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# IN VITRO ANTIOXIDANT EFFICACY OF *TURBINARIA CONOIDES*

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## ABSTRACT

Phytochemicals are active components found in marine Sea weeds that act against many diseases. Sea weeds of genus *Turbinaria* has been widely used by the traditional healers to cure many disease. *Turbinaria conoides* was used for study the phytochemicals. The extraction of phytochemicals from *Turbinaria conoides* were carried out with different solvent such as Water, Ethanol, methanol. The preliminary screening of *Turbinaria conoides* shows the presence of Alkaloids, Flavonoids, Carbohydrates, Phenolic Compounds, Proteins and Amino acids.It was found that Methanol shows better results than the other. The in vitro antioxident activity of *Turbinaria conoides* was measured by Total antioxidant activity method, DPPH radicals scavenging assay activity, superoxide anion scavenging activity, ion chelating activity and reducing power assay. The results revealed that the crude extract of the *Turbinaria conoides* has a wide range of antioxidant activity and it's suggested that it is useful in the treatment of infections caused by many microorganisms.

**KEYWORDS**: Phytochemicals, Antioxident, *Turbinaria conoides.*

## INTRODUCTION

Oxygen is essential for the survival of all on this earth. All living systems have evolved to survive in the presence of molecular oxygen. Oxidative property of oxygen plays a vital role in many biological phenomena. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals. Free radicals are reactive atom or group of atoms that has one

or more unpaired electrons which are highly reactive and unstable. They play an important role in the biological system. Humans are impacted by many free radicals both from inside and our surrounding environment, particularly reactive oxygen species (ROS).ROS can be generated by endogenous sources like metabolic pathways in cells which constitutes superoxide ($O_2$), hydroxyl ($OH^-$), nitric oxide radical ($NO^-$) and hydrogen peroxide ($H_2O_2$) and exogenous sources like other several pathways like ionizing radiation, UV light, cigarette smoke, industrial waste and pollutants. Faulkner, D.J.(2005)[1], Once formed these highly reactive radicals can start a chain reaction. Free radicals formed by these reactions are capable of attacking the healthy cells of body, causing them to lose their structure and function. Reactive oxygen species (ROS) and free radicals can only be eliminated efficiently under normal conditions but pathological conditions it induces various chronic diseases.

Antioxidants are substances that are capable of stabilizing or deactivating free radicals before they attack cells.[2] They are able to prevent or retard oxidation of lipids, proteins and DNA and to protect the compounds or tissues from damage caused by oxygen or free radicals. Naturally there is a dynamic balance between the amount of free radicals produced by the body and the antioxidant to scavenge or quench them to protect the body against deleterious effects. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. To overcome these problems a wide range of synthetic antioxidants. Several synthetic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) are commercially available and are currently in use.[3]

## MATERIALS AND METHODS

### Collection of sample

The brown algae, *Turbinaria conoides* (Fucales/phaeophyta) (T. conoides) was collected from the intertidal region of Mandapam coast (Lat. 09 ˚ 17'N; Long 79 ˚ 07'E) of Gulf of Manner, South east coast of India. Gulf of Mannar is a marine biosphere which harbour biodiversity of global significance and unique for coral reef, seaweed and sea grass ecosystems.

### Processing of Brown seaweeds

*Turbinaria conoides* grows by attaching to coral substratum. Algal sample was cleaned of epiphytes and extraneous matter and necrotic parts were removed. Plants were washed with

seawater and then in fresh water. The seaweeds were transported to the laboratory in sterile polythene bags at 20℃ temperature.

**Preparation of extract**

25gms of the powder of *Turbinaria conoides* was transferred into different conical flask (250ml).The conical flask containing 100ml of different solvents viz. ethanol, methanol and water. The conical flask containing plant powder and solvent was shacked it well for 48 hours by free hand. After 3 days, the extracts were filtered using Whatman filter paper No.1. and was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45$^{o}$C.The obtained extracts were stored at 4°c in air tight bottle until further use**.**

**Preliminary phytochemical screening**

The crude extraction of seaweed were analysed for the presence of various phytoconstituents by phytochemical tests.[6]

***In vitro* Antioxidant Activity of seaweeds**

The In vitro antioxidant activity of the sample was measured by the following preparation of extract and determination method.

