Phytochemical Analysis, In Vitro Antioxidant and Wound Healing Activities of Turbinaria ornata (Turner) J. Agardh from Gulf of Mannar, India

Mohamed Saibi K M
Bharath University: Bharath Institute of Higher Education and Research
Leeba Balan (leebabalan86@gmail.com)
Bionyme Laboratories Pvt. Ltd. https://orcid.org/0000-0002-0345-5110
Sankar Jamuna
CSIR-CLRI: Central Leather Research Institute CSIR
Ramesh Babu
Bharath Institute of Science and Technology School of Bio Sciences

Research Article

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Abstract

Turbinaria ornata, tropical brown algae found in the South pacific and Indian Ocean ecosystems. In accordance with recent studies, Turbinaria ornata J. has potent anti-inflammatory effects. Therefore, this study is aimed to explore the biological activities of ethanolic extract of Turbinaria ornata J by analyzing the presence of phytochemical components, antioxidant property, antimicrobial activity and the wound healing activity. From the results, phytochemical analysis of ethanolic extract of T. ornata showed the presence of alkaloids, saponins, oils, total phenolic and total flavanoid content were estimated to be 0.683 Abs and 0.433 Abs respectively. Anti-oxidant activity of the ethanolic extract of T. ornata extract showed remarkable DPPH radical scavenging activity of about 58.8% at 200µg/ml and total anti-oxidant activity of 0.257 absorbance at 100µg/ml concentration, as compared to that of their respective controls. The ethanolic extract of T. ornata exhibited the maximum zone of inhibition against the clinical pathogens like Pseudomonas aeuruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans and Methicillin-resistant Staphylococcus aureus with their potent anti-microbial activity. Wound healing effects of the ethanolic extract of T. ornata was analysed by using Zebra fish model. The results showed the rapidand significant regeneration of the wounded caudal fin on day 14. Therefore, the preliminary results of this study strongly supports the ethanolic extract of T. ornata may be effective in wound healing and regeneration of the wounded tissues.

Highlights

- Antioxidant and Antimicrobial activities ethanolic extract of *T. ornata* J. supports the *in vitro* biological properties.
- The ethanolic extract of *T. ornata* J. reveals the promising regenerative property in zebra fish model.
- *T. ornata* J. possess strong antioxidant, antimicrobial and regenerative properties.

Introduction

Wound healing is the disruption of the normal biological organs and tissues. It regenerate healing in a dynamic, regulated and molecular mechanisms of cells to the wounded areas [1]. Emerging trends in healthy economy amongst the innovations with in the field of wound healing is growing expeditiously [2]. Many researchers widely use natural products for the design of therapeutic drugs and its shows potential wound healing compounds [3]. Zebrafish exhibits the same wound repair property as that of mammalian wound repairing steps [4]. Seaweeds play an emerging trend in the field of wound healing property against Zebrafish. Earlier studies by researchers have showed that most of the biological therapeutics such as immunomodulatory, stress tolerance, wound healing [5] anti-inflammatory, antiviral, antitumor, antithrombotic, anticoagulant and antioxidant bioactivities have been reported in seaweeds [6].

Turbinaria ornata (Turner) J. Agardh, is a marine green algae belongs to the family phaeophyceae which is rich in fucoids and sulphated polysaccharides and are commonly distributed on southeast coast of Tamilnadu, India, tropical and subtropical areas of Central and Western Pacific and also in Indian Ocean

(Fig. 1a., 1b.). The algae is commonly used in animal food, in fertilizers and also used as food ingredients [7].

T. ornata possess wide range of biological properties such as anti-coagulant, antioxidant [8], anti-inflammatory [9], antibacterial [10], and wound healing [11] properties have been reported. The present study focus on the invitro antioxidant and wound healing effects of *T. ornata* ethanol extract against zebrafish model.

Experimental Methods

Qualitative Phytochemical Analysis

The ethanolic extract of *T. ornata* J. was assessed for the presence of phytochemical components by the following standard methods like Dragendorff's method, Fehling's method, Borntrager's test, Foam test, Biuret's method, Ninhydrin's method, Ferric chloride method, Spot test and Terpenoids test [12][13][14][15].

Quantitative Analysis

Total Phenolic Content

The ethanolic extract of *Turbinaria ornata* was soaked in different solvents such as hexane, chloroform, ethyl acetate, methanol and water were kept in the orbital shaker for 24 hrs. The residues were then filtered and the filtrate was evaporated. The different extracts of plant material were then centrifuged at 10,000 rpm for 15 min at 4°C. Twenty µL of extracts was prepared using the supernatant and made up to 3 mL of distilled water. Then, 0.5 mL of Folin- Ciocalteu's phenol reagent was added to all the tubes. The tubes were then placed in the incubator for 3 min at 45°C. After 3 min, 2 mL of 20% Na_2CO_3 was added to all the tubes and kept for incubation after which, its absorbance was measured at 650 nm [16].

