

**ANTI DIABETIC COMPOUNDS ISOLATED FROM *TURBINARIA*
CONOIDES AND *ULVA LACTUCA***

Submitted in partial fulfillment of the requirements for the award of the
Bachelor of Technology degree in Biotechnology.

By

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DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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This is to certify that this Project Report is the bonafide work of Vindhya.D (37230097) who carried out the project entitled "Anti diabetic compounds isolated from *Turbinaria conoides* and *Ulva lactuca* " under my supervision from January 2021 to March 2021.

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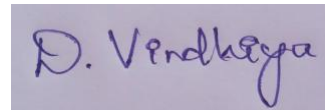
DECLARATION

I Vindhiya.D (37230097) here by declare that the Project Report entitled “**Anti diabetic compounds isolation from *Turbinaria conoides* and *Ulva lactuca***” done by me under the guidance of **Dr.V Ramesh Kumar** and

Dr. Aneesh Nair at Biozone research technologies Pvt. Ltd. Submitted in partial fulfillment of their requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE : 16.04.21

PLACE: Chennai

A handwritten signature in blue ink on a light purple rectangular background. The signature appears to read "D. Vindhya".

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Marine algae are an important source of bioactive metabolites in drug development. Diabetic mellitus is a metabolic disorder and world-wide death disease due to life style changes associated with urbanization. Due to the side effects of currently available antidiabetic drugs, search for effective plant based anti-diabetic drug is important combat diabetes and its complication. It's important to find the potential benefits of seaweeds for type two diabetes. Marine seaweeds are abundant bioactive components in brown algae and green algae. In the bioactive compound leads in drug and neurocritical application from the marine algae derived secondary metabolites which contributes various biological and chemical properties. Phlorotannin's present in brown algae exhibit anti-diabetic activities inhibiting the α - amylase, glucose uptake by yeast cell, hydrogen peroxide radical scavenging activity. The present review focus on anti-diabetic mechanism of secondary bioactive compounds presents in brown and green algae.

Keywords: anti-diabetic, marine algae, α -amylase, secondary metabolites, etc.

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LIST OF ABBREVIATION

SL.NO	ABBREVIATION	FULL FORM
1.	NaOH	Sodium hydroxide
2.	H ₂ SO ₄	Sulphuric acid
3.	g	grams
4.	mg	milli gram
5.	ml	milli litre
6.	°C	Degree Celsius
7.	mm	milli metre
8.	mM	milli Molar
9.	Hrs	hours
11.	N	Normality
12.	(v/v)	Volume/volume
13.	Mins	Minutes
14.	µg	microgram
15	µl	microlitre
16	L	Litre
17	%	percentage
18	M	Molarity
19	HCl	Hydrochloric acid

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CHAPTER 1 - INTRODUCTION

1.1 Diabetes Mellitus

Diabetic mellitus is metabolic disease caused by increased blood sugar level. Pancreas of diabetic individual does not produce enough insulin to control glucose in blood. when body doesn't respond to insulin causing blood glucose level to abnormally high. Insulin is a hormone which controls blood glucose level in body.it is a disease associated with "sweet urine". Glucose gets elevates (after eating food) insulin is released from the pancreas to normalize glucose in blood by uptake of glucose into the body cells. Lack of insulin is caused hyper glycemia.(Thilina L. Gunathilaka, et al., 2020)

Diabetes is an important factor which accelerating the hardening of the arteries, leading to stroke, coronary heart disease, blood vessel disease is known as macrovascular disease.(Chiara Lauritano 2016)

1.2 Types

Diabetes is two types which are type1 and type 2. In other term insulin dependent diabetes and non-insulin dependent diabetes. Type 1 diabetes is a insulin producing beta cells are destroyed when the immune system fighting for infection. It is caused by the genes, environmental factor such as virus, disease. symptoms are extreme thirst, increased hunger, frequent urination, fatigue.(Diabetes care.2010)

Type 1diabetes complication are cardiovascular disease, skin problem, gum disease, pregnancy problem, kidney damage, retinopathy, nerve damage.(Diabetes care 1997)

Type 2 diabetes are the lifelong disease. It is an insulin resistance which is body cell doesn't respond to the insulin in blood glucose level People who are in the middle aged or older are likely get this type of diabetes. It is called as a "adult-onset diabetes"(Frank B.Hu,1999). Pancreas secret hormone called insulin. Insulin is helped in the conversion of the food into energy. Pancreas help to make more insulin to try to get glucose in the cells, it can't keep up, glucose is built up into blood.(Hegard FG,2015)

Type 2 is caused by the genes, extra weight, metabolic syndrome, too much glucose from liver, low communication between cells, breakage of beta cells.

The symptoms in the type 2 diabetes are very thirsty, blurry vision, fatigue, yeast infection, weight loss, wounds that don't heal, peeing a lot. It has a complication are heart and blood vessel disease, high triglycerides, gestational diabetes when pregnancy, depression.(Kathleen M.fox, et al., 2005)

1.3 SEaweEDS

Seeds is the species of marine plants, algae that grows in water bodies, ocean, rivers, lakes. Seaweed or macroalgae refers to more specie of macroscopic, multicellular, marine algae. Seaweed contains a wide range of vitamins, minerals, iodine, iron and calcium, high amount of vitamin B12.Good source of omega-3 fat.

1.3.1. Seaweed types:

The types of seaweed are

- 1.Rhodophyta (red seaweed)
- 2.Phaeophyta (brown seaweed)
- 3.Chlorophyta (green seaweed)
- 4.Chrysophyceae (gold seaweed)(Mitali Priyadarshini Pati, et al., 2016).

1.3.2. Seaweed importance

Seaweed produces foods, oxygen, energy, pharmaceuticals, habitats. It is important for marine food chain. It is autotroph so its produce food for itself.it is balance ecosystem, bioindicator of marine chemical damage, it absorbs the harmful or excess nutrients in the ocean Excess iron ion is damage the autotroph organism in the marine ecosystem, seaweeds are protecting from the excess iron ion by trapping iron ion, all heavy metal in the marine can trapped by the seaweeds. pathogen in seaweed change seaweed colour or smell it cause very dangerous to marine environment.(Saranraj .P,2014)

1.3.3. Seaweed uses

Seaweed is used as food, industrial application, fertilizer, cosmetics, extraction of chemical gums and chemicals. Source of agar, medicine and minerals. Seaweed is having more potential activity as such as anti-diabetic, anti-microbial, medicine. Seaweed have bioactive components are phenol, phlorotannin's, peptides, vitamins.

Seaweeds are rich source of poly unsaturated fatty acid (PUFA) which play a major role in the cellular and tissue metabolism. Mostly PUFA present in seaweeds in the form of omega-3 fatty acid or omega-6 fatty acid.(Shilpi Gupta,2011)

1.4 *Ulva lactuca* (Chlorophyta)

Ulva lactuca is type of green seaweeds.it is a high potential activity of anti-diabetic in diabetic rats. It is a cholesterol lowering effects in rats. Therapeutic action of poly saccharide from *Ulva lactuca* on diabetic status activities of alpha amylase, maltose, lipase in the small intestinal and liver kidney. *Ulva* species are high source of omega-3 fatty acid.(Herminia Dominguez,et al.,2019)



Figure 1.1 *Ulva lactuca*

1.5 *Turbinaria conoides*

Turbinaria conoides type of brown seaweeds. It is also called as Phaeophyta. It is a major nutritional resource. Risk of developing T2DM reduce by the consumption of seaweeds. Seaweeds are regulation of insulin and sensitivity. Seaweeds are rich in fatty Acid which have a methylene. Methylene is important for the cellular function which against to T2DM. (Sheu et al., 1999).



Figure 1.2 *Turbinaria conoides*

1.6 GAS CHROMOTAGRAPY AND MASS SPECTROSCOPY(GCMS)

GCMS is an analytical technique which is combination of gas chromatography and mass spectroscopy. It is identifying the different substance in the test sample. It is used in the drug detection, identification of the test sample, environmental analysis, explosive investigation. It is used in the identification of the presence of specific substance in the sample. High temperature used in the GCMS is 300°C for thermal degradation of sample. (Jeffrey R. Gilbert et al., 2013)

GCMS is used to identify the sample in the molecular level, when heat is supplied the component are separated, the heat is carried out the molecule through column which flow into the MS. The computer with MS is detect the component in the analyte molecules. It can be used to detect, quantify, identify chemicals in plant, soil, water. It is

most commonly used to detect hydrocarbons in the crude oil, petroleum product.(Shusha Cheriye dath,2019).

Insulin resistance due to overproduction of glucose by the liver and underutilization of glucose by muscle and adipose tissue. Insulin resistance is caused by an unhealthy diet, sedentary lifestyle, and obesity. Insulin resistance is cause type 2 diabetic mellitus. Type 2 diabetic mellitus such as oral insulin administration or oral anti-diabetic drugs have either limited efficacy or detrimental side effects. It is keeping searching of the new plant compound yielded and potential drug for therapeutic. Plant phytochemical compounds contain secondary metabolites that can be used control various disease by diabetic mellitus.(Abdulfatima B. Olokoba et al.,2012).

In additional bioactive compound are abundant in the marine algae with high potential ingredient the treatment of type 2 diabetes mellitus. Most brown algae are rich in the secondary metabolites, such as phlorotannin's which h have anti-diabetic activity. Anti-diabetic activity from the brown algal compound and green algal compound in the purpose of the review.(Unnikrishnan P.S et al.,2015).

1.7 DOCKING

Molecular docking is study of how two or more molecular structure fit together. It is molecular modelling technique is used to predict how enzyme interact with small molecules. Molecular docking procedure involves either ligand/target flexible or rigid based upon objectives of docking like flexible docking (target as a rigid molecule) rigid body docking (both target and rigid molecule are rigid molecule).Software used as a GemDock which was a generic evolutionary method of molecular docking(Kai-cheng Hsu,2011).

CHAPTER 2 - LITERATURE SURVEY

Reka.p(2017) work on seaweeds namely *Acanthoporphyras*, *Gracilariasp*, *Ulva lactuca* and *ulva reticulate* where used for alpha amylase and alpha glucosidase inhibition activities of edible seaweeds. It was potential to act as a potent inhibitor of the carbohydrates hydrolysing enzymes. Zeng Sun and Feng Chen reported that anti-diabetic agents from natural source have attracted more and more attention because the clinical trials of several synthetic anti-diabetic drugs have been terminated due to safety concerns.

Lucy Mohapatra, et.al, (2016) works on edible seaweeds *Sargassum wightii* and *Ulva* sp controlling anti-diabetics which is fat diet and multi low dose streptozotocin induced type 2 diabetic mice in this work it was demonstrated that ethyl acetate extract is effective in lowering blood lipids. This is also help full in improving anti-oxidant activities.

L.Gunathilaka, Kalpasamarakoon, et.al, (2020) works on anti-diabetic's potential of marine brown algae. Investigated the inhibitory effect of enzymatic protein hydrolysate of brown seaweeds *Fucus spiralis* on ACE. Three ultrafiltrate fraction of *F. spiralis* were obtained after obtaining the protein hydrolysate with enzymatic digestion by mixture two enzymes cellulase and bromelain.

Cha et al. reported the ACE inhibitory activity of some Korean brown seaweeds *Sargassum fusiforme*, *Sargassum horneri*. Among these methanolic extract have highest inhibitory activity compare to others for further information, undergone a taxonomic revision and it has become *Sargassum fusiforme*.

Vijayan et al. founded that ethyl acetate extract *Sargassum wightii* exhibited inhibitory activity on ACE. During further analysis phloroglucinol was isolated from the ethyl acetate fraction of *S. wightii* showed that the highest inhibitory activity. According to the literature, phlorotannin's showed that higher molecular weight such as exhibited significantly higher ACE inhibition.

Zhanyi Sun et al. (2018) works on the antiobesity, antidiabetic, antioxidant and antihyperlipidemic activities of bioactive seaweed substance. An effective strategy for reducing postprandial hyperglycaemia is by inhibition of pancreatic alpha amylase and alpha glucosidase which delays the carbohydrate digestion and glucose absorption significantly.

Tarling et al. (2008) antioxidant compound also plays a vital role in the control of oxidant stress-disease including diabetes, cancer, cardio vascular disease.

Lavanya Rayapu , Kajal Chakraborty(2020)Marine Algae as a Potential Source for Anti-diabetic Compounds,marine algae, red and brown algae are reported to show anti-diabetic activity.

Shubham Kumar (2020), worked on Herbal medicines for diabetes management and its secondary complications,the presence of phenolic compounds, flavonoids, terpenoids and coumarins are responsible for the antidiabetic nature of the medicinal plants.which include inhibition of α -glucosidase, α -amylase, and protein tyrosine phosphatase 1B activities.

Ngan Tran,(2020) worked on Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery on antiallergic, anticancer, antimicrobial, anti-inflammatory, antidiabetic and antioxidant activities Diabetes mellitus is a chronic disease result of metabolic disorders in pancreas β -cells that have hyperglycemia.

Malvia.N (2010) worked on Antidiabetic potential of medicinal plants,Harman Ece(2010) worked on Use of Oral Antidiabetic Drugs (Metformin and Pioglitazone) in Diabetic Patients with Breast Cancer, There was not statistically significant difference in terms of mean age between treatment groups. In our study, there was statistically significant difference in terms of HIF-1 α levels between patients and control group.

Venkataiah Gudise(2019), worked on In vitro free radical scavenging and antidiabetic activity of aqueous and ethanolic leaf extracts,The ethanolic leaf extract of *Matelea denticulata* exhibited superior in vitro free radical scavenging and antidiabetic activity amongst the other extracts.

CHAPTER 3 - AIM AND OBJECTIVE

3.1 AIM

To investigate antidiabetic and hydrogen peroxide scavenging activity of the extraction from the *Ulva lactuca* and *Turbinaria conoides*.

3.2 OBJECTIVE

- 3.2.1 Identification of anti-diabetic activity of the brown seaweed.
- 3.2.2. Identification of the anti-diabetic activity of the green seaweed.
- 3.2.3. Compare the activity of the green and brown seaweed.
- 3.2.4. Identify the compounds present in the brown and green seaweed by GCMS.
- 3.2.5. Docking the compound in the seaweed.

CHAPTER 4 - MATERIAL AND METHODS

4.1 COLLECTION OF THE SAMPLE:

Fresh and live brown seaweed, *Turbinaria conoides* and green seaweed *Ulva lactuca* were collected from the coastal area of R.K. algae production in Rameswaram. The fresh brown seaweed and green seaweed was washed with the marine water to remove debris, epiphytic organism and holdfast were removed. The seaweeds were washed with the distilled water remove impurities still present in the sample.

Before extraction, the seaweeds were shade dried for 2 weeks. The Dried seaweeds were powdered and stored in the air tight cover for prevention of epiphytic organism and the damage.



Figure 4.1 *Ulva lactuca* live sample



Figure 4.2 *Turbinaria conoides* live sample

4.1.1 After one week of shade dry



Figure 4.3 *Turbinaria conoides*



Figure 4.4 *Ulva lactuca*

4.1.2 Powered sample

After 2 weeks of shade dry sample was powdered.



Fig 4.5 *Turbinaria conoides* powder



Figure 4.6 *Ulva lactuca* powder

4.2 PREPARATION OF SEAWEED EXTRACT

79g of powdered brown seaweed was weighed and dissolved in solvents in the ratio of (1:3) and 85g of green seaweed were weighed and dissolved in solvent in the ratio of (1:6) and sealed with aluminium foil stored for 48 hours in the room temperature

After 48 hours, each extract was filtered using the filter paper. The filtrate was evaporated to dryness under room temperature. The obtained filtrate was dried using vacuum rotary evaporator under reduced pressure at 40°C until the filtrate was reduced to one third of the starting filtrate volume. Then the remaining extracts were transferred to a pre-weighed Petri dishes and kept for complete dryness and weighed again to get yield of those extracts.

4.2.1 Hexane extract of Seaweed

Turbinaria conoids & *Ulva lactuca* mixed with hexane in (1:3) ratio.

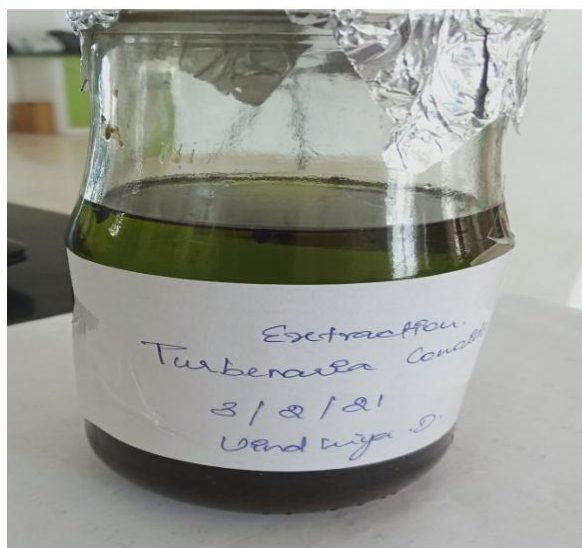


Figure 4.7 Hexane extract of *Turbinaria conoides*

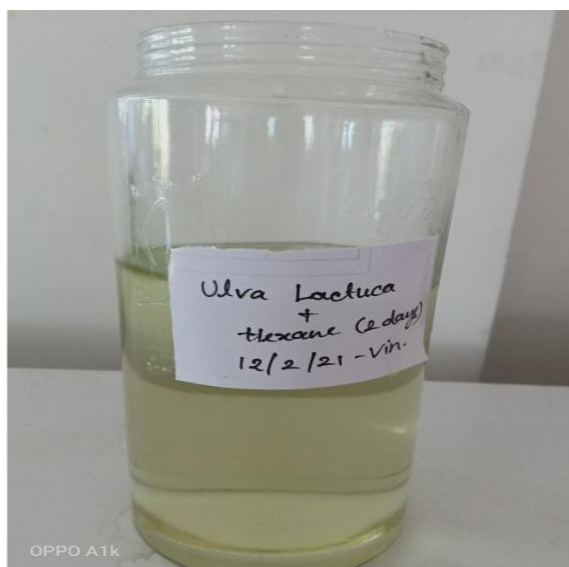


Figure 4.8 Hexane extract of *Ulva lactuca*

4.2.2 Ethyl acetate extract of Seaweed

Turbinaria conoides & *Ulva lactuca* was mixed with ethyl acetate in 1:3 ratio.