**Preparation of extract**

Different concentrations of *Turbinaria conoides* (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

**DPPH radical-scavenging activity**

DPPH radical-scavenging activity was determined by the method of Shimada,[7]

**Determination of Total Antioxidant Capacity**

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method.

**Superoxide anion scavenging activity assay**

The superoxide anion radicals scavenging activity was measured by the method of Liu.[8]

**$Fe^{2+}$ chelating activity assay**

The chelating activity of the extracts for ferrous ions $Fe^{2+}$ was measured according to the method of Dinis.[9]

**Reducing power assay**

The $Fe^{3+}$ reducing power of the extract was determined by the method of Oyaizu.[10]

## RESULTS

**Preliminary screening of Phytochemicals**

In this study, we found that polar and non-polar solvents can extracts the phytochemicals present in the *Turbinaria Conoides*, the preliminary screening of the phytochemicals reveals the presence of flavonoides, phenolic compounds, tannins, saponins, carbohydrates and oil compounds. The various part of the plant contains the different types of the phytochemicals. The whole algae contain flavonoides, phenolic compounds, tannins, sopanins at notable level. The various phytochemicals present in the dirrerent extracts of *Turbinaria conoids* are presented in Table 1. in our present study we found that aqueous ethanol and methanol extracts of this seaweeds contains important phytochemicals such as.

**Table 1. Phytochemical screening of different extract of algae**

| Secondary Metabolites | Ethanol | Methanol | Water |
|---|---|---|---|
| Tannin | ++ | + | ++ |
| Phslobatannins | _ | _ | _ |
| Saponin | + | ++ | ++ |
| Flavonoids | + | ++ | + |
| Steroids | + | ++ | ++ |
| Terpenoids | + | + | + |
| Triterpenoids | + | + | ++ |
| Alkaloids | _ | + | _ |
| Carbohydrate | + | + | + |
| Amino acid | _ | + | + |
| Anthroquinone | + | ++ | ++ |
| Polyphenol | _ | ++ | + |
| Glycoside | + | + | + |

(+) Presence: (-)Absence (++) High concentration

**Antioxidant properties of seaweed polyphenols**

The antioxidant activity of the *Turbinaria conoides* was measured by the following methods by the different concentration of sample.

**(a) Total antioxidant activity**

The total antioxidant activity capacity of *Turbinaria conoides* was measured by phosphomolybdenum method and the results were presented in Table 2 and Fig.1.The

antioxidant activities increase with increasing concentration of the sample (at the concentration of 80µg/ml.)

**Table 2. Percentage of Total antioxidant activity of mehanolic extract of *Turbinaria conoides* at different concentrations.**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| **Total antioxidant** | 25.64 ± 4.45 | 47.19 ± 4.63 | 70.19 ± 5.61 | 89.68 ± 6.37 | 42.62 |
| **Standard (Ascorbic acid)** | 22.35± 1.80 | 51.23± 4.09 | 72.54± 5.80 | 86.35± 6.91 | 42.41 |