Total Flavonoids

Flavonoid contents were determined by slightly modified spectrophotometry method of Karadeniz*et al*. (2005). One gram Ethanolic extract of *Turbinaria ornata* was weighed and ground with 200 mL of 80 % aqueous methanol in a mortar and pestle. The ground sample was filtered and a clear filtrate was obtained. The aliquot of the sample (0.5 mL) was taken in a test tube add 3 mL of distilled water and 0.3 mL of 5% sodium nitrite were added. The solution was vortexed and allowed to stand at room temperature for 5 min and 0.6 mL of 10% aluminium chloride was added to the solution. After 6 min, 2 mL of 1 M sodium hydroxide was added to the test tube. The solution was made up to 10 mL with distilled water. The absorbance was read at 510 nm [17].

Total Proteins

Add an equal volume of 1 M NaOH to 100µg sample and vortex. Add NaOH to standards as well if this option is used. Add 5 ml dye reagent and measure the absorbance at 595 nm [18].

Total Lipids

The ethanolic extract of *Turbianria ornata* (2 gm) was placed in a porous thimble of a Soxhlet extractor with cotton plug at its mouthed and thimble was placed in an extraction chamber which was suspended to previously weighed flask containing methanol, methanol-chloroform or petroleum ether. The whole assembly was adjusted and flask was heated using heating mental for 2 hrs to extract crude lipid. After the extraction, thimble was removed from the Soxhlet apparatus and the solvent was removed under reduced pressure to afford crude lipid. Furthermore, the flask containing lipid was placed in oven at 100°C for 30 minutes to remove residual solvent, cooled in a desiccator and weighed. The amount of crude lipid was calculated and expressed as percentage crude lipid content (AOAC. 1990) [19].

Total Carbohydrates

To 1 mL of the sample, 1 ml of phenol and 5 ml of concentrated Sulphuric acid was added and the mixture was mixed thoroughly. The solution is allowed to stand for 15 min in a boiling water bath and the OD for the solution was read at 490 nm. The amount of total carbohydrates was calculated using standard graph prepared by D-glucose and the values are expressed as µg/Ml [19].

Antioxidant Activity

DPPH Assay

The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Szabo*et al*. (2007). The samples were reacted with the stable DPPH radical in methanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 1 mL of methanol and 1 mL of DPPH radical solution 0.5 mM in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer. The control solution was prepared by mixing methanol (1.0 mL) and DPPH radical solution (1.0 mL) and 1 mL of methanol serves as blank. The scavenging activity percentage was determined according to % of inhibition = Control O.D – Sample O.D/Control O.D X 100 [20].

Total Antioxidant Assay

Extracts in different concentration ranging from 10 to 100 µg/mL were added to each test tube individually containing 1 ml of distilled water and 1 ml of Molybdate reagent solution, 1mL of Sodium phosphate and 1 mL of Sulphuric acid were added separately. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm. The values were recorded [21].

Antimicrobial Activity

Antimicrobial assay of different samples was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU/ml. MHA plates was cultured with standardized microbial culture broth. Each well was filled with varying concentrations from 100, 125, 150 µg/ml of the samples with positive control as streptomycin 25 mcg and negative/solvent control as DMSO, respectively. The plate was allowed to diffuse for about 30 minutes at room temperature and incubated for 18–24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the tested samples. The zone of inhibition (ZOI) was observed and measured in mm [22].

Wound Healing Activity

6–12 month old wild-type fish of the TL/Ek strain was used for adult wounding experiments. Puncture wounds in embryonic median fins were manually introduced with a sterilized blade with a diameter of approximately 2 mm (Richardson et al., 2013).The treated and control fishes were weighed, measured before the puncture. The fins were carefully collected and stored in 10% formalin for further study. The fishes were fed with the feed combined with extracts for the regeneration study. They were watched carefully and after 14 days of treatment, the grown fins were again measured and collected for histochemical parameters [23].

Results And Discussion

Phytochemical Screening

The phytochemical screening of *Turbinaria ornata* revealed the presence of
Alkaloids, Saponins and fixed oils. Also revealed the absence of carbohydrates, glycosides, proteins, aminoacids, phenols and terpenoids (Table 1). Three types of extracts such as hexane, acetone and methanol was subjected to T. ornata and the results showed that the presence of alkaloids, terpenoids, flavonoids, polyphenols and quinones in hexane extract whereas alkaloids, terpenoids and flavonoids were found to be absent in both acetone and methanol extracts [24].