Figure 4.9 Ethyl acetate extract of *Turbinaria conoides*



Figure 4.10 Ethyl acetate extract of *Ulva lactuca*

4.2.3 Ethanol extract of seaweed

Turbinaria conoides and *Ulva lactuca* was mixed with ethanol in 1:3 ratio.

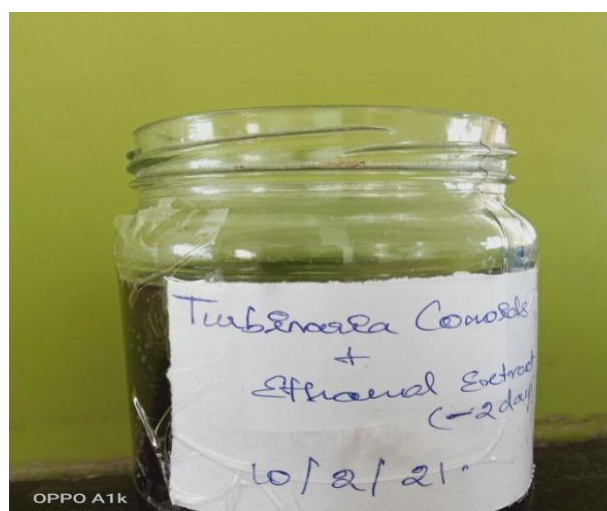


Figure 4.11 Ethanol extract of *Turbinaria conoides*



Figure 4.12. Ethanol extract of *Ulva lactuca*

After evaporation of extract was transfer to petri dish for future analysis.

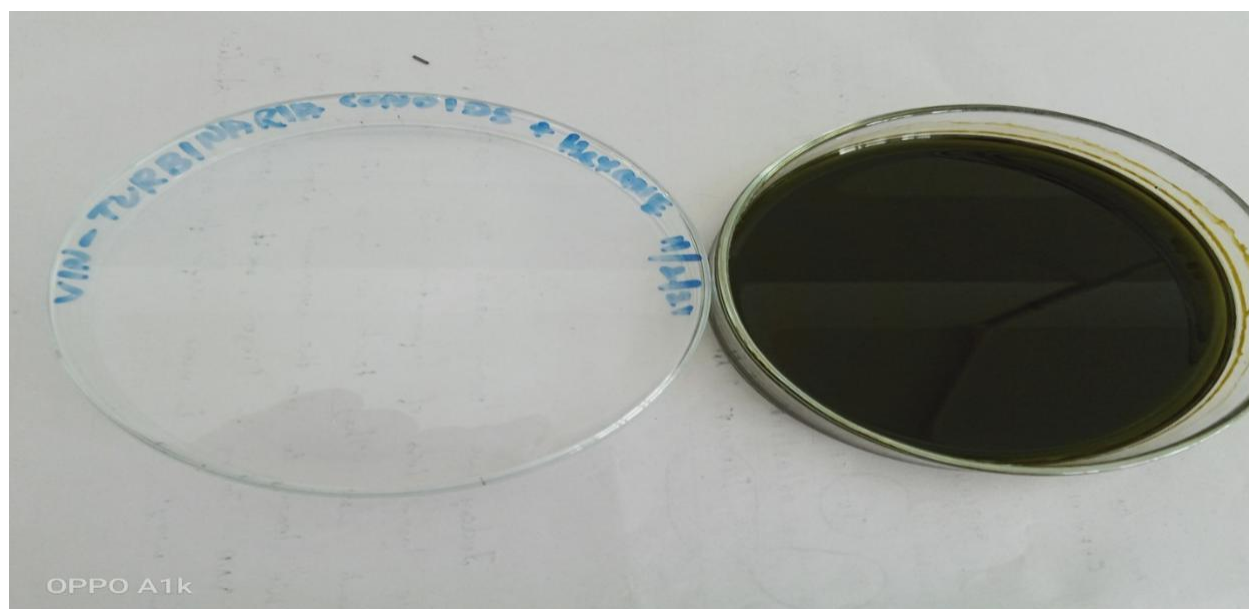


Figure 4.2.4. Hexane extract of *Turbinaria conoides*

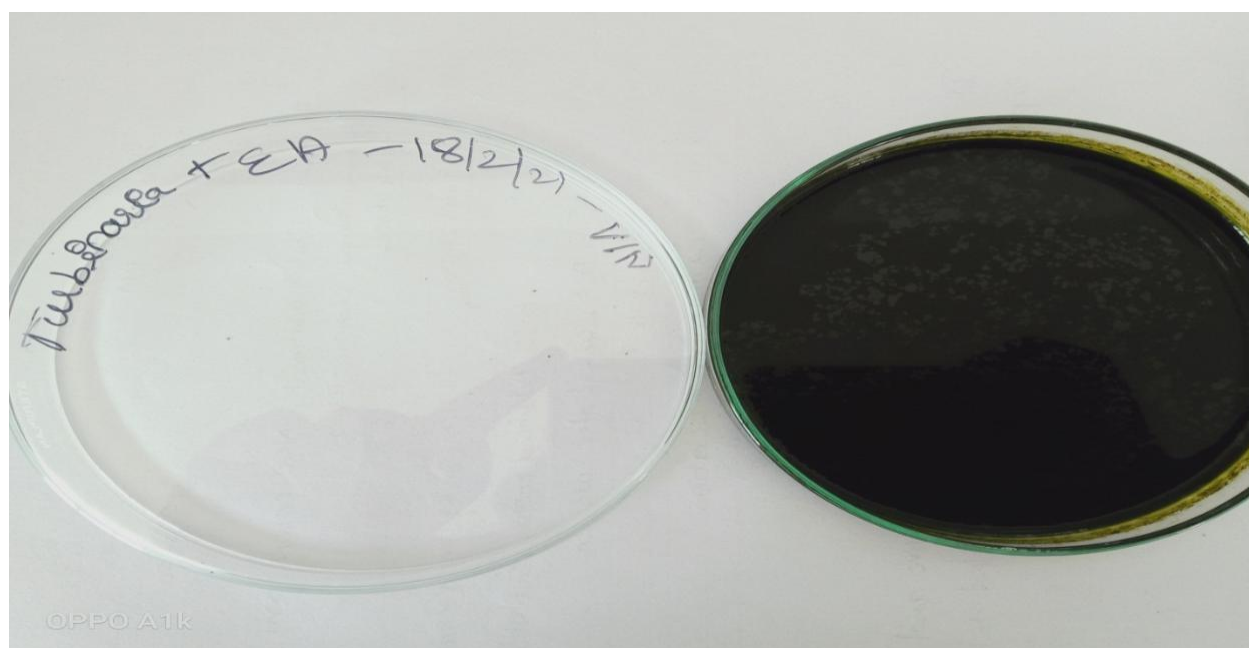


Figure 4.2.5. Ethyl acetate extract of *Turbinaria conoides*

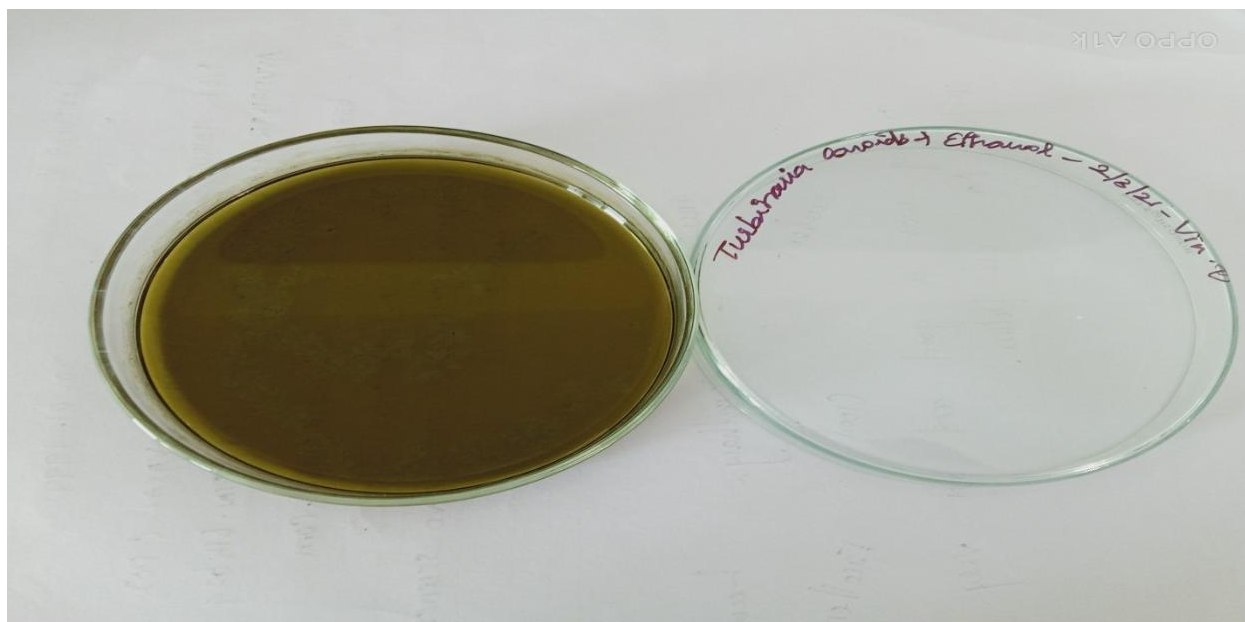


Figure 4.2.6 Ethanol extract of *Turbinaria conoides*

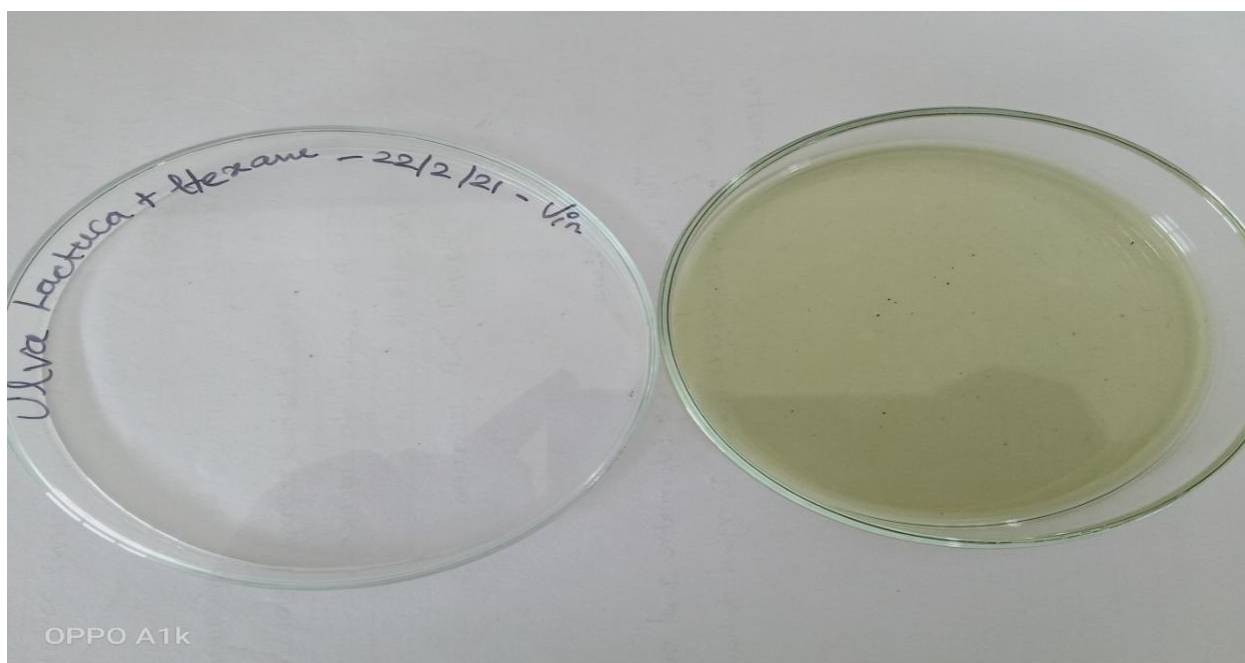


Figure 4.2.7 Hexane extract of *Ulva lactuca*

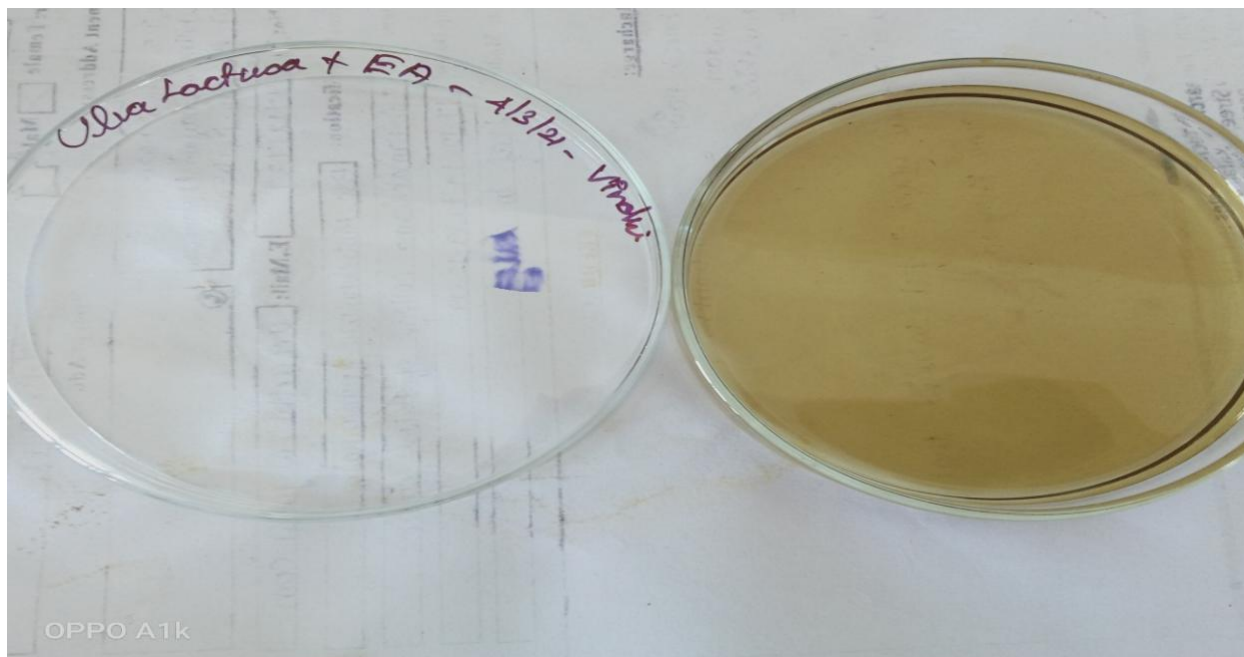


Figure 4.2.8. Ethyl acetate extract of *Ulva lactuca*

4.3 QUALITATIVE ANALYSIS

Qualitative phytochemical analysis was carried out to find out the presence of secondary metabolites in the various solvent extract of green seaweed and brown seaweed.

4.3.1 CARBOHYDRATE

To 2 ml of the seaweed extract, 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrate.

4.3.2 TANNIS

To 1 ml of the seaweed extract, 2 ml of the 0.7M NaOH and few drops of phenol Denis reagent was added formation of dark blue or greenish black indicates presence of tannins.

4.3.3 SAPONINS

To 2ml of seaweed extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. Lengthwise formation of foam in 1cm layer indicates presence of saponins.

4.3.4 FLAVANOIDS

To 2ml of seaweed extract, 1ml of 2N sodium hydroxide was added, Presence of yellow colour indicates the presence of Flavonoids.

4.3.5 ALKALOIDS

To 2ml of seaweed extract, 2ml of concentrated hydrochloric acid was added. Few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates presence of alkaloids.

4.3.6 QUINONES

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicates presence of Quinone's.

4.3.7 GLYCOSIDES

To 2ml of seaweed extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates presence of glycosides.

4.3.8 CARDIAC GLYCOSIDES

To 0.5ml of plant extract, 2ml of glacial acetic acid and few drops 5% ferric chloride were added. This was under layered with 1ml of concentrated sulphuric acid. Formation of brown ring indicates presence of cardiac glycosides.

4.3.9 TERPENOIDS

To 0.5ml of seaweed extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour indicates presence of terpenoids.

4.3.10 PHENOL

To 1ml of seaweed extract, a few drops of phenol Dennis reagent was added followed by 2 ml of 15% sodium carbonate solution. Formation of blue or green colour indicates presence of phenols.

4.3.11 COUMARINS

To 1ml of seaweed extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates presence of coumarins.

4.3.12 STEROIDS AND PHYTO STERIONDS

To 1ml of seaweed extract equal volume of chloroforms added and subjected with few drops of concentrated sulphuric acid were added, appearance of brown ring indicates the presence of steroids, appearance of bluish brown ring indicates presence of Phyto steroids.

4.3.13 PHLOBATANNIS

To 1ml of seaweed extract and few drops of 2% HCl was added, appearance of red colour precipitate indicate presence of phlobatannis.

4.3.14 ANTHRAQUINONES

To 1ml of seaweed extract few drops of 10% ammonia solution added, appearance of pink colour precipitates the presence of anthraquinones.