Values were expressed as Mean ± SD for triplicates

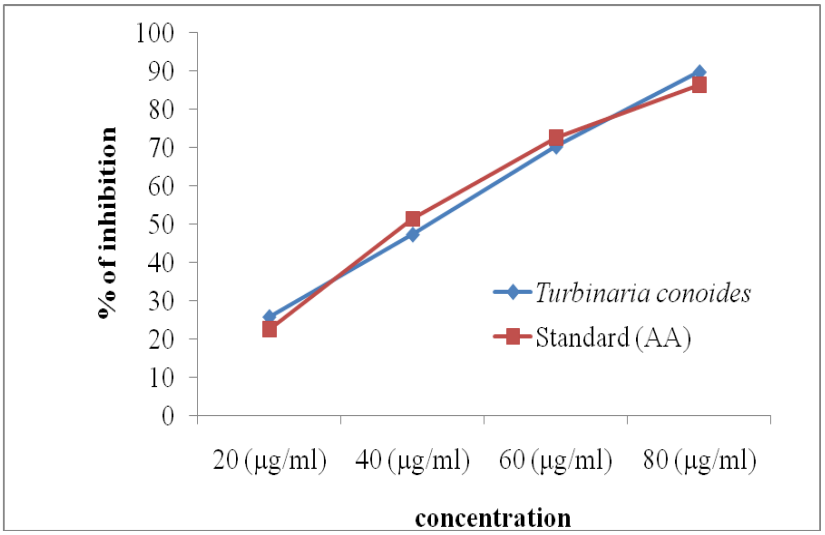


**Fig .1 Total antioxidant assay of *Turbinaria conoides***

**(b) DPPH radical scavenging activity**

The effect of seaweed extracts and standard on DPPH radical was compared and shown in Table 3 & Fig.2. The scavenging effect increases with the concentration of standard and samples (at 80 µg/ml concentration,) *Turbinaria conoides* possessed (99.34±7.94) % scavenging activity on DPPH.

**Table. 3.Percentage of DPPH Radical scavenging activity of methanolic extract of *Turbinaria conoides* at different concentration**

| Table 1-% of parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| DPPH | 23.56 ± 1.83 | 36.25 ± 2.87 | 67.89 ± 5.29 | 87.45 ± 6.82 | 46.63 |
| Standard (Ascorbic acid) | 25.6±2.04 | 61.26±4.90 | 88.98±7.11 | 99.34±7.94 | 35.03 |

Values were expressed as Mean ± SD for triplicates

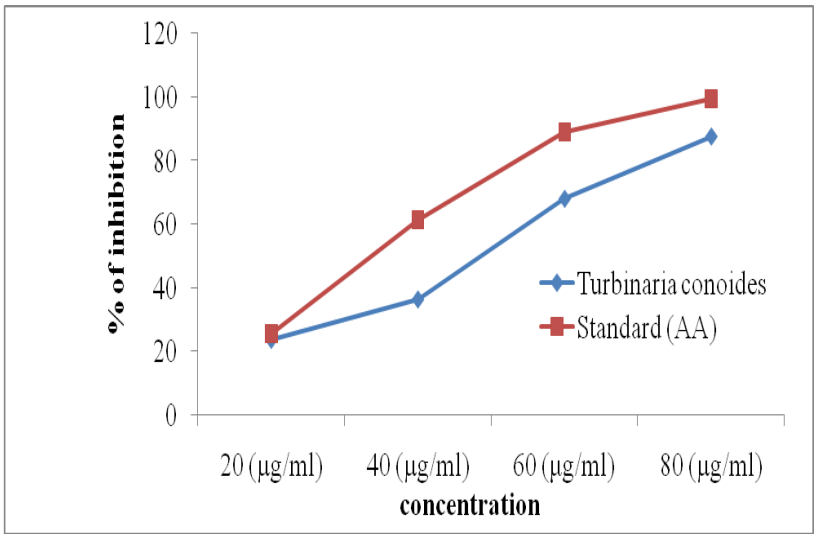


**Fig .2. DPPH radical-scavenging activity**

**(c)Superoxide anion radical scavenging activity**

The seaweed extracts were subjected to be superoxide scavenging assay and the results were shown in Table.4 & Fig.3 It indicates that *T.conoides* (80 µg/ml) exhibited the maximum superoxide scavenging activity of (87.04±6.69).

**Table 4. percentage of Superoxide Radical scavengig activity of methanolic extract of *Turbinaria conoides* at different concentration**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| Superoxide | 28.32 ± 2.20 | 47.59 ± 3.71 | 65.56 ± 5.11 | 87.04 ± 6.69 | 42.69 |
| Standard (Ascorbic acid) | 31.25 ± 2.50 | 64.23 ± 5.13 | 89.54 ± 7.16 | 98.51 ± 7.88 | 31.62 |