Table 1 Quantitative Analysis of ethanol extract of *T. ornata*

S.No.	Phytochemical Test	Observation
1.	Alkaloids	**+**
2.	Carbohydrates	**-**
3.	Glycosides	**-**
4.	Saponins	**+**
5.	Protein	**-**
6.	Amino acids	**-**
7.	Phenolic compounds	**-**
8.	Fixed oil	**+**
9.	Terpenoids	**-**
(**+**) – Positive; (**-**) – Negative		

Quantitative Analysis

The ethanolic extract of *Turbinaria ornata* contains the highest amount of phenols and flavanoids of 0.684nm and 0.434nm, whereas the amount of carbohydrates and proteins were found to be 0.274nm and 0.242nm respectively and total lipids was found to be in meager amount as 0.01mg/g. Earlier work have supported our data that among the two extracts used for the analysis, aqueous extract contained the highest amount of phenol and flavanoid compounds of about 1.187 and 1.020. The difference in the results obtained might possibly be due to the different method of extraction and solvents polarities [25].

Table 2 Quantitative analysis of ethanol extract *T. ornata*

S.No	Quantitative Analysis	Absorbance/Weight
1.	Total phenolic compounds	0.683±0.001
2.	Total flavanoid	0.433±0.001
3.	Total carbohydrates	0.273±0.002
4.	Total proteins	0.243±0.001
5.	Total lipids	0.01 g

Antioxidant Assay

DPPH Assay

The DPPH radical scavenging activity of the ethanol extract of *Turbinaria ornata* showed dose-dependent with maximum percentage of inhibition of 58.80 µg/mL at 200 µg/mL concentration and minimum percentage of inhibition of 17.30% at 100 µg/mL concentration (Fig. 2) when compared with that of standard quercetin. The half maximal inhibitory concentration (IC_{50}) of the concentration is 175.98µg/ml. Studies have reported that acetone extract showed significant scavenging ability on DPPH (65%) at a concentration of 1000 *µ*g/mL when compared with that of a standard BHT (97%). However, none of the extracts exhibited higher activity than BHT at the same concentration [7].

Total Antioxidant Activity

The total antioxidant activity in the ethanol extract of *Turbinaria ornata* is presented in Fig. 3 showed that maximum absorbance of 0.257 at 100µg/ml and minimum of 0.028 at 10µg/ml concentration when compared with that of standard ascorbic acid. The reducing capacity of various concentrations of ethanol extract at different concentrations along with the standard (Ascorbic acid) showed significant phosphomolybnedic activity presented in Fig. 3. The reducing capacity of the extract was found to be increased with the concentration of the sample. Work on CSP of *T. ornata* has the total antioxidant activity of 22.21 0.88 mg of ascorbic acid equivalents per g of the sample [26].

Antimicrobial Activity

The antimicrobial activity of ethanolic extract of *Turbinaria ornata* showed maximum zone of inhibition against *Pseudomonas aeruginosa* and Methicillin Resistant *Staphylococcus aureus* (MRSA) strains used and moderate inhibition of organism against *Staphylococcus aureus.* The least level of zone inhibition was observed in the organisms *Escherichia coli* and *Candida albicans* (Table 5, Fig. 4). According to the earlier reports the inhibition zone of 19mm, 23mm and 24mm was observed for hexane, acetonic and methanolic extracts, respectively. The positive control (amoxicillin) produced 21-24mm zone of inhibition. The negative controls (hexane, acetone and methanol) did not show any inhibition for marine algae [24].

Table 5. Antimicrobial activity of ethanol extract of *T. ornata*

S.No	Organisms	Zone of Inhibition			Antibiotic (streptomycin)
		100 µg	125 µg	150 µg	
1	*Pseudomonas aeruginosa*	0.336± 0.03 mm	0.533± 0.012 mm	0.623± 0.032 mm	0.92 mm
2	*Escherichia coli*	0.13± 0.016 mm	0.27± 0.016 mm	0.316± 0.012 mm	0.86 mm
3	*Staphylococcus aureus*	0.226± 0.009 mm	0.346± 0.02 mm	0.486± 0.02 mm	0.94 mm
4	*Candida albicans*	0.243± 0.028 mm	0.326± 0.012 mm	0.34± 0.021 mm	1.5 mm
5	Methicillin-resistant *Staphylococcus aureus*	0.333± 0.012 mm	0.53± 0.037 mm	0.626± 0.024 mm	1.2 mm
Mean ± SD					

Wound Healing

The fin growth measurement was observed on days 3, 7, 10 and 14 were compared with that of control. The fishes were grouped in two groups, with each group consisting of 3 fishes. First group was treated with ethanolic extract of

T. ornata and the second group was treated as control. First group showed better regeneration of fin compared to those of control fishes (Fig. 5). The fishes were treated with 500 µg of the ethanol extract of *T. ornata* showed maximum fin regeneration on day 14 when compared to the control fishes. Tissue regeneration was observed by histochemical analysis where, the control fishes recruited less number of neutrophils compared to the ones treated with the ethanol extract (Fig. 6). Earlier work reveals that the seaweeds of *Turbinaria ornata*, *Gracillaria crassa* and *Laurencia papillosa*, collected from the Tuticorin coast of the Southeast coast of India and selected based on preliminary screening, were extracted with acetone and evaluated for antiulcer, wound healing and hepatoprotective activities among the seaweeds studied *Laurencia papillosa* showed the greatest activity [11].