4.4 QUANTITATIVE ANALYSIS

Sample preparation: 10 mg of *Turbinaria conoids* and *Ulva lactuca* was weighed and diluted in 1 ml of solvents and used as a sample.

1. Reagent's preparation

5% sodium nitrate: 5g of sodium nitrate in 10 ml of the distilled water.

10% ammonium chloride: 8 ml of ammonium nitrate in 20 ml of distilled water.

1M NaOH: 0.4 g in 10 ml of distilled water.

2. Stock standard solution

500mg of quercetin was weighed accurately and dissolved in 250ml of distilled water using standard measuring flask.

3. Working standard solution

2.5 ml of stock standard solution was made up to 50ml using distilled water.

Procedure:

Standard was taken in S1 to S5, test sample was taken in T1 to T3. Working standard was taken in 200µl to 1000µl. Test sample was taken 100µl, 200µl and 500µl, in the test labelling test tube. 75µl of 5% sodium nitrate was added in the standard and test sample and blank. In blank without test sample, reagent is added. Incubation at room temperature for 6 minutes. 150µl of 10% ammonium chloride were added in each tube. Incubation at room temperature for 5 minutes. 500µl of 1M NaOH was added in each tube. The content was made into 2.5 ml using distilled water. Absorbance was absorbed at 510nm.

4.4.2 PHENOL TEST

Sample preparation:

10mg of *Ulva lactuca* and *Turbinaria conoids* was weighed and diluted in 1 ml of solvents and used as sample in testing phenol.

Reagents:

Sodium carbonate: 3g of Na₂CO₃ in 40 ml of distilled water.

Stock standard solution:

500 mg of gallic acid was weighed and dissolved in 100 ml of distilled water using standard measuring flask. (concentration 2mg/ml).

Working standard solution:

2.5ml of stock standard solution was made up to 50ml using distilled water. (Concentration 100µg/ml).

Procedure

Working standard was added in 200µl to 1000µl in S1 to S5 labelled test tubes. 100µl, 200µl and 500µl of testing sample was mixed. Subsequently adding 5ml of phenol Dennis reagent. Keep it in dark place for 3 minutes. Adding 5ml of Sodium carbonate and incubated. Absorbance in 765nm at wavelength.

4.4.3 TANNINS

Sample preparation:

10 mg of *Turbinaria conoids* and *Ulva lactuca* crude extract was weighed and diluted in 1 ml of solvents.

Reagent:

15% sodium carbonate: 1.5 ml of sodium carbonate in 10ml of distilled water.

Stock standard solution:

500mg of tannic acid was weighed accurately and dissolved in 250ml of distilled water using standard flask.

Working standard solution:

2.5ml of stock standard solution was made up to 50ml of distilled water.

Procedure:

Different concentration of working standard solution (200µl to 1000µl) added in S1 to S5 in the labelled test tube and 100µl, 200µl and 500µl of test sample was added. Subsequently, adding 1ml of distilled water and 0.5 ml of phenol Dennis reagent was added. In the mixture adding 1ml of 15% sodium carbonate and incubated in room temperature for 30 minutes. Absorbance was calculated in 700nm at wavelength.

4.5 ALPHA AMYLASE INHIBITION ASSAY:

Sample preparation: 10 mg of *Turbinaria conoids* and *Ulva lactuca* extract was weighed and diluted in 1 ml of solvents and used as a sample.

Alpha amylase:

5ml of phosphate buffer, 0.029 g of sodium chloride was added. Adjust the pH to 6.5, 1ml of Poly vinyl pyrrolidone. 1g of Rajma was weighed and grinded. Grinded rajma was diluted in phosphate buffer and polyvinylpyrrolidone.

1% starch solution with phosphate buffer: 0.1g of starch diluted in 10 ml of phosphate buffer.

Dinitro alicyclic acid: 0.02g of dinitro alicyclic acid in 14ml of 0.5N NaOH. 6g of sodium potassium tartrate, 6ml of distilled water.

0.5N NaOH: 0.02g sodium hydroxide was diluted in 14ml of distilled water.

Procedure:

Different concentration of seaweed extract (20µl, 30µl and 40µl) and 500µl of α-amylase enzyme (0.5mg/ml) were incubated at 25°C for 10 minutes. After the incubation, 500µl of 1% starch solution in 0.02 mol/L sodium phosphate buffer was added. Subsequently the reaction mixture was incubated at 25°C for 10 minutes followed by addition of 1.0 ml of Dinitro salicylic acid (DNS). Finally, reaction was stopped by incubation in boiling water for 5 minute and cooled to room temperature. The reaction mixture was diluted with 10ml distilled water, and the absorbance was measured at 540 nm. The mixture of all other reagents and enzyme except the test sample was used as a control and the results of α-amylase inhibition activity were expressed in terms of inhibition percentage.

The experiments were performed in triplicates, and the alpha-amylase inhibitory Activity was calculated as percentage inhibition, using formula.

$$\% \text{ inhibition} = ((\text{Abs control} - \text{abs sample}) / \text{abs control}) \times 100$$

4.6 GLUCOSE UPTAKE BY YEAST CELL:

Sample preparation: 10mg of *Turbinaria conoid* extract and *Ulva lactuca* extract was weighed in Eppendorf tube and diluted in 1 ml of solvents.

Yeast preparation:

0.25g of yeast cell was weighed and diluted in 5ml of distilled water. The suspension was centrifuged in 4000 rpm for 5 minutes. The supernatant was transfer to fresh Eppendorf tubes. The centrifuged was continue until the supernatant was appear in clear. The precipitate was diluted in distilled water.

100mM glucose:

0.54g glucose was weighed in 30 ml of distilled water.

Anthrone reagent:

100mg of anthrone was weighed and diluted in 50 ml of concentrated sulphuric acid.

Procedure:

Three concentration of (20µl,30µl and 40µl) seaweed extract was added in labelled test tubes. A standard glucose solution of concentration 100mM/L was prepared. Various concentration of seaweed extract added in 1 ml glucose solution and Incubate at 37 ° C for 10 minutes. The reaction was started by adding 100µl of yeast suspension, vortexed and further incubated at 37 ° C for 60 minutes. After incubation, Centrifuged (3800rpm ,5 minutes). Glucose was estimated, Supernatant was collected. From the supernatant, 1ml of supernatant was diluted in 1ml of anthrone, heated at water bath for 10 minutes and thaw in room temperature. From the mixture sample was diluted in (1:9) ratio with distilled water.

Increase in glucose uptake (%) = $\frac{\text{Abs control} - \text{Abs sample}}{\text{abs control}}$

Where abs control is the absorbance of the control reaction (containing all reagents except test sample), Abs sample is the absorbance of the test sample. From the mixture 100µl was collected and diluted in 900µl. The dilution was added in 2 ml of distilled water. Absorbance was calculated in 620nm at wavelength.

4.7 HYDROGEN PEROXIDE (H₂O₂) RADICAL SCAVENING ACTIVITY:

Sample preparation:

10 mg of *Turbinaria conoids* and *Ulva lactuca* was weighed and diluted with 1 ml of solvents. Mixed thoroughly, used as sample.

Hydrogen peroxide:

68µl of hydrogen peroxide solution diluted in 20ml of phosphate buffer in the concentration of 40 mM.

Procedure

Different concentration of sample (50µl,100µl,150µl and 200µl) mixed with 0.6 ml of hydrogen peroxide concentration in 40 mM. The mixture was incubated at room temperature for 10minutes. After, the incubation adding 2ml of distilled water. Absorbance was calculated in 600nm at wavelength.

4.8 EXTRACTION:

Fresh green seaweed, *Turbinaria conoids* and green seaweed, *Ulva lactuca* was collected and washed in distilled water, shade dried in 15 days, powdered and weighed. The powdered sample were extracted with hexane ethyl acetate, ethanol. The extract was filtered using what man No.1 filter aper and the filtrate was concentrated in room temperature and rotary evaporator.

4.8.1 Yield:

The yield of extract was calculated using the formula:

$$\text{Percentage of yield} = (\text{amount of extract obtained} / \text{amount of seaweed used for extraction}) \times 100.$$

4.9 DOCKING:

4.9.1 iGEMDOCK

A graphical environment for recognizing pharmacological interaction and virtual screening.

Pharmacological interaction is useful for identifying lead compound and understanding ligand binding mechanism for a therapeutic target. Currently, these interactions are often inferred from a set of active compounds that were acquired experimentally. Moreover, most of docking programs loosely coupled the stages of structure-based virtual-screening(vs) from preparations through to post-screening analysis. An integrated with VS environment which provides their friendly interface to seamlessly combine different stage programs for VS and identifying the pharmacological interaction from screening compounds, is valuable for drug discovery. iGEMDOCK is an easy-to-use graphic environment for docking, virtual screening and post-screening analysis. iGEMDOCK can enrich the hit rate and provide biological insights by driving the pharmacological interaction from screening compound. The pharmacological interaction represents conserved interacting residues that often form binding pocket with specific physio-chemical properties to play essential functions of the target protein. Experiment results show that the success rate of iGEMDOCK is 78% (root-mean-square derivations below 2.0 Armstrong) on 305 protein-compound complexes. For virtual screening, pharmacological interactions derived by Igemdock often involve biological function and enrich the hit rate on 3 public sets (I. Estrogenic receptor α for antagonist (ER) and agonists (ERA) and thymidine kinase (TK)). iGEMDOCK is useful for understanding the ligand binding mechanism and discovery lead compound.

4.9.2 Molecular docking interaction analysis:

Fitness is the total energy of a predicted pose in the binding site. The empirical scoring function igemdock is estimate as:

$$\text{Fitness} = \text{vdW} + \text{H bond} + \text{Elec.}$$

Here, the vdW term is van der Waal energy. H bond and Elec terms are hydrogen bonding energy and electro statistic energy, respectively.

CHAPTER 5 - RESULT AND DISCUSSION

5.1 QUALITATIVE TEST

Table 5.1.1: Hexane extract of *Turbinaria conoides*

Hexane extract of <i>Turbinaria conoides</i>	Inference
Carbohydrate	+
Tannins	+
Saponins	-
Flavonoids	-
Alkaloids	+
Quinones	-
Glycosides	-
Cardiac glycoside	+
Terpenoids	+
Phenols	+
Coumarins	-
Steroids	-
Phlobatannins	-
Anthraquinones	-

In the table (+) represents presents of the compound, (-) represents absents of the compound.

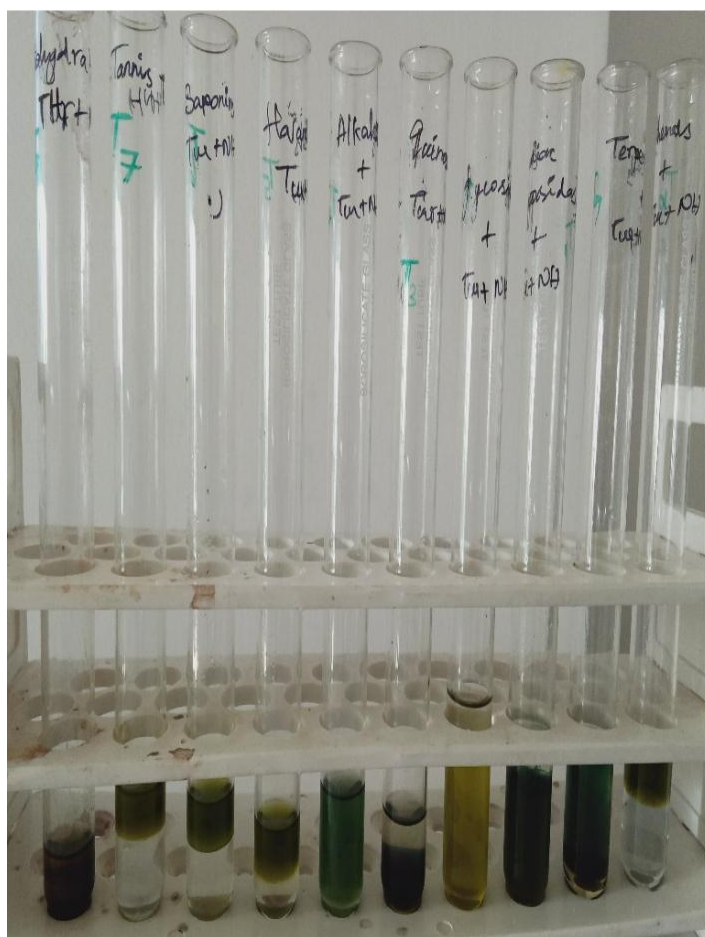


Figure 5.1.1 Hexane extract of *Turbinaria conoides*

Table 5.1.2. Ethyl acetate extract of *Turbinaria conoids*

Ethyl acetate extract of <i>Turbinaria conoids</i>	present	absent
Carbohydrate	-	+
Tannins	+	-
Saponins	+	-
Flavanoids	-	+
Alkaloids	+	-
Quinones	-	+
Glycosides	-	+
Cardiac glycoside	+	-
Terpenoids	+	-
Phenols	+	-
Coumarins	-	+
Steroids	+	-
Phlobatannins	-	+
Anthraquinons	-	+

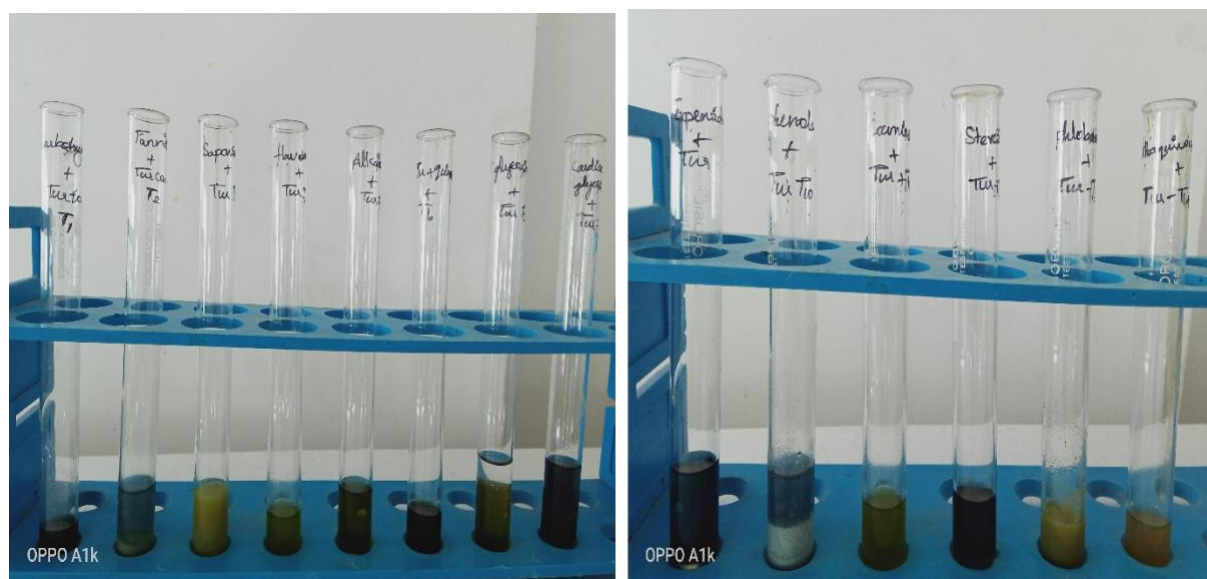


Figure 5.1.2. Ethyl acetate extract of *Turbinaria conoids*

Table 5.1.3.Ethanol extract of *Turbinaria conoids*

Ethanol extract of <i>Turbinaria conoids</i>	Present	Absents
Carbohydrate	+	-
Tannins	+	-
Saponins	-	+
Flavonoids	-	+
Alkaloids	-	+
Quinones	+	-
Glycosides	-	+
Cardiac glycosides	-	+
Terpenoids	+	-
Phenols	+	-
Coumarins	-	+
Steroids	+	-
Phlobatannins	-	+
Anthraquinones	-	+

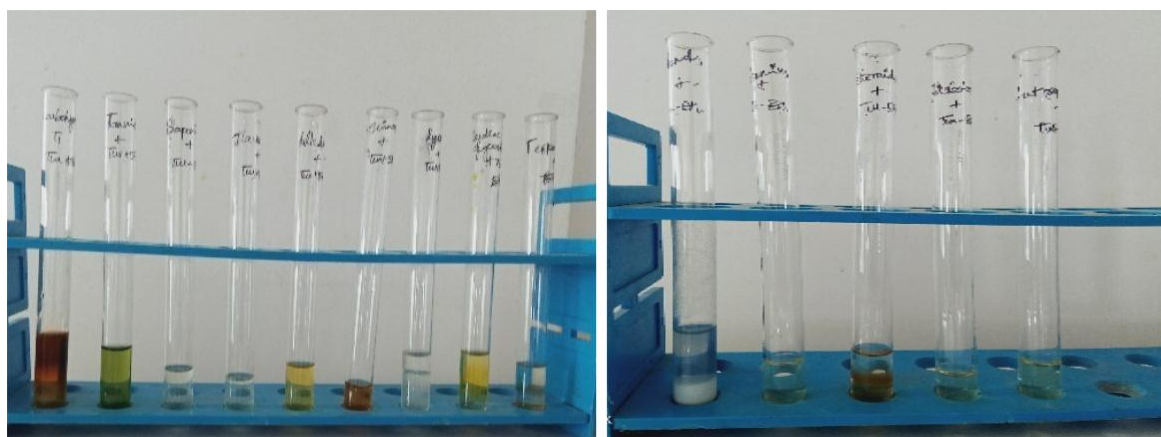


Figure 5.1.3 Ethanol extract of *Turbinaria conoids*

Table 5.1.4 Hexane extract of *Ulva lactuca*

Hexane extract of <i>Ulva lactuca</i>	Present	Absents
Carbohydrate	+	-
Tannins	+	-
Saponins	+	-
Flavonoids	-	+
Alkaloids	+	-
Quinones	+	-
Glycosides	-	+
Cardiac glycosides	+	-
Terpenoids	+	-
Phenols	+	-
Coumarins	-	+
Steroids	+	-
Phlobatannins	-	+
Anthraquinones	-	+