Values were expressed as Mean ± SD for triplicates

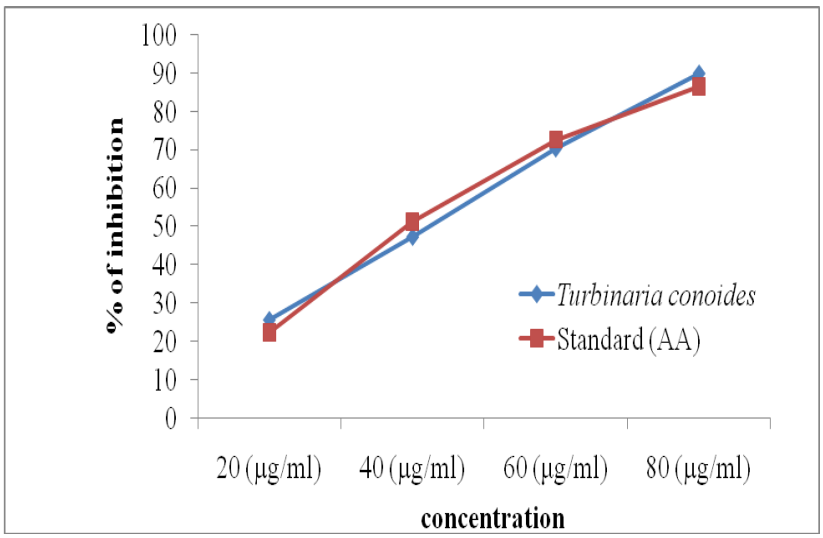


**Fig . 3. Super oxide scavenging activity of *Turbinaria conoides***

**Iron chelating activity**

The chelating activity of the extracts for ferrous ions $Fe2^{+}$ was shown in fig.It indicates that *Turbinaria conoides* (80µg/ml) exhibited the maximum iron chelating activity of (84.25±6.4)

**Table 5. percentage of Iron chelating activity of mehanolic extract of *Turbinaria conoides* at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| $Fe^{2+}$chelating activity | 23.54 ± 1.6 | 48.16 ± 3.73 | 72.86 ± 5.6 | 84.25 ± 6.4 | 43.04 |
| Standard (Ascorbic acid) | 35.23 ± 2.81 | 65.21 ± 5.28 | 78.51± 6.28 | 98.65 ± 7.89 | 30.96 |

Values were expressed as Mean ± SD for triplicates

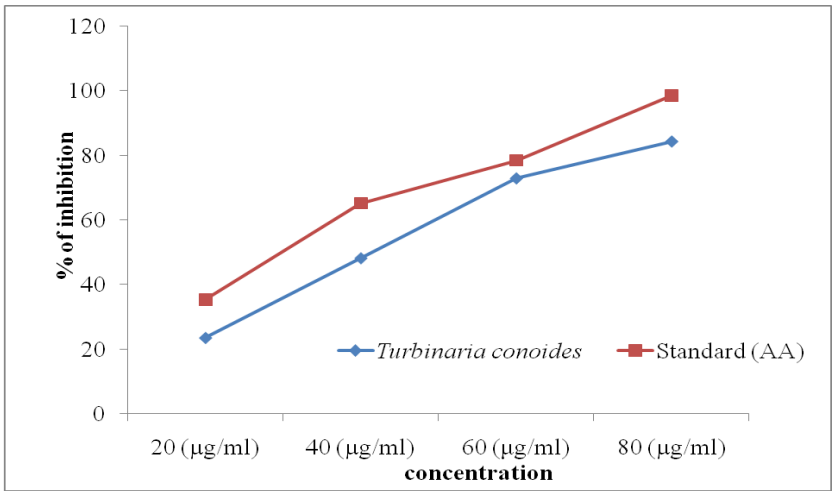


**Fig.4. Ferrous iron chelating activity of *Turbinaria conoides***

**(e) Reducing power**

The reducing power of T*urbinaria.Conoides* was compared with the standard gallic acid. The reducing power of the sample was shown in Fig.5.

**Table.6.Reducing power assay of mehanolic extract of *Turbinaria conoides* at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) |
|---|---|---|---|---|
| Reducing power assay | 0.28±0.03 | 0.57 ± 0.04 | 0.73 ± 0.05 | 0.87 ± 0.064 |
| Standard (Ascorbic acid) | 0.41± 0.03 | 0.71 ± 0.05 | 0.89± 0.07 | 0.98 ± 0.08 |