Conclusion

There is a constant endeavor amongst scientist to acquire new therapeutic agents from the marine algae. The present work is devoted to the determination of the yield, chemical composition, antioxidant, radical

scavenging properties and antibacterial effect of ethanolic extract of *Turbinaria ornata.*

Phytochemical screening revealed the richness in alkaloids, saponins and fixed oils. In addition, the quantitative analysis revealed significant levels of phenols and flavanoids are presented and the subsequent minimal levels of carbohydrates, proteins and lipids are revealed.

With regards to the results the total antioxidant activity revealed the tremendously increased antioxidant activity to the concentration of the sample on the one hand and on the another hand it shows the highest free radical scavenging abilities on DPPH which is very powerful, close to the positive control respectively.

Antimicrobial tests show the highest inhibition against the *Pseudomonas aureus* and Methicillin-resistant *Staphylococcus aureus.* Whereas, it shows strongest activity against the antibiotic-resistant bacterial strain. So we further proceed for the drug development, and furthermost, it reveals the immense response on the wound healing and also better regeneration of the wounded tissues on zebra fish.

Declarations

Author Contribution M.S.K.M and B.L. contributed in data gathering and designing this study. M.S.K.M and S.J. contributed in preparation of the manuscript. R.B. contributed in revision of the manuscript. All the authors are responsible for the final manuscript.

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Data Availability Not Applicable

Declarations

Ethical Approval and Consent to participate Not applicable

Consent to publish Not applicable

Conflict of Interest The authors report no competing interest.