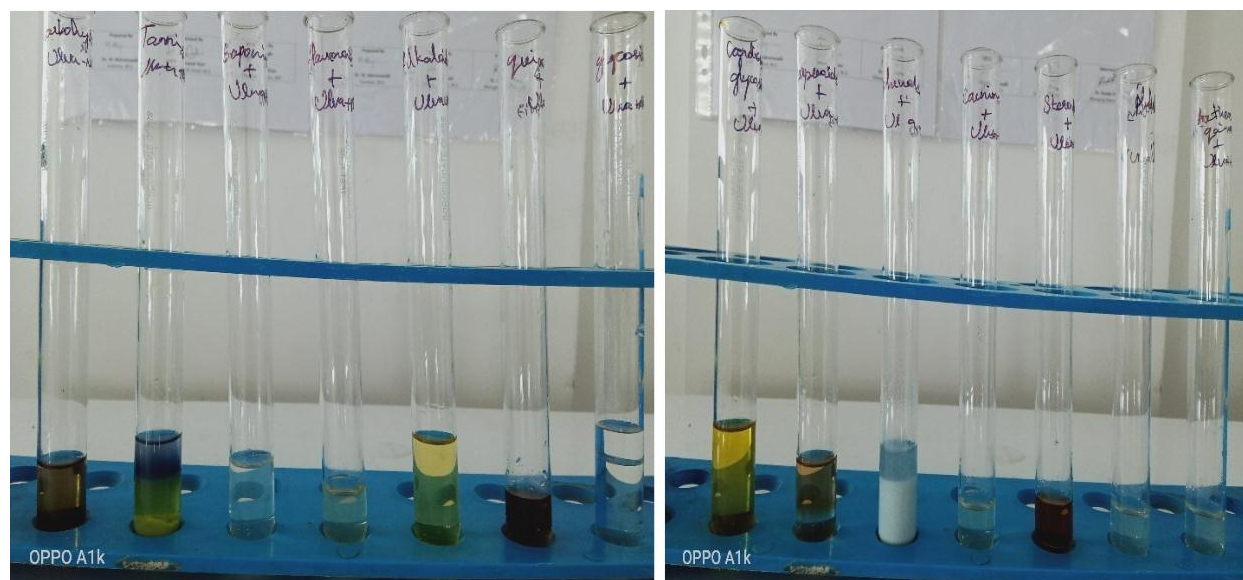


Figure 5.1.4 Hexane extract of *Ulva lactuca*

Table 5.1.5 Ethyl acetate extract of *Ulva lactuca*

Ethyl acetate extract of <i>Ulva lactuca</i>	present	absent
Carbohydrate	+	-
Tannins	+	-
Saponins	+	-
Flavonoids	-	+
Alkaloids	+	-
Quinones	-	-
Glycosides	-	+
Cardiac glycosides	+	-
Terpenoids	-	+
Phenols	-	+
Coumarins	-	+
Steroids	-	+
Phlobatannins	-	+
Anthraquinones	-	+

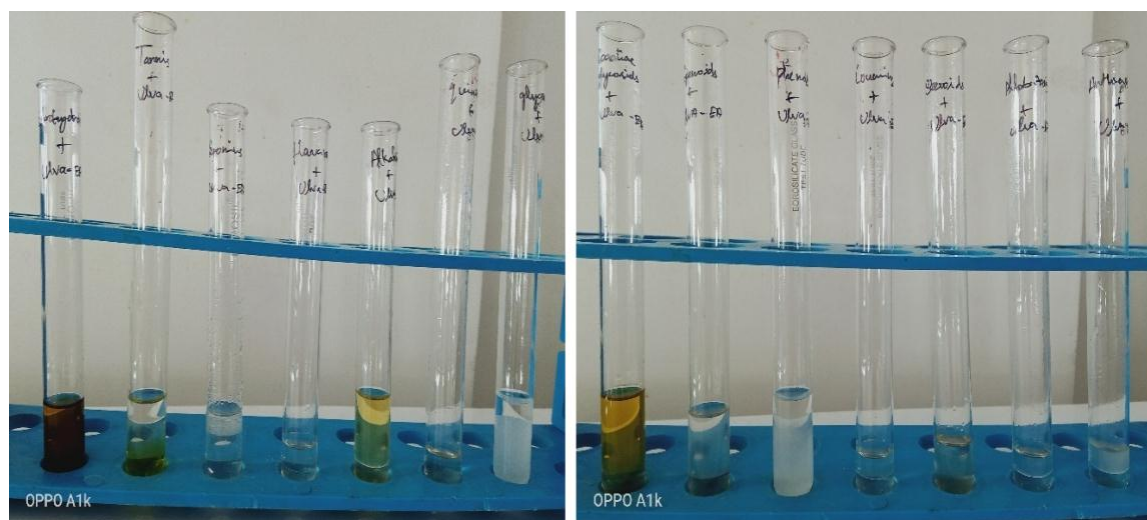


Figure 5.1.5 Ethyl acetate extract of *Ulva lactuca*

Table 5.1.6 Ethanol extract of *Ulva lactuca*

Ethanol extract of <i>Ulva lactuca</i>	Present	Absents
Carbohydrate	+	-
Tannins	+	-
Saponins	-	+
Flavonoids	-	+
Alkaloids	+	-
Quinones	-	+
Glycosides	-	+
Cardiac glycosides	+	-
Terpenoids	-	+
Phenols	+	-
Coumarins	-	+
Steroids	-	+
Phlobatannins	-	+
Anthraquinones	-	+

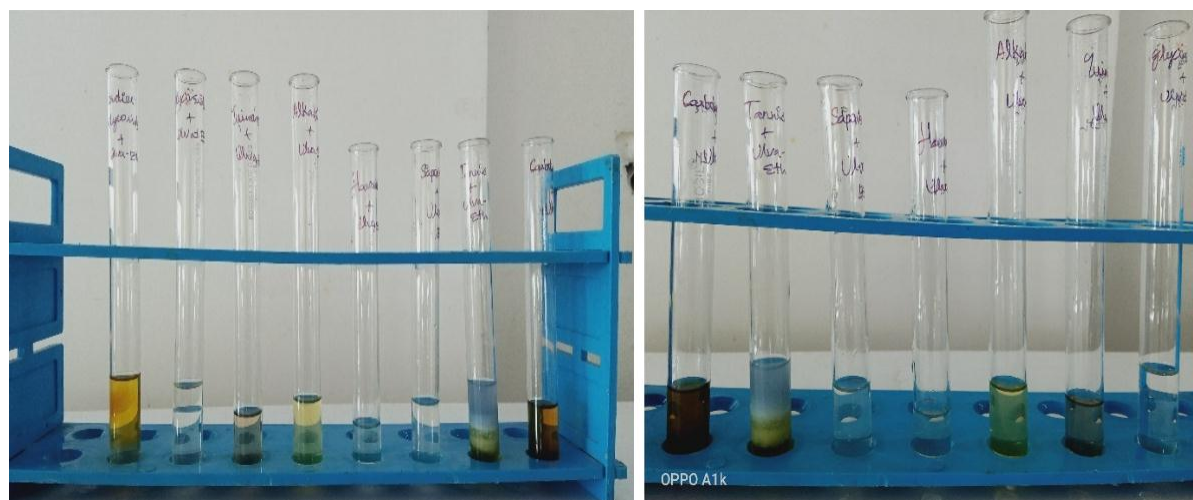


Figure 5.1.6 Ethanol extract of *Ulva lactuca*

5.2 QUANTITATIVE ANALYSIS

From the qualitative analysis presents and absents of phenol, flavonoids and tannins was identified and analysis quantitative for the extracts.

Sample+ extract	Flavonoids	phenol	tannins
Hexane extract of <i>Turbinaria conoides</i>	Present	present	present
Ethyl acetate extract of <i>Turbinaria conoides</i>	Absent	present	present
Ethanol extract of <i>Turbinaria conoides</i>	Absent	present	present
Hexane extract of <i>Ulva lactuca</i>	Absent	present	present
Ethyl acetate extract of <i>Ulva lactuca</i>	Absent	absent	present
Ethanol extract of <i>Ulva lactuca</i>	Absent	present	present

5.2.1. FLAVANOIDS

Table 5.2.1.1.Hexane extract of *Turbinaria conoides*

Content	Concentration	Absorbance at 510 nm
B	-	0
S1	20	0.0039
S2	40	0.0028
S3	60	0.0002
S4	80	0.0009
S5	100	0.0015
T1	-	0.4131
T2	-	0.5206
T3	-	1.3262

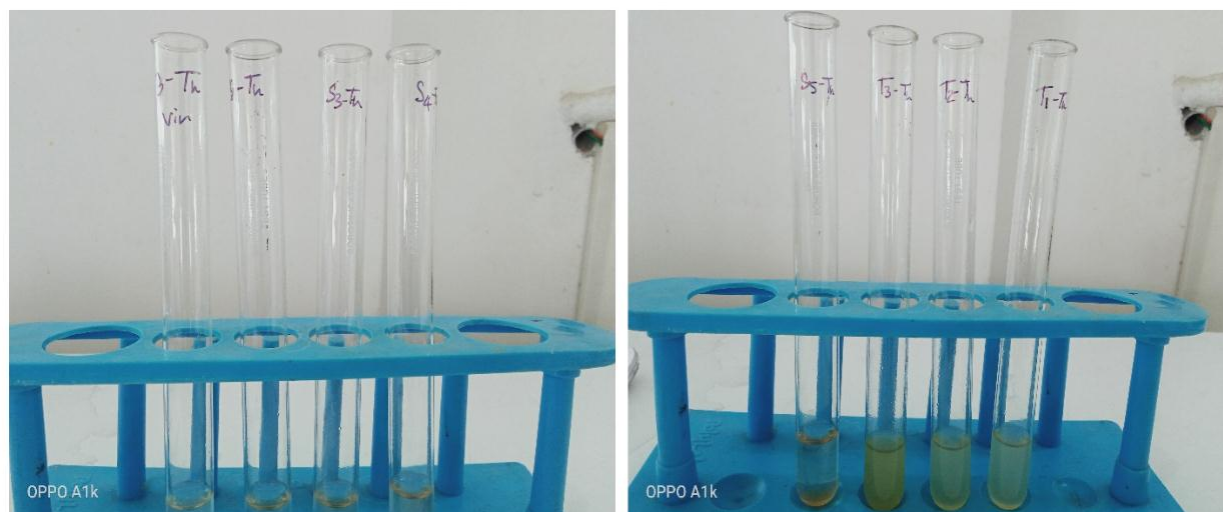


Figure 5.2.1.1 Hexane extract of *Turbinaria conoides*

5.2.2 TANNINS

Table 5.2.2.1.Hexane extract of *Turbinaria conoides*

Content	Concentration	Absorbance at 700 nm
B	-	0
S1	20	0.0075
S2	40	0.0062
S3	60	0.0157
S4	80	0.0673
S5	100	3.0000
T1	-	0.0049
T2	-	0.0080
T3	-	3.0000

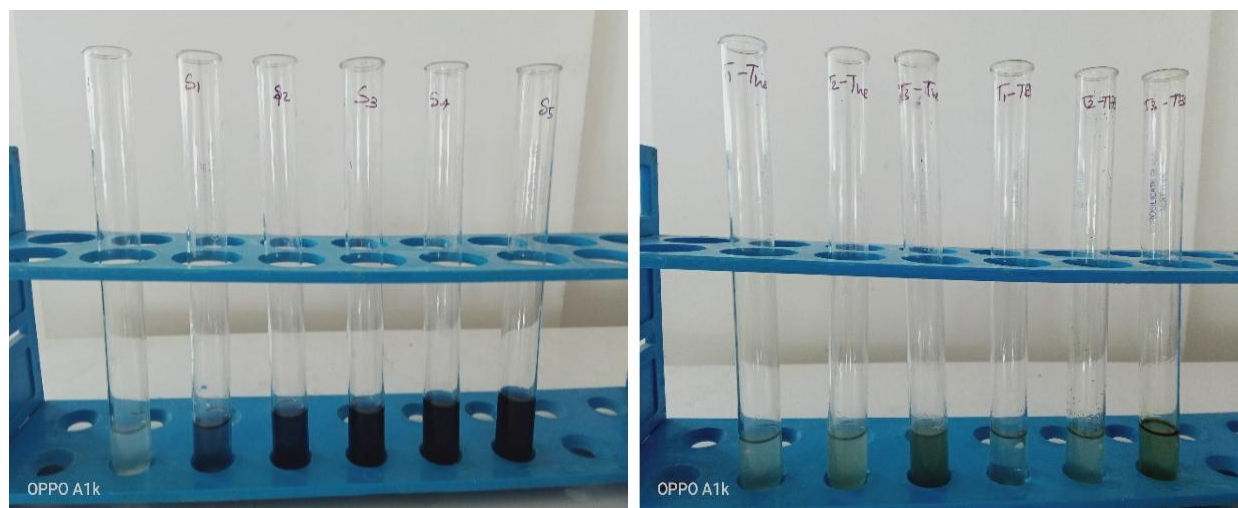


Figure 5.2.2.1.Hexane extract of *Turbinaria conoides*

Table 5.2.2.2. Ethyl acetate extract of *Turbinaria conoids*

Content	Concentration	Absorbance at 700 nm
B	-	0
S1	20	0.0075
S2	40	0.0062
S3	60	0.0157
S4	80	0.0673
S5	100	3.0000
T1	-	0.0071
T2	-	0.0110
T3	-	0.0330

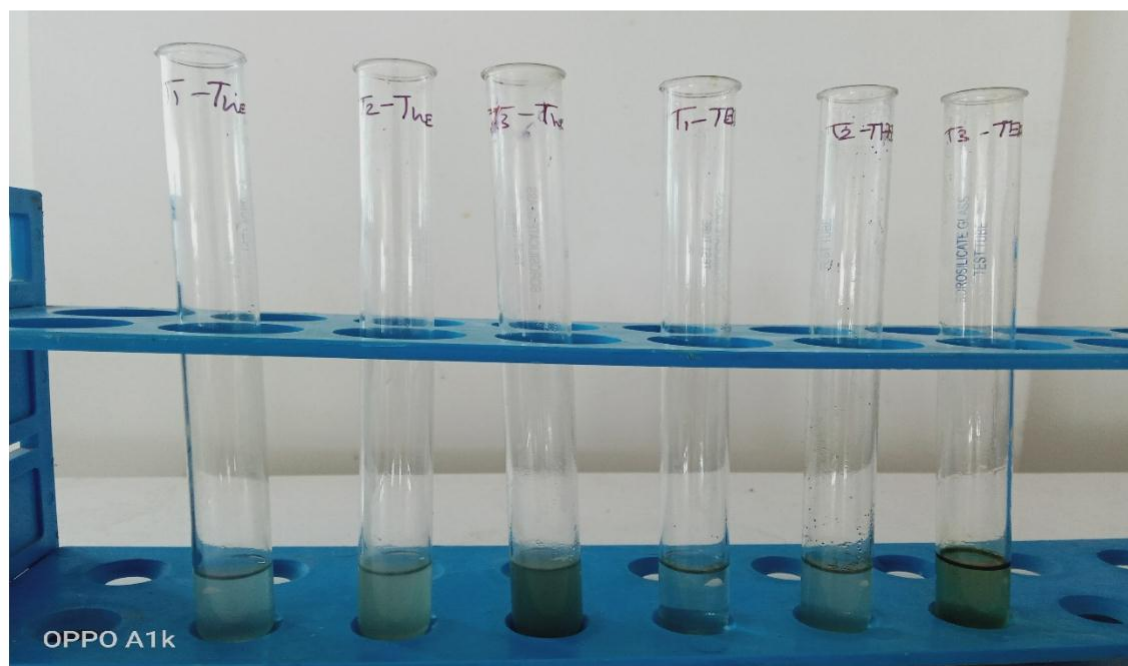


Figure 5.2.2.2. Ethyl acetate extract of *Turbinaria conoids*

Table 5.2.2.3 Ethanol extract of *Turbinaria conoids*

Content	Concentration	Absorbance at 700 nm
B	-	0
S1	20	0.0075
S2	40	0.0062
S3	60	0.0157
S4	80	0.0673
S5	100	3.0000
T1	-	0.0002
T2	-	0.0132
T3	-	0.0205

