-derived bioactive compounds from marine products that make them important candidates for further pharmaceutical studies for feeding the anticancer drug pipeline.

Thus, isolation or modification of novel marine products as well as their analogs and the subsequent evaluation of their bioactivity will propel the discovery of novel promising chemotherapeutic drugs.

Table. 1: Properties of the compound

S.	Retention time	Name	Molecular weight (g/mol)	Molecular formula
1	12.23	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	534.595	$C_{28}H_{38}O_{10}$
2	14.02 and 19.72	Isoxazole, 5-[3,3-dicyano-1-cyclohexylidene-2-morpholino-prop-2-enyl)]-3-p-methoxyphenyl-	430.498	$C_{25}H_{26}N_4O_3$
3	15.63	Trans-4-Nitro-4'-(octadecyloxy)chalcone	521.731	$C_{33}H_{47}NO_4$
4	17.12	7,8,Epoxylanostan-11-ol,3-acetoxy	502.768	$C_{32}H_{54}O_4$
5	18.43	Pregnane-3,11,12,14,20-pentol, 3,12,20-triacetate 11-(hydroxyacetate), (3.beta.,11.alpha.,12.beta.,14.beta.)-	552.654	$C_{29}H_{44}O_{10}$

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