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Figures

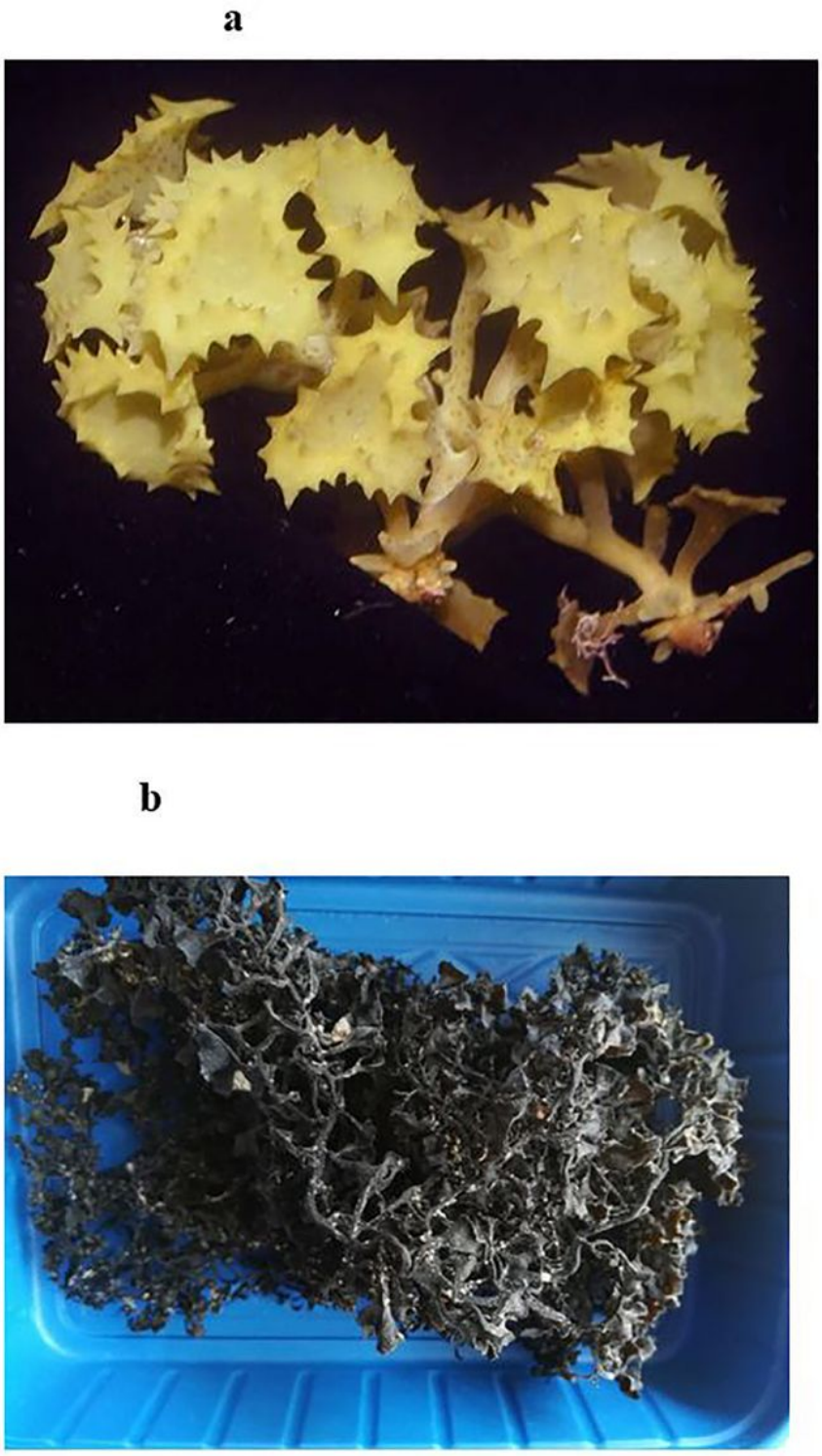


Figure 1

a. Habit of Turbinaria ornata. b. Dried form of Turbinaria ornata.