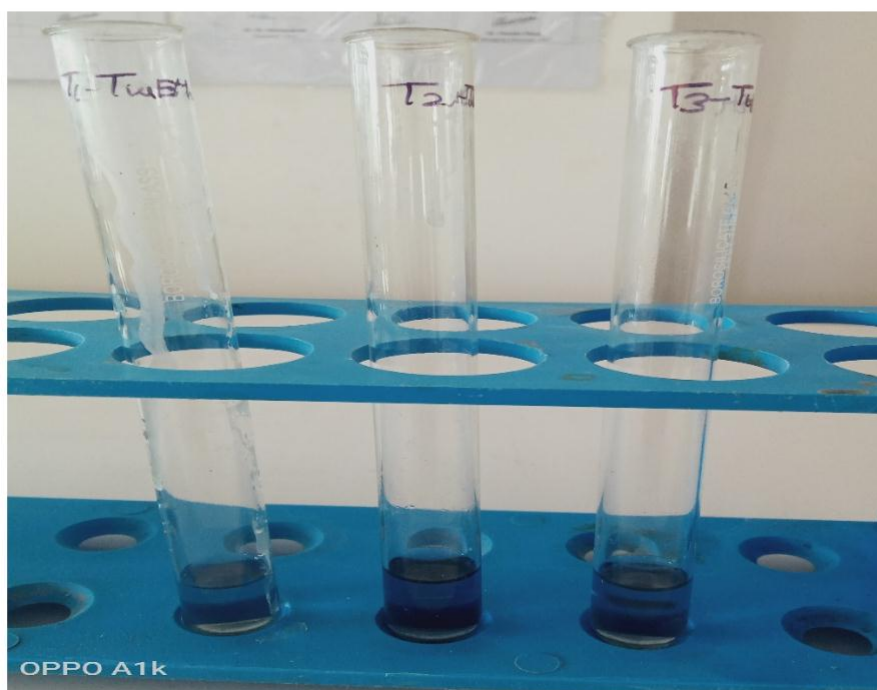


Figure 5.2.2.3 Ethanol extract of *Turbinaria conoids*

Table 5.2.2.4 Hexane extract of *Ulva lactuca*

Content	Concentration	Absorbance at 700 nm
B	-	0
S1	20	0.0075
S2	40	0.0062
S3	60	0.0157
S4	80	0.0673
S5	100	3.0000
T1	-	0.0095
T2	-	0.0288
T3	-	0.4974

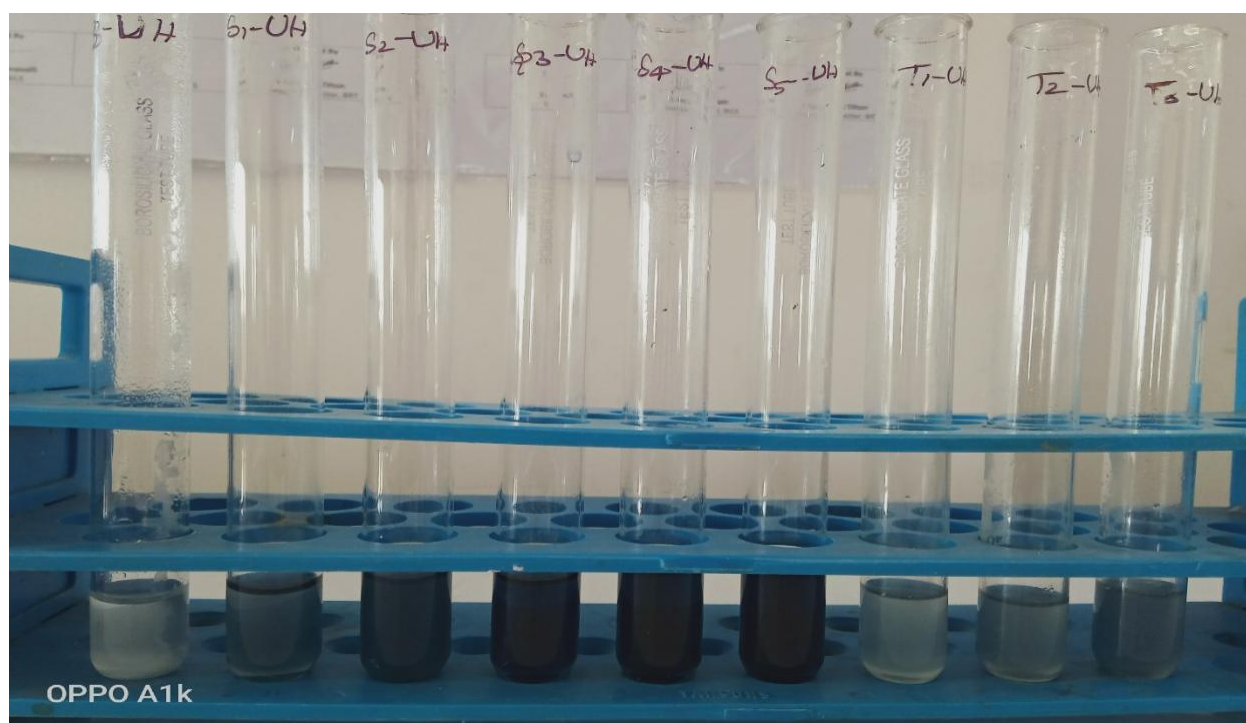


Figure 5.2.2.4 Hexane extract of *Ulva lactuca*

Table 5.2.2.5 Ethyl acetate extract of *Ulva lactuca*

Content	Concentration	Absorbance at 700 nm
B	-	0
S1	20	0.0075
S2	40	0.0062
S3	60	0.0157
S4	80	0.0673
S5	100	3.0000
T1	-	1.5300
T2	-	1.8853
T3	-	3.0000

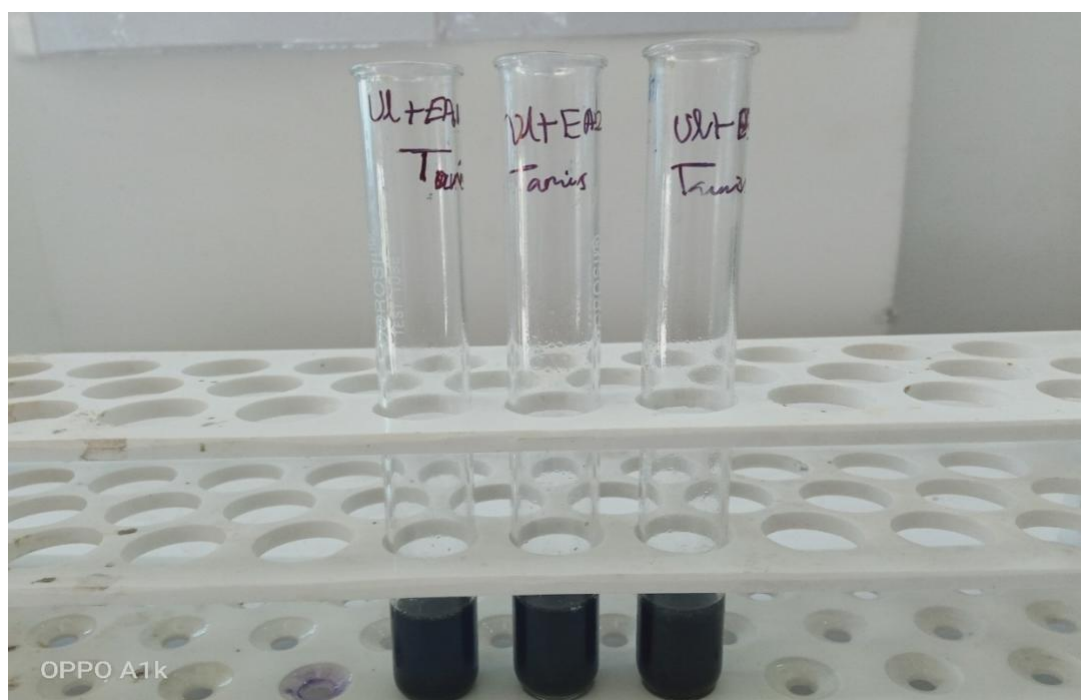


Figure 5.2.2.5 Ethyl acetate extract of *Ulva lactuca*

Table 5.2.2.6 Ethanol extract of *Ulva lactuca*

Content	Concentration	Absorbance at 700 nm
B	-	0
S1	20	0.0075
S2	40	0.0062
S3	60	0.0157
S4	80	0.0673
S5	100	3.0000
T1	-	0.5542
T2	-	0.9116
T3	-	1.2219

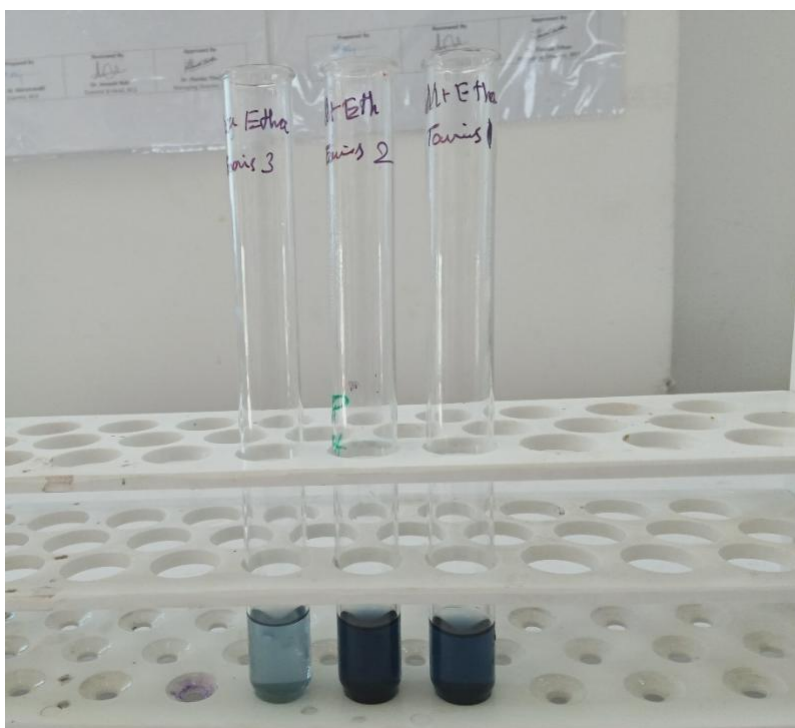


Figure 5.2.2.6 Ethanol extract of *Ulva lactuca*

5.3 PHENOL TEST

Table 5.3.1.Hexane extract of *Turbinaria conoides*

Content	Concentration	Absorbance at 765 nm
B	-	0
S1	20	0.0006
S2	40	0.0009
S3	60	0.0041
S4	80	1.7972
S5	100	3.0000
T1	-	0.0146
T2	-	0.0203
T3	-	0.0847

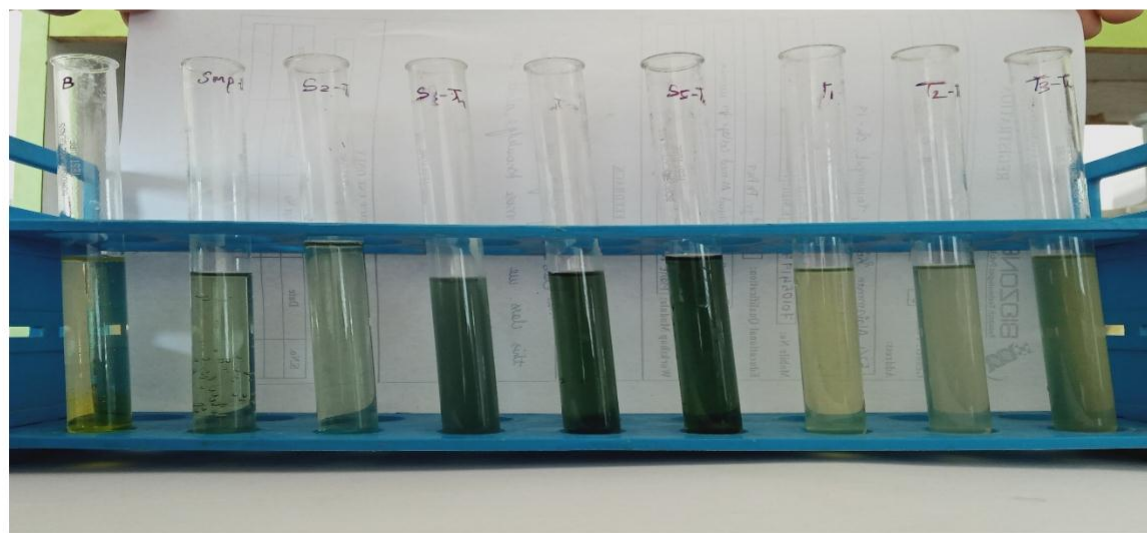


Figure 5.3.1.Hexane extract of *Turbinaria conoides*

Table 5.3.2. Ethyl acetate extract of *Turbinaria conoids*

Content	Concentration	Absorbance at 765 nm
B	-	0
S1	20	0.0006
S2	40	0.0009
S3	60	0.0041
S4	80	1.7972
S5	100	3.0000
T1	-	0.0034
T2	-	0.0915
T3	-	0.1837

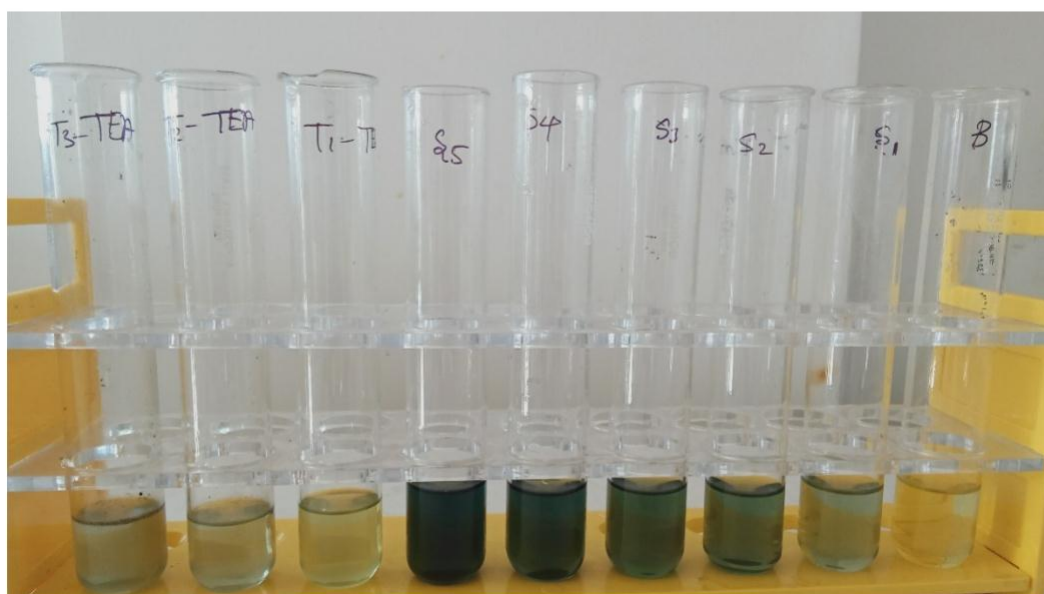


Figure 5.3.2. Ethyl acetate extract of *Turbinaria conoids*

Table 5.3.3. Ethanol extract of *Turbinaria conoides*

Content	Concentration	Absorbance at 765 nm
B	-	0
S1	20	0.0006
S2	40	0.0009
S3	60	0.0041
S4	80	1.7972
S5	100	3.0000
T1	-	1.2085
T2	-	3.0000
T3	-	3.0891

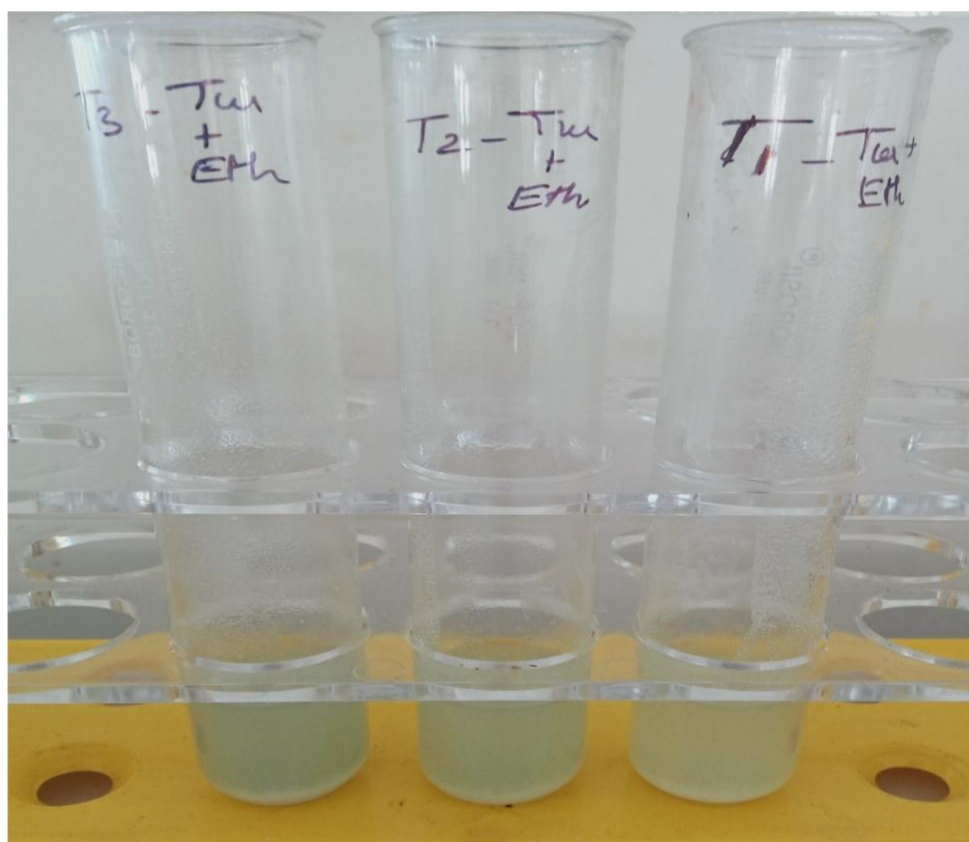


Figure 5.3.3. Ethanol extract of *Turbinaria conoids*

Table 5.3.4 Hexane extract of *Ulva lactuca*

Content	Concentration	Absorbance at 765 nm
B	-	0
S1	20	0.0006
S2	40	0.0009
S3	60	0.0041
S4	80	1.7972
S5	100	3.0000
T1	-	0.1392
T2	-	0.1817
T3	-	0.4137