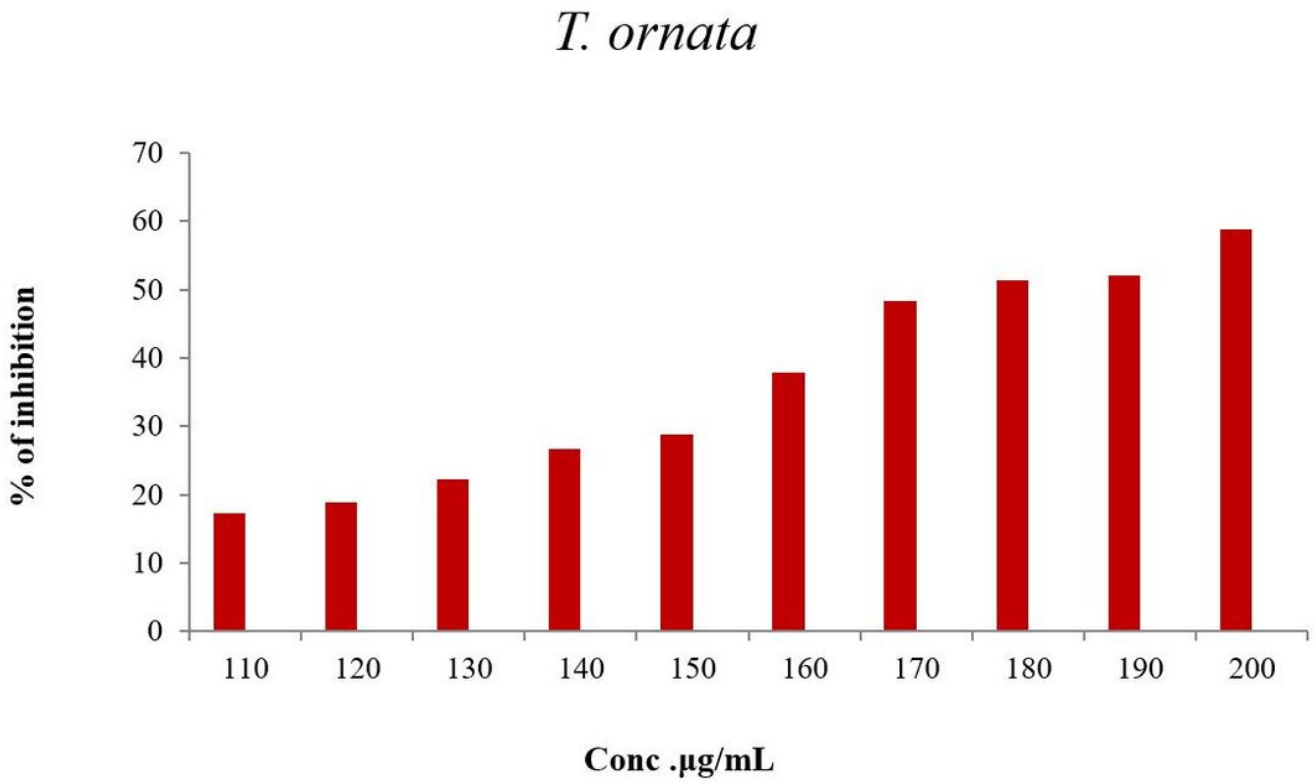


Figure 2

Graphical Representation of DPPH assay of ethanol extract of T. ornata

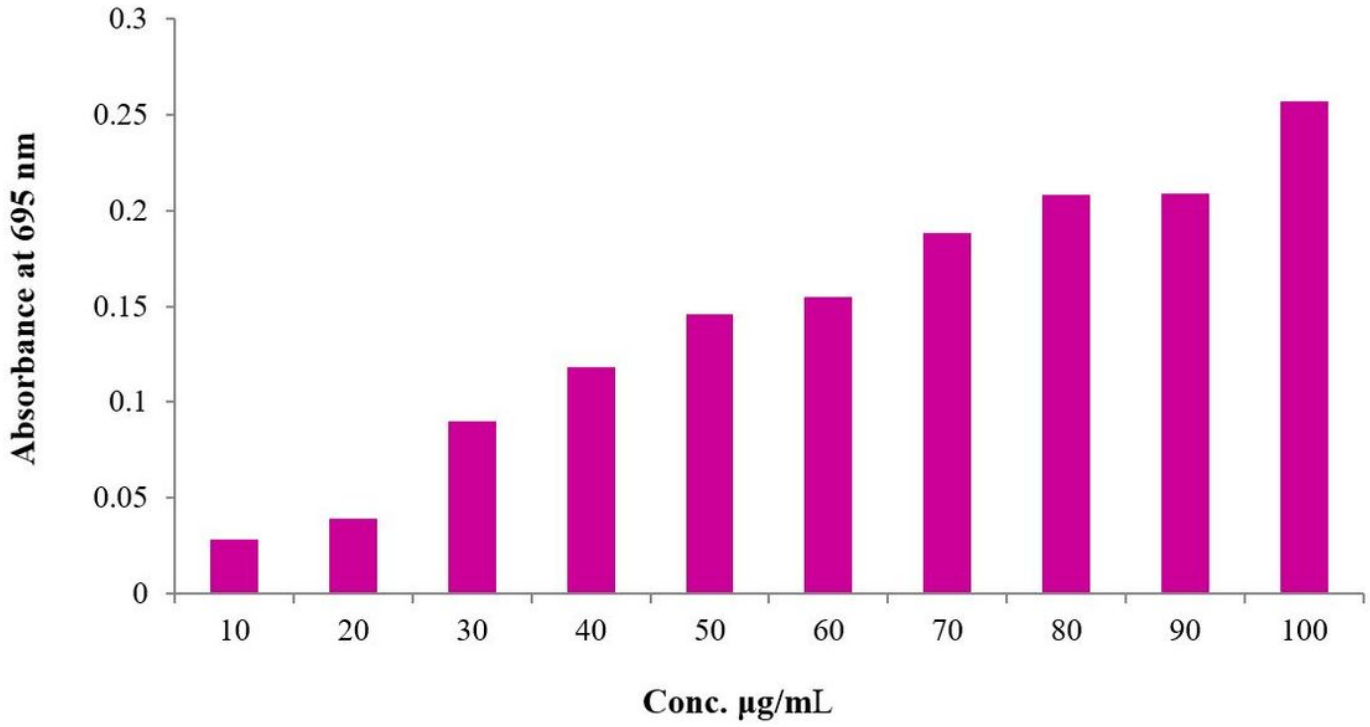
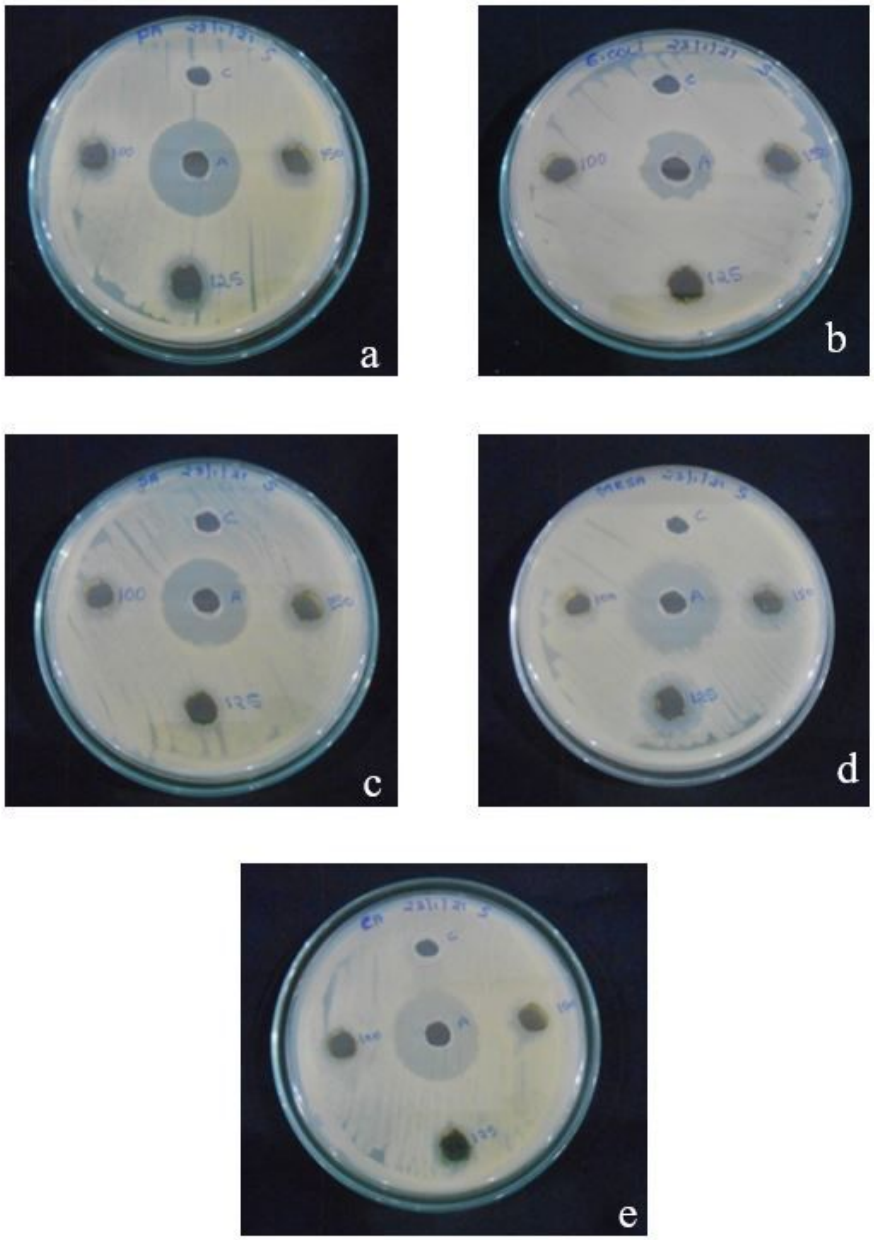