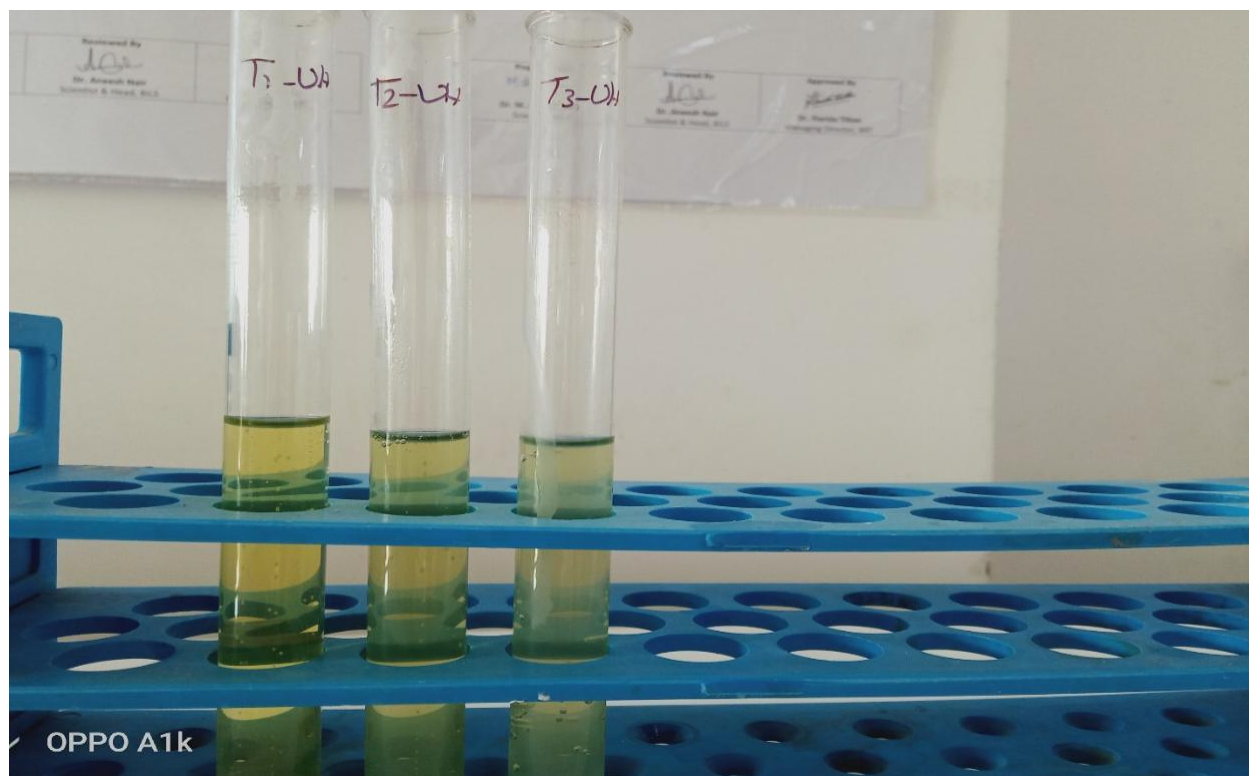


Figure 5.3.4 Hexane extract of *Ulva lactuca*

Table 5.3.5 Ethanol extract of *Ulva lactuca*

Content	Concentration	Absorbance at 765 nm
B	-	0
S1	20	0.0006
S2	40	0.0009
S3	60	0.0041
S4	80	1.7972
S5	100	3.0000
T1	-	0.1624
T2	-	0.2045
T3	-	1.2667

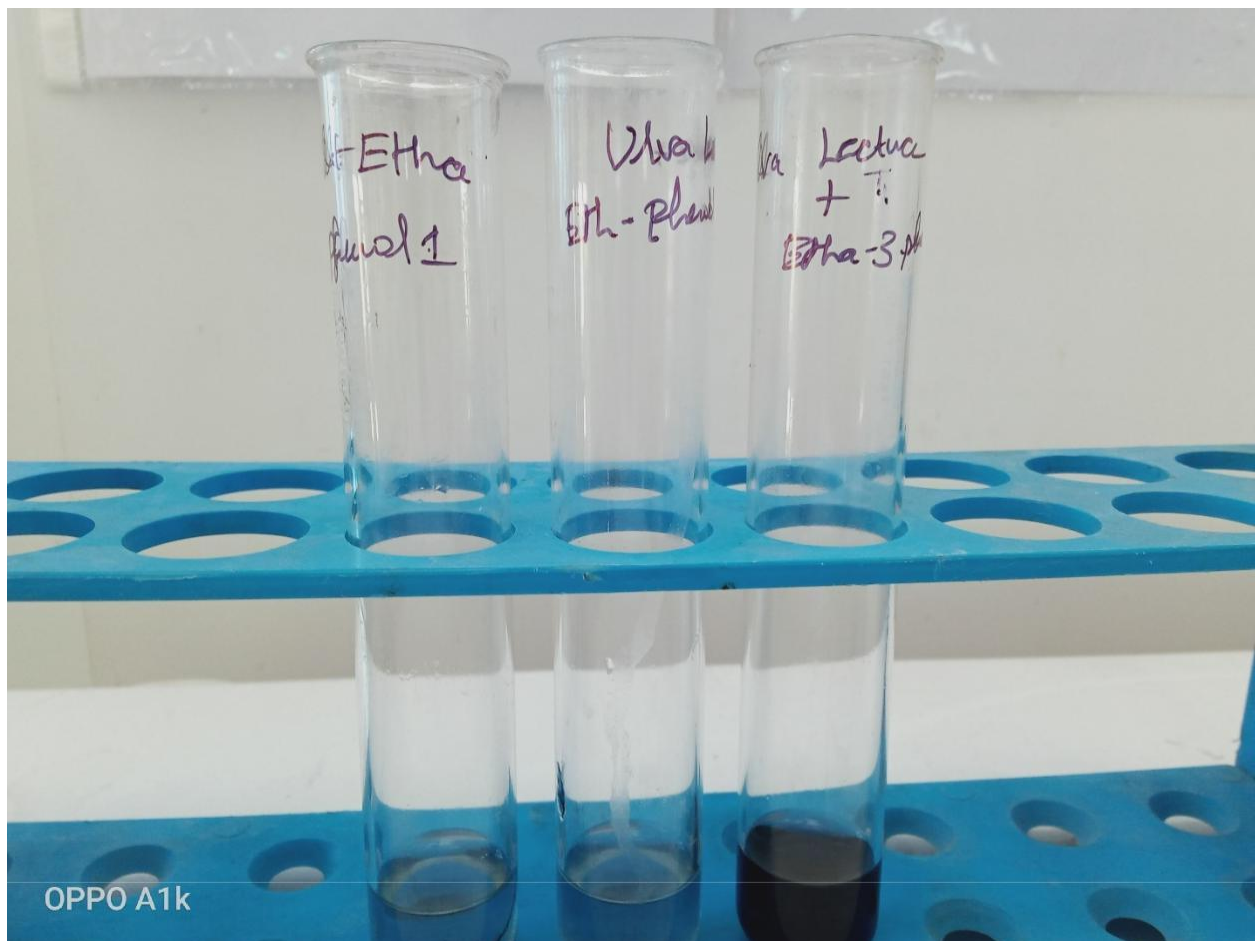


Figure 5.3.5 Ethanol extract of *Ulva lactuca*

5.4 GLUCOSE UPTAKE BY YEAST CELL

Table 5.4.1 *Turbinaria conoids* (% of inhibition)

Turbinaria conoids	Concentration(μ g)	Hexane	Ethyl acetate	ethanol
T1	200	99.78	99.88	99.76
T2	300	99.70	99.95	99.87
T3	400	99.65	99.82	99.80

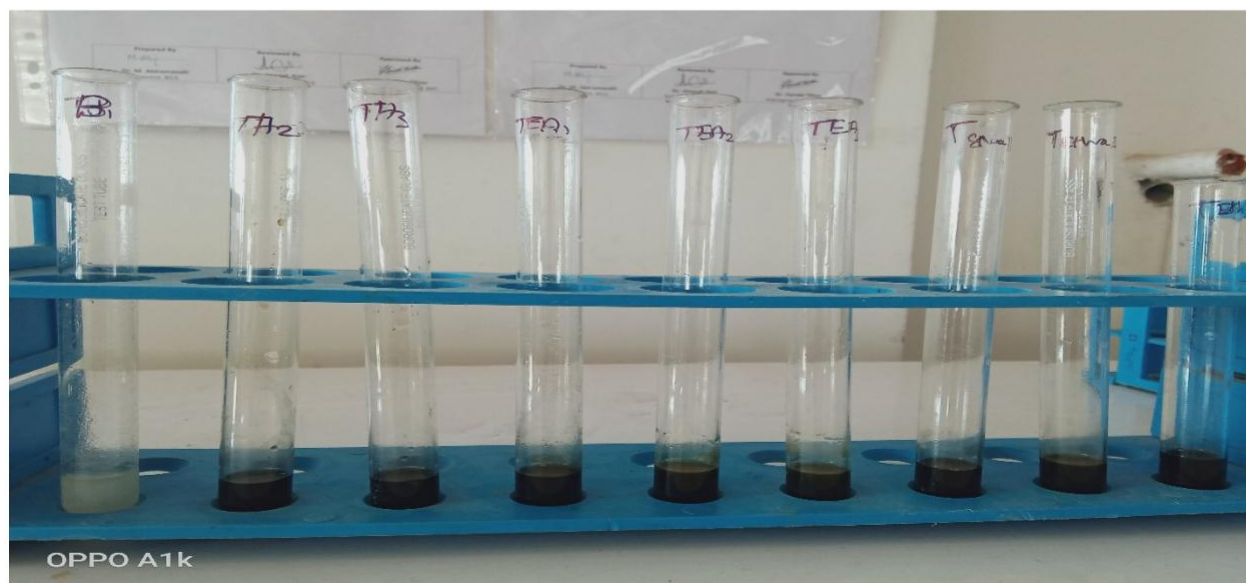
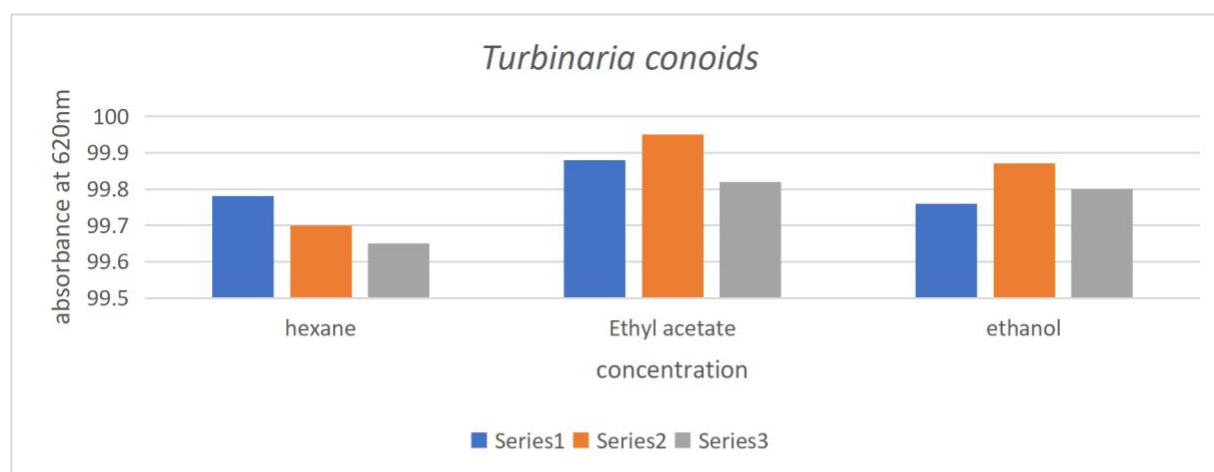


Figure 5.4.1 *Turbinaria conoids* (% of inhibition)

Table 5.4.2 *Ulva lactuca* (% of inhibition)

Ulva lactuca	Concentration(μ g)	Hexane	Ethyl acetate	ethanol
T1	200	84.42	90.83	95.13
T2	300	89.89	91.33	96.73
T3	400	91.26	91.98	97.75

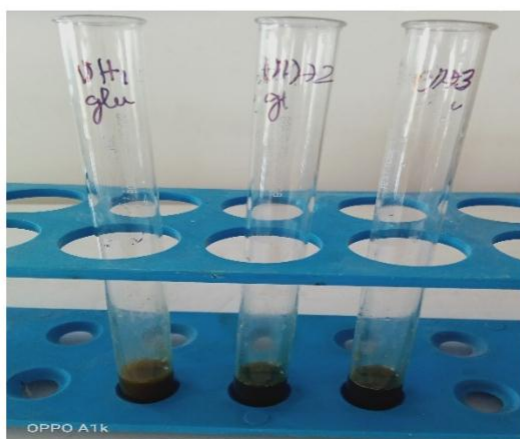
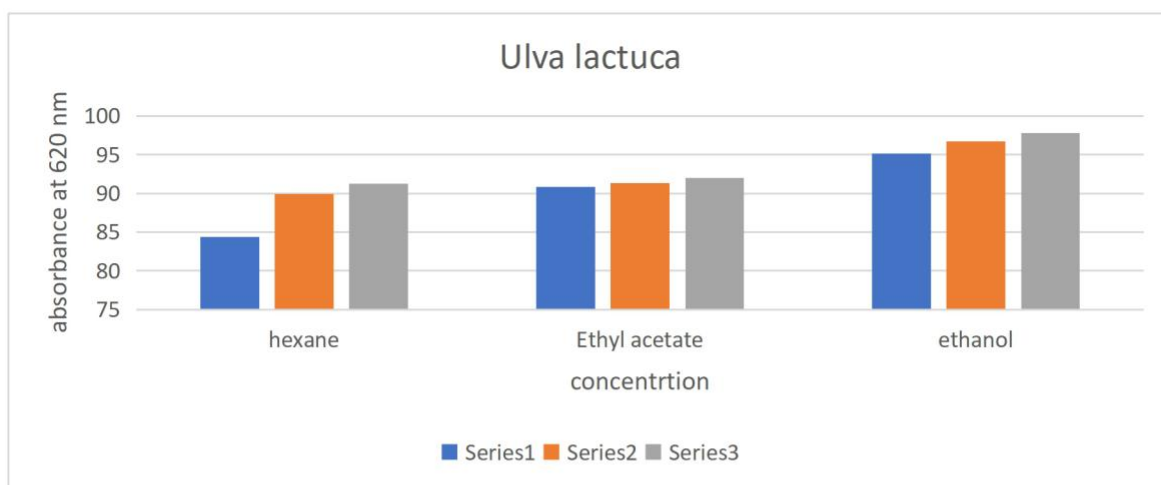


Figure 5.4.2 Hexane extract of *Ulva lactuca* (% of inhibition)

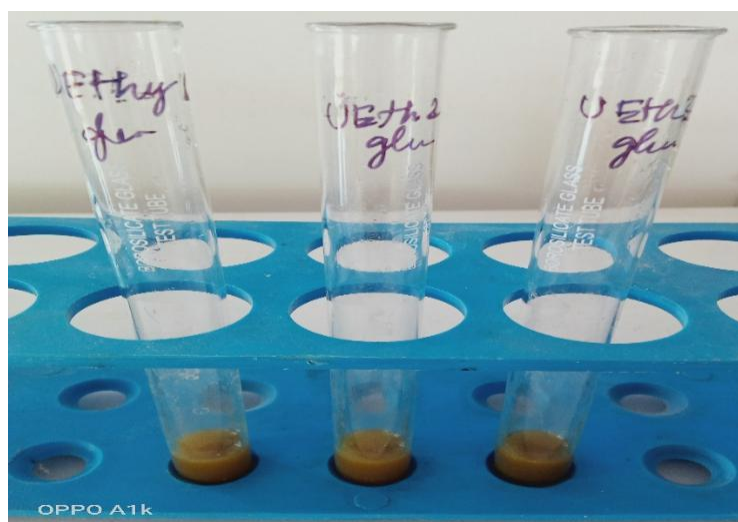


Figure 5.4.3 Ethanol extract of *Ulva lactuca* (% of inhibition)

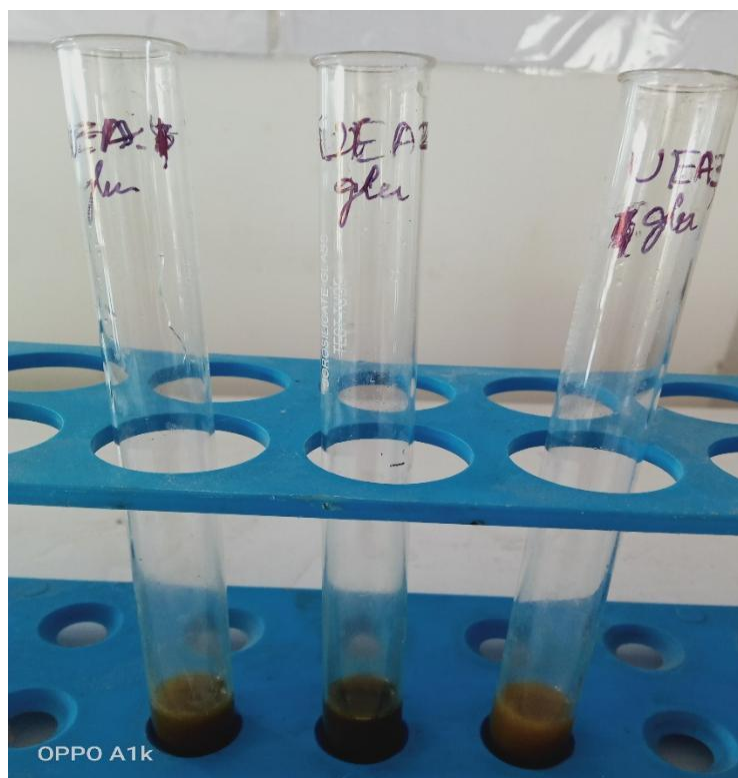


Figure 5.4.4 Ethyl acetate extract of *Ulva lactuca* (% of inhibition)

5.5 ALPHA AMYLASE INHIBITION ASSAY

Table 5.5.1 *Turbinaria conoids* (% of inhibition)

Turbinaria conoids	Concentration(μ g)	Hexane	Ethyl acetate	ethanol
T1	200	99.32	99.82	99.62
T2	300	99.52	99.92	99.79
T3	400	99.46	99.73	99.69

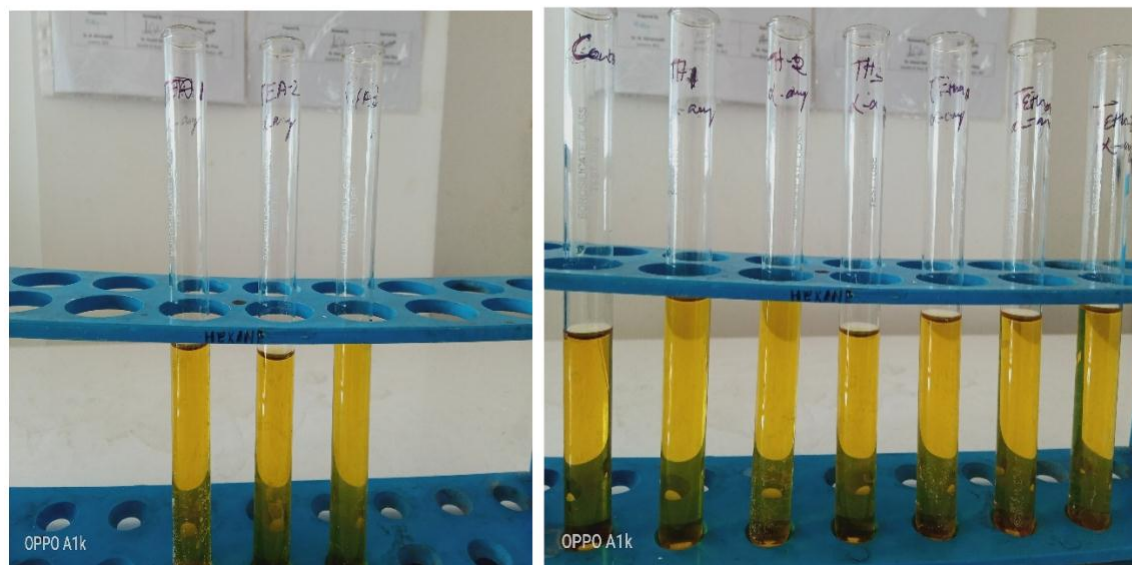
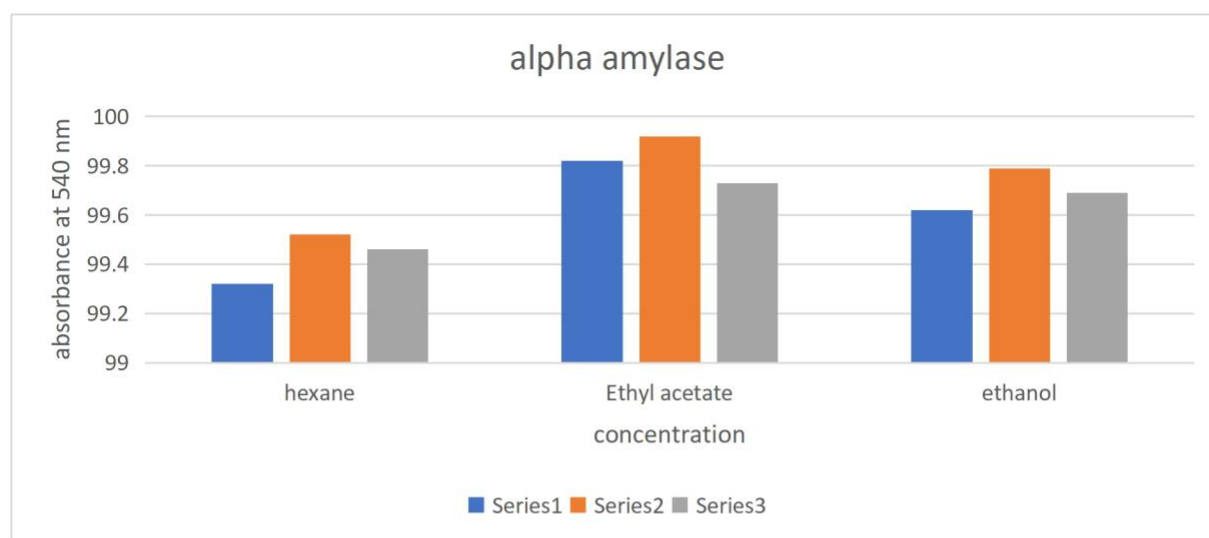


Figure 5.5.1 *Turbinaria conoids* (% of inhibition)

Table 5.5.2 *Ulva lactuca* (% of inhibition)

Ulva lactuca	Concentration(μ g)	Hexane	Ethyl acetate	ethanol
T1	200	89.31	89.806	87.58
T2	300	91.71	92.12	89.69
T3	400	89.43	91.10	88.30

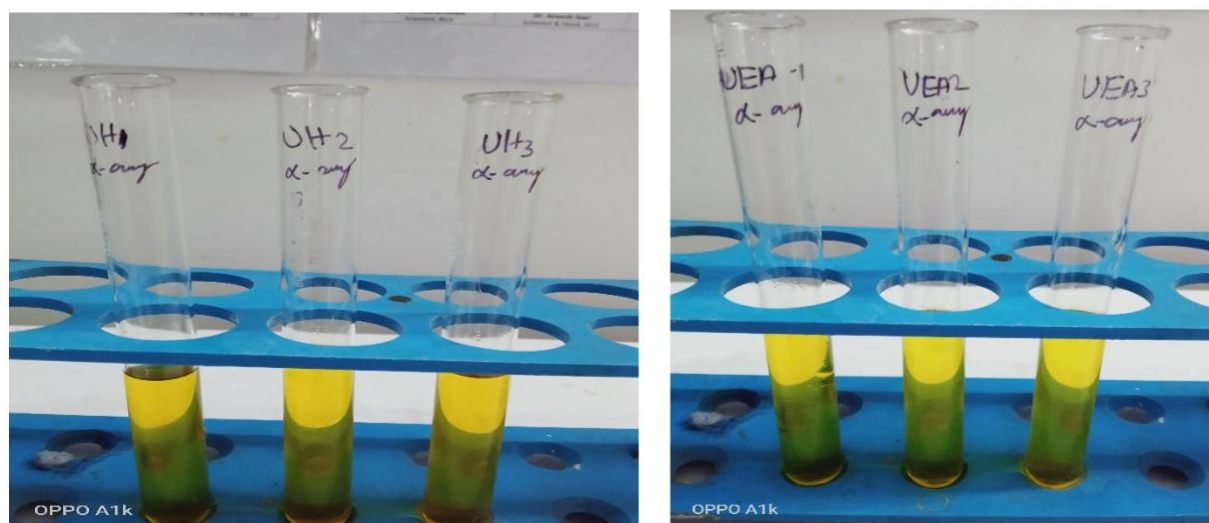
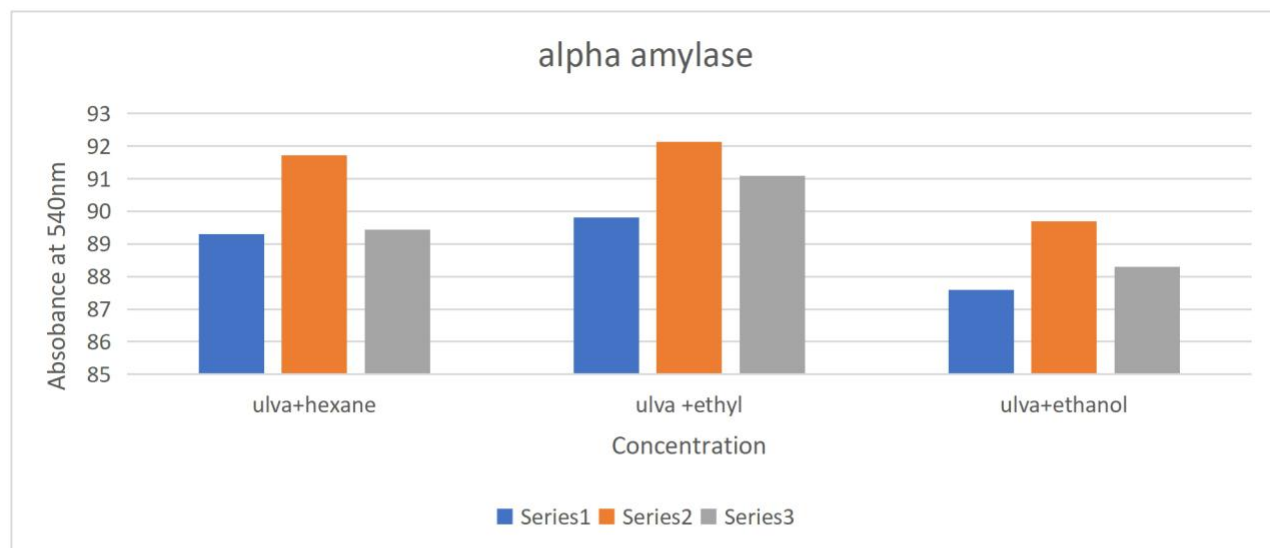


Figure 5.5.2 Hexane & Ethyl acetate extract of *Ulva lactuca* (% of inhibition)

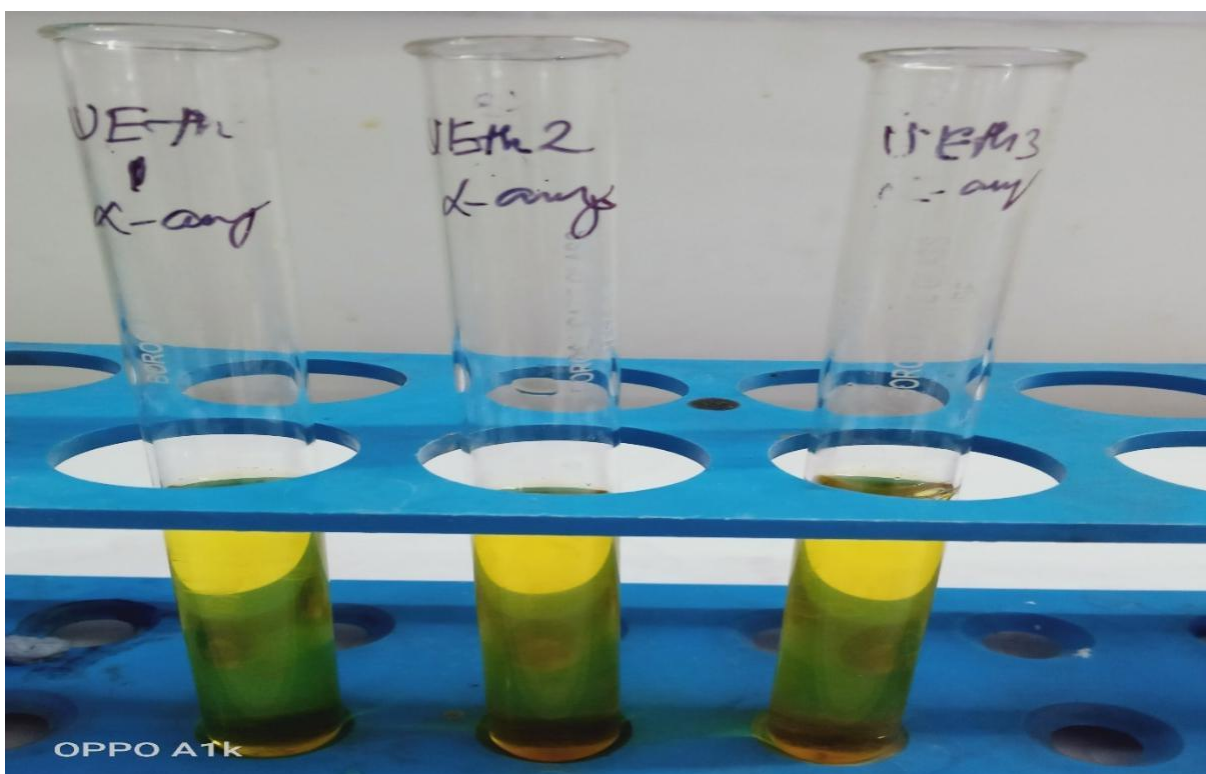


Figure 5.5.3 Ethanol extract of *Ulva lactuca* (% of inhibition)

5.6 HYDROGEN PEROXIDE (H₂O₂) RADICAL SCAVENGING ACTIVITY:

Table 5.6.1 *Turbinaria conoids*

Turbinaria conoids	Concentration(µg)	Hexane	Ethyl acetate	ethanol
T1	500	99.99	99.18	97.30
T2	1000	99.58	96.76	99.99
T3	1500	89.42	76.66	90.33
T4	2000	94.22	89.54	98.92

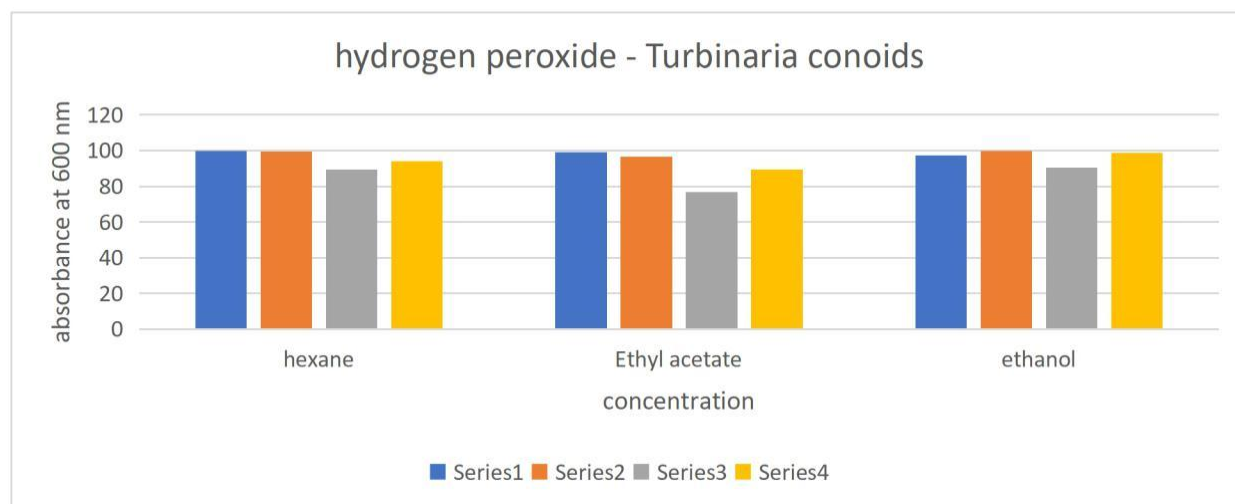


Figure 5.6.1 Ethyl acetate & ethanol extract of *Turbinaria conoids*

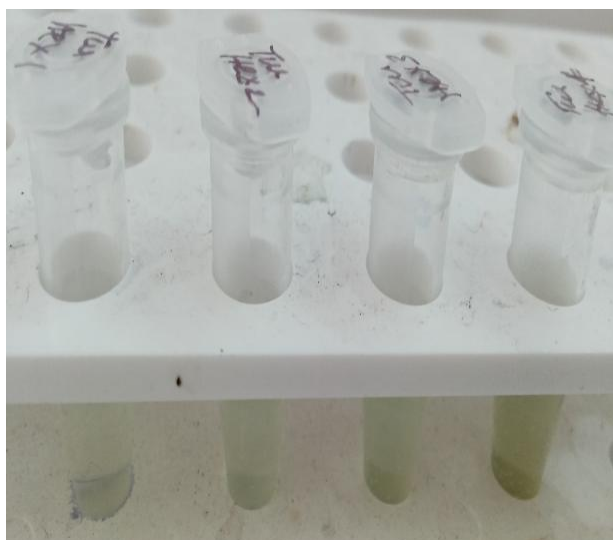
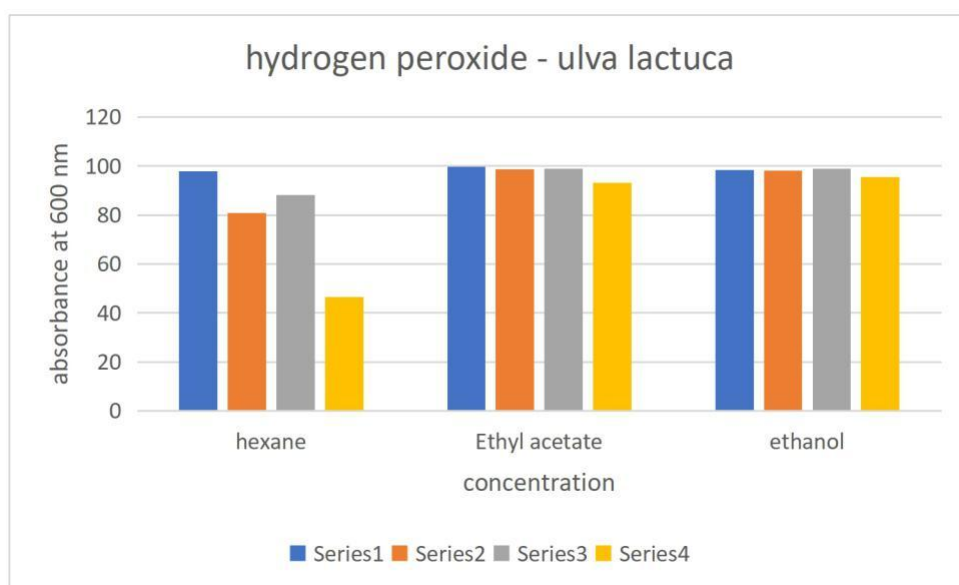