Figure 3

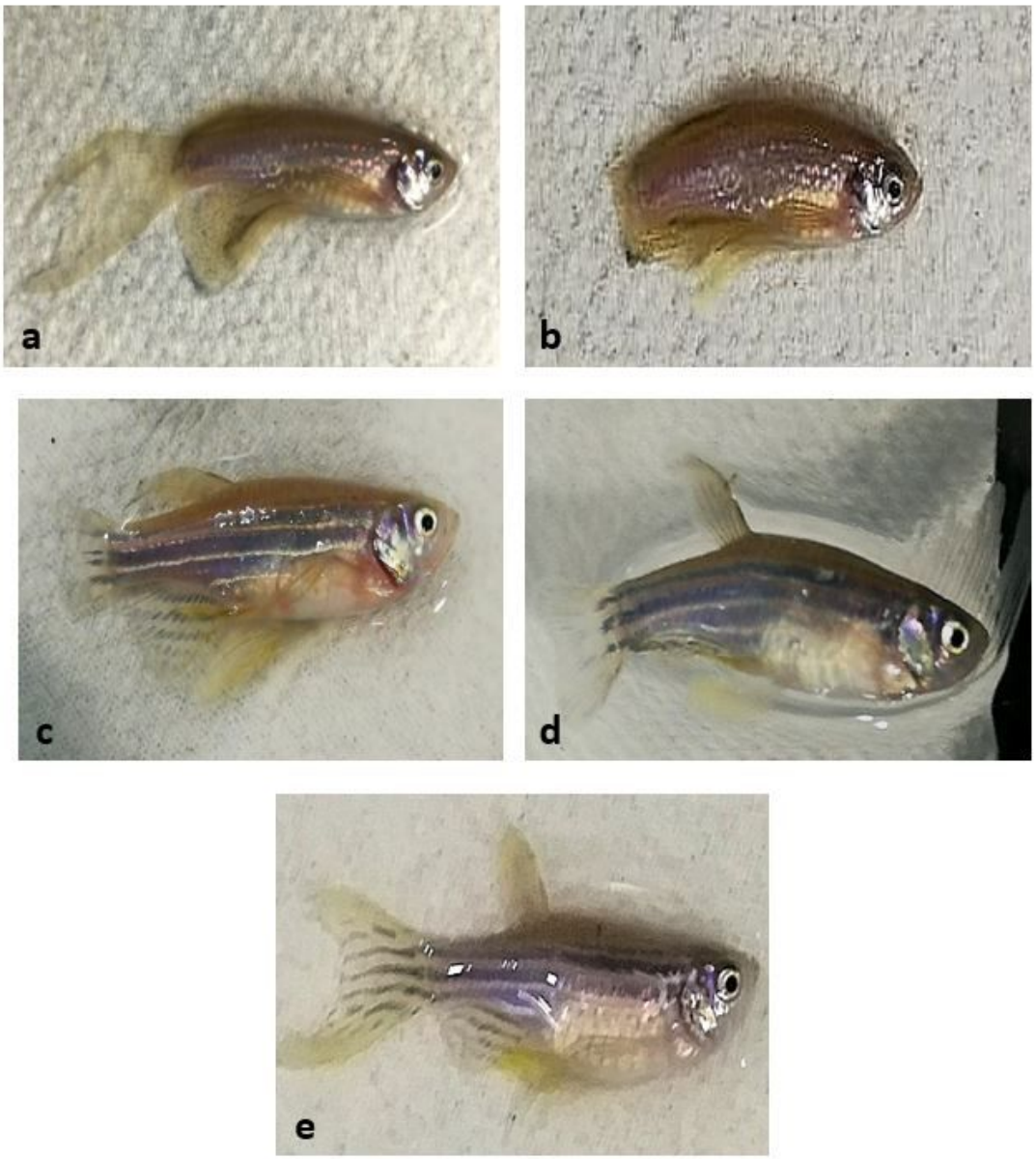
Graphical Representation of Total antioxidant assay of ethanol extract of T. ornata



a. *Pseudomonas aeuruginosa*
b. *Escherichia coli*
c. *Staphylococcus aureus*
d. Methicillin-resistant *Staphylococcus aureus*
e. *Candida albicans*