Figure 5.6.2 Hexane extract of *Turbinaria conoides*

Table 5.6.2 *Ulva lactuca*

<i>Ulva lactuca</i>	Concentration($\mu\text{g/ml}$)	Hexane	Ethyl acetate	ethanol
T1	500	97.92	99.74	98.33
T2	1000	80.81	98.63	98.21
T3	1500	88.21	98.96	98.80
T4	2000	46.50	93.177	95.35



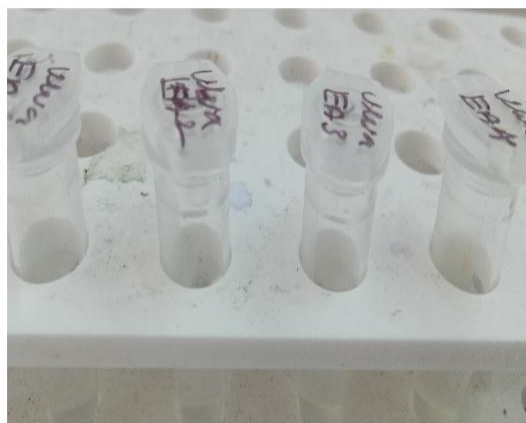
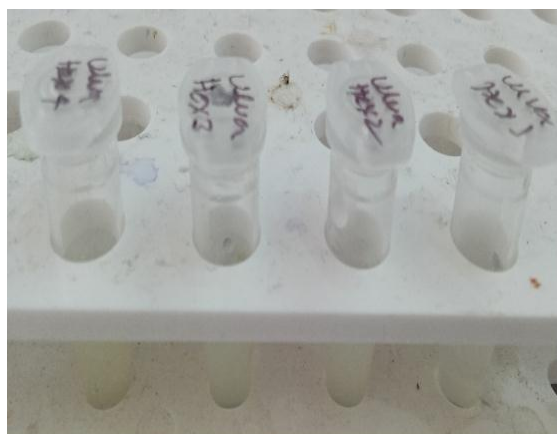


Figure 5.6.3 Hexane & ethyl acetate extract of *Ulva lactuca*

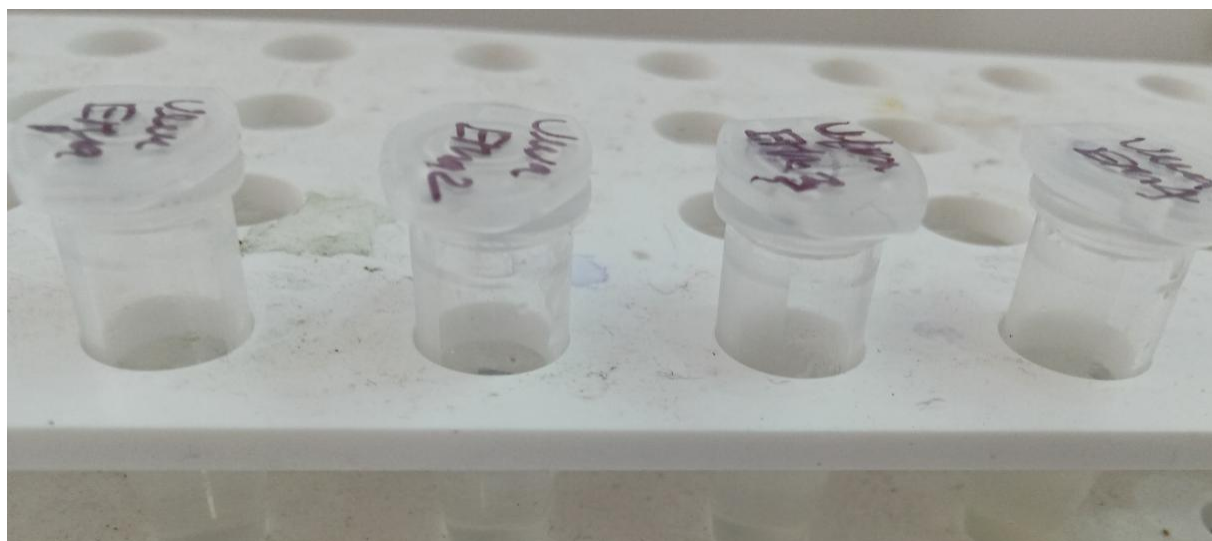


Figure 5.6.4 Ethanol extract of *Ulva lactuca*

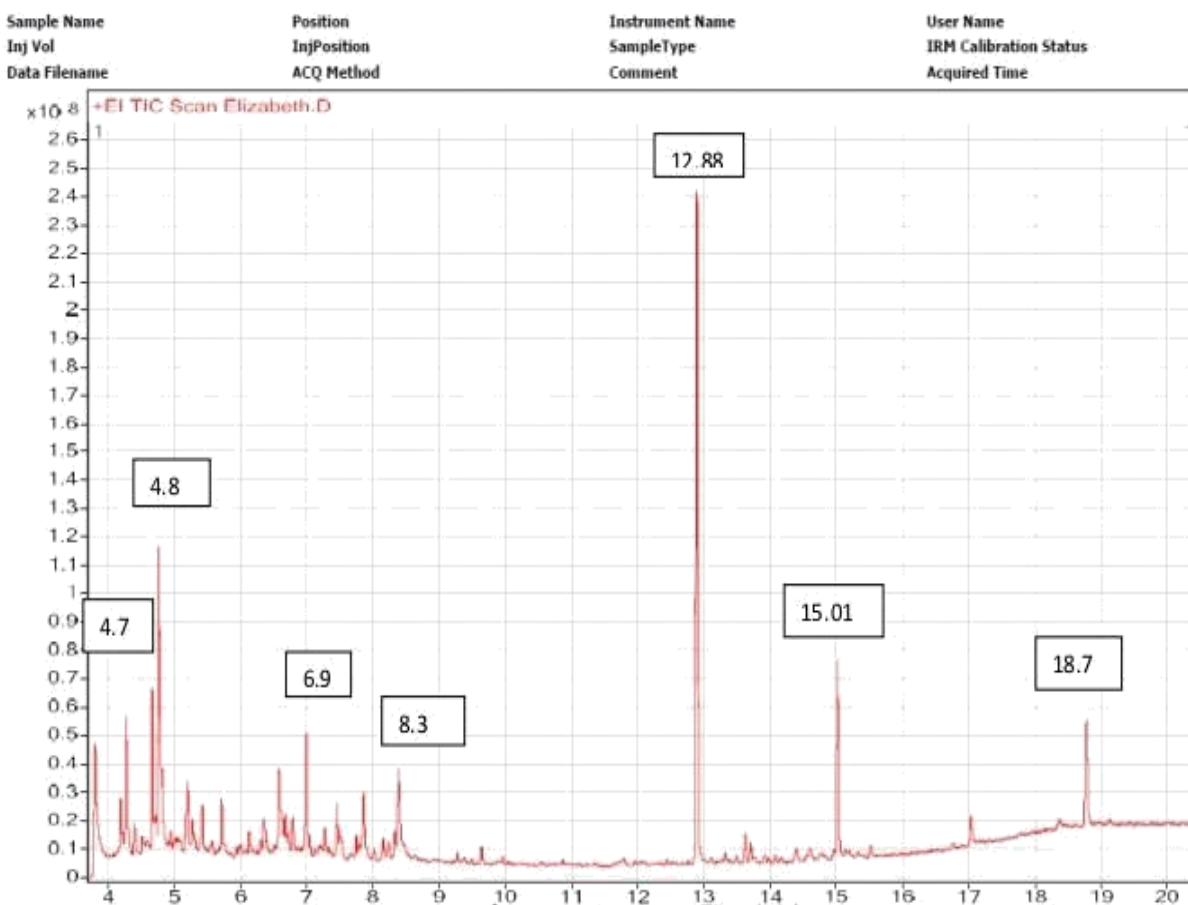
Table 5.7 EXTRACTION (Yield Percentage)

Sample name	Yield Percentage
Hexane extract of <i>Turbinaria conoids</i>	1.6202
Ethyl acetate extract of <i>Turbinaria conoids</i>	0.7253
Ethanol extract of <i>Turbinaria conoids</i>	0.1569
Hexane extract of <i>Ulva lactuca</i>	0.5882
Ethyl acetate extract of <i>Ulva lactuca</i>	0.8705
Ethanol extract of <i>Ulva lactuca</i>	0.7058

5.8 GAS CHROMATOGRAPHY MASS SPECTROSCOPY(GCMS)

Compound No	RT	Compound Name	Molecular formula	Molecular weight	CAS
1	4.189	(6-Hydroxymethyl-2,3-dimethylphenyl)methanol	C ₁₀ H ₁₄ O ₂	166.21	
2	4.271	Tridecane	C ₁₃ H ₂₈	184.36	629-50-5
3	4.666	9-Oxabicyclo[3.3.1]nonan-2-ol, 5-methoxy-, endo-	C ₉ H ₁₆ O ₃	172.22	35377-89-0
4	4.767	Vanillin	C ₈ H ₈ O ₃	152.15	121-33-5
5	4.812	Vanillin lactoside	C ₂₀ H ₂₈ O ₁₃	476.42	
6	6.575	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C ₁₀ H ₁₂ O ₃	180.2	
7	6.991	2,4-Diphenyl-4-methyl-1-pentene	C ₁₈ H ₂₀	236.35	
8	7.856	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	276.37	82304-66-3
9	8.383	5,6-Dimethoxyphthalaldehydic acid	C ₁₀ H ₁₀ O ₅	210.18	519-05-1
10	12.885	Cedran-diol, (8S,14)-	C ₁₅ H ₂₆ O ₂	238.36	62600-05-9
11	15.017	Squalene	C ₃₀ H ₅₀	410.71	111-02-4
12	18.781	Di-n-octyl phthalate	C ₂₄ H ₃₈ O ₄	390.56	117-84-0

Instrument Name: Agilent Triple Quad GC/MS
Model Number: GC- 7890A, MS-7000



5.8.1 Graph of gas chromatography and mass spectroscopy(GCMS)

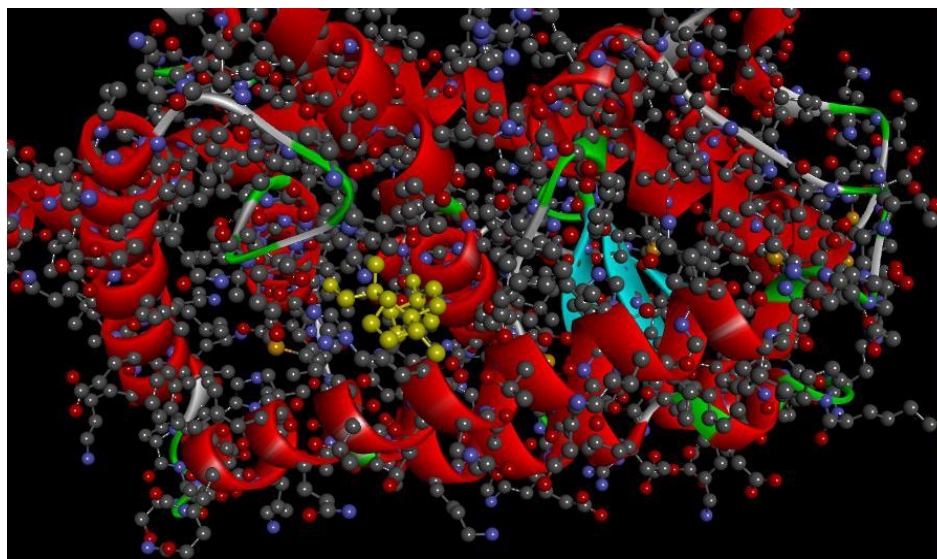
5.9 DOCKING

Name	Visible	Color	Parent	X	Y	Z	Distance	Category	Types	From	From Chemistry	To	To Chemistry
1 A:ARG397:NH1 - Z:UNK999:O	☒ Yes	Green	Ligand Non-bond Monitor	20.943	11.9...	5.86	2.73972	Hydrogen Bond	Conventi...	A:ARG3...	H-Donor	Z:UN...	H-Ac
2 A:ARG397:NH1 - Z:UNK999:O	☒ Yes	Green	Ligand Non-bond Monitor	19.9...	13.9...	6.651	3.09738	Hydrogen Bond	Conventi...	A:ARG3...	H-Donor	Z:UN...	H-Ac
3 A:ARG443:NH2 - Z:UNK999:O	☒ Yes	Green	Ligand Non-bond Monitor	19.8...	15.0...	7.964	2.84965	Hydrogen Bond	Conventi...	A:ARG4...	H-Donor	Z:UN...	H-Ac
4 Z:UNK999:O - A:TYR320:O	☒ Yes	Green	Ligand Non-bond Monitor	20.835	10.5...	4.8235	3.07756	Hydrogen Bond	Conventi...	Z:UNK99...	H-Donor	A:TY...	H-Ac
5 Z:UNK999:O - A:GLU324:OE2	☒ Yes	Green	Ligand Non-bond Monitor	21.798	10.749	6.294	2.99655	Hydrogen Bond	Conventi...	Z:UNK99...	H-Donor	A:GL...	H-Ac
6 Z:UNK999:O - A:ASP396:OD1	☒ Yes	Green	Ligand Non-bond Monitor	19.2...	16.2...	7.548	2.69969	Hydrogen Bond	Conventi...	Z:UNK99...	H-Donor	A:AS...	H-Ac
7 A:ARG397:CD - Z:UNK999:O	☒ Yes	Light Green	Ligand Non-bond Monitor	19.054	14.735	5.969	2.68949	Hydrogen Bond	Carbon H...	A:ARG3...	H-Donor	Z:UN...	H-Ac
8 A:PRO398 - Z:UNK999	☒ Yes	Pink	Ligand Non-bond Monitor	22.6...	16.524	2.90...	4.68822	Hydrophobic	Alkyl	A:PRO398	Alkyl	Z:UN...	Alky
9 A:ARG443 - Z:UNK999	☒ Yes	Pink	Ligand Non-bond Monitor	24.0...	12.6...	7.256	5.33318	Hydrophobic	Alkyl	A:ARG443	Alkyl	Z:UN...	Alky

Visible	Color	Parent	X	Y	Z	Distance	Category	Types	From	From Chemistry	To	To Chemistry	Angle XDA	Angle DAY
☒ Yes	Green	Ligand Non-bond Monitor	20.943	11.9...	5.86	2.73972	Hydrogen Bond	Conventi...	A:ARG3...	H-Donor	Z:UN...	H-Acceptor	66.359	113.957
☒ Yes	Green	Ligand Non-bond Monitor	19.9...	13.9...	6.651	3.09738	Hydrogen Bond	Conventi...	A:ARG3...	H-Donor	Z:UN...	H-Acceptor	32.647	105.673
☒ Yes	Green	Ligand Non-bond Monitor	19.8...	15.0...	7.964	2.84965	Hydrogen Bond	Conventi...	A:ARG4...	H-Donor	Z:UN...	H-Acceptor	63.146	116.536
☒ Yes	Green	Ligand Non-bond Monitor	20.835	10.5...	4.8235	3.07756	Hydrogen Bond	Conventi...	Z:UNK99...	H-Donor	A:TY...	H-Acceptor	170.911	130.515
☒ Yes	Green	Ligand Non-bond Monitor	21.798	10.749	6.294	2.99655	Hydrogen Bond	Conventi...	Z:UNK99...	H-Donor	A:GL...	H-Acceptor	111.567	156.503
☒ Yes	Green	Ligand Non-bond Monitor	19.2...	16.2...	7.548	2.69969	Hydrogen Bond	Conventi...	Z:UNK99...	H-Donor	A:AS...	H-Acceptor	123.104	108.004
☒ Yes	Light Green	Ligand Non-bond Monitor	19.054	14.735	5.969	2.68949	Hydrogen Bond	Carbon H...	A:ARG3...	H-Donor	Z:UN...	H-Acceptor	14.447	126.205
☒ Yes	Pink	Ligand Non-bond Monitor	22.6...	16.524	2.90...	4.68822	Hydrophobic	Alkyl	A:PRO398	Alkyl	Z:UN...	Alkyl		
☒ Yes	Pink	Ligand Non-bond Monitor	24.0...	12.6...	7.256	5.33318	Hydrophobic	Alkyl	A:ARG443	Alkyl	Z:UN...	Alkyl		



5.9.1 2HFP ligand molecule structure



5.9.2 Interaction of 2HFP + PPAR gamma

CHAPTER 6 - CONCLUSION

Natural compounds that can reduce postprandial hyperglycaemia by inhibiting certain enzymes, such as alpha amylase and glucose uptake by yeast cells have been found to be controlling diabetes. *Turbinaria conoids* and *Ulva lactuca* extracted and screened for presence of polar and nonpolar compound. Extraction prepared using different solvents such as hexane, ethyl acetate and ethanol. Major bioactive compounds alkaloids, flavonoids, phenols, tannins, glycosides. From the testing of bioactive compounds, alpha amylase and glucose uptake by yeast cells were analysed and compare the activity of each extracted sample. Compare the activity of extraction in the alpha amylase and glucose uptake by yeast cell, Hydrogen peroxide scavenging activity test and identified high activity of sample. ethyl acetate in *Turbinaria conoids* were shown high activity. Ethyl acetate extract of *Turbinaria conoids* was analyze to identify presence of compounds using gas chromatography and mass spectroscopy. In GCMS higher amount of cedran diol was present and docking for this molecule using PPAR gamma receptor.

CHAPTER 7- REFERENCE

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