Figure 4

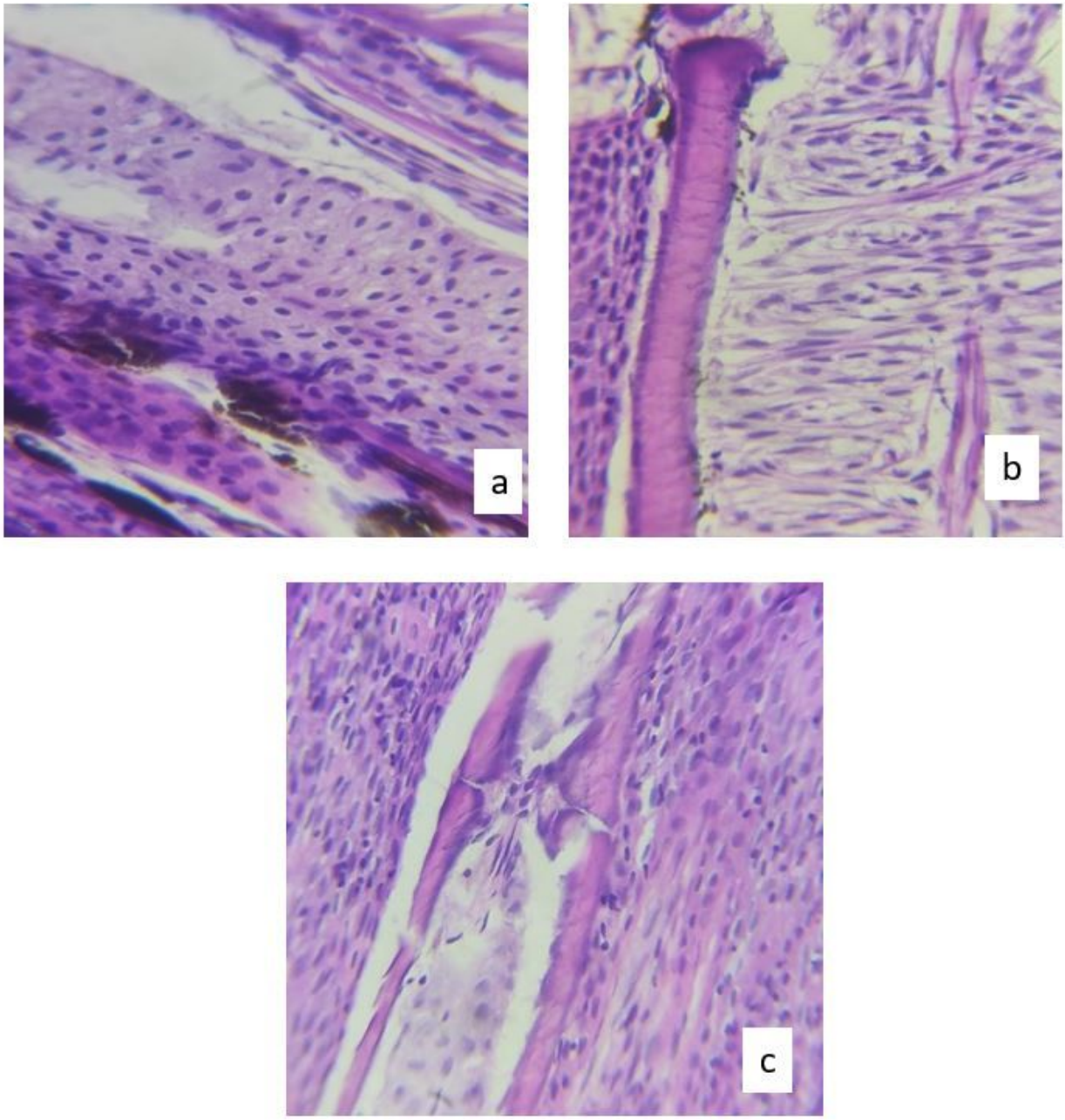
Antimicrobial Activity on Ethanolic Extract of Turbinaria ornata



a. Control – 0th day (Before tail cutting)
b. 3rd day
c. 7th day
d. 10th day
e. 14th day

Figure 5

Regeneration of Zebra fish Caudal Fin Treated with Ethanol Extract of Turbinaria ornata



a. Control
b. Control treated
c. *Turbinaria ornata* treated

Figure 6

Histochemical parameters of regenerative tissues of Zebrafish