Values were expressed as Mean ± SD (Optical density) for triplicates

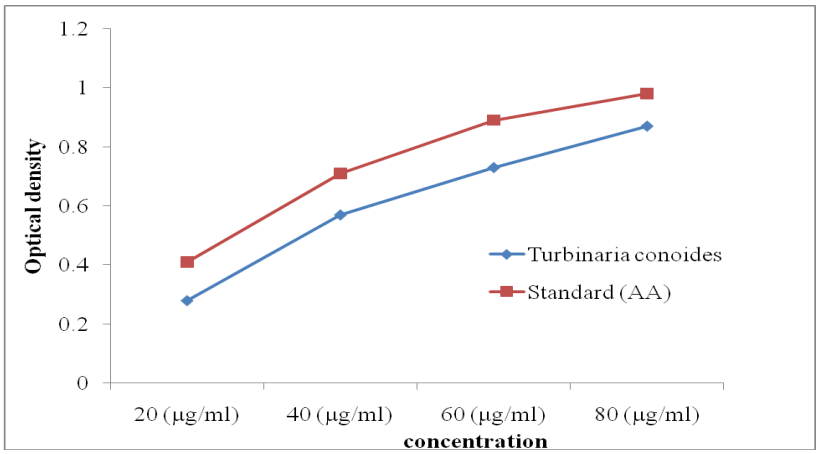


**Fig.5.Reducing power assay of *Turbinaria conoides***

## DISCUSSION

The antioxidant activitiy of several naturally occurring compounds have been known for decades. Recently, many types of seaweed have been considered as source of reactive oxygen species inhibitors. They can be used as food additives and can also provide protection against tissue oxidation. The present investigation has also proved that seaweed Polyphenols (T. conoides) possess antioxidant activity to scavenge free radicals. Dietary natural antioxidants are reported to help in preventing aging and other diseases. There are some evidences that seaweeds contain compounds with a relatively high antioxidant and antiproliferative activity. Seaweeds are low in fat but contain vitamins and bioactive compounds like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants.

Antioxidant compounds scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress related diseases. Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring antioxidants. A great number of naturally occurring substances like seaweeds have been recognized to have antioxidant abilities.[11]

Total phenol content by Folin Ciocalteau Reagent and in vitro antioxidant capacity assays, such as the DPPH, ABTS and free radical scavenging assays (which were used in this study), represent convenient methods for the identification of potential sources of antioxidant compounds. As already mentioned, seaweed polyphenols have significant potential health benefits; they may protect cell constituents against oxidative damage and therefore limit the

risk of various degenerative diseases associated to oxidative stress such as cancer, cardiovascular disease and osteoporosis. However, the value of in vitro antioxidant capacity assays for assessing the health-related implications of a food extract has been limited for a number of reasons, mainly due to the lack of standardization amongst these methods, the changes in the antioxidant activity of Polyphenols. Nevertheless, this does not exclude antioxidant properties from being one of the key parameters in determining their biological effects.

The total phenolic content of *T. conoides* was of 43.72 mg 1.63 mg gallic acid equivalents/g extract.

In the present work, DPPH, $OH^-$, NO., $O_2$.- free radical assay systems were successfully used for the evaluation on the antioxidant activity of the crude extracts derived from *Turbinaria conoides*. 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) is stable nitrogen centered free radical which can be effectively scavenged by antioxidants. Hence it has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts relative to other methods.

The present investigation has shown that both the seaweed extracts being T. conoides which exhibited significantly higher DPPH scavenging activity [(84 ± 1.78) % inhibition] when compared with the highest concentration of standard Absorbic acid [(43.39 ± 1.31) % inhibition]. The result is indicative of the hydrogen donating ability of *T. conoides.*

The results show that Turbinaria conoides has superoxide scavenging activity which can be of potential health interest as it may be effective in reducing the level of $O_2^{\cdot -}$ which is elevated during oxidative stress in the body. $O^{2-}$ mediated oxidative stress is believed to be involved in the pathogenesis of cardiovascular disorders, diabetes mellitus, acute respiratory distress syndrome, neurodegenerative disorders like Alzheimer's and Parkinson's diseases. The methanolic extract of *Turbinaria conoides* was higher than the standard absorbic acid Phenol, 2-[(1-phenylethyl)thio] represent a diverse group of pigment, widely distributed in nature. They serve as accessory pigments to harvest light for photosynthesis.[13] Moreover these types of pigments can give rise to rich in polyphenol compounds. Norisoprenoids resulting from the oxidative cleavage of carotenoids are signals in algae development, serve as antifungal and antibacterial agents and contribute to their flavor and aroma. Some other

Polyphenols such as ionone, dihyro actinidiode, 2, 3-Epoxy ionone and carotene have been reported as the most common marine seaweed norisoprenoids.

It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors and free radical quenchers and phenolics may act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals. It is possible that the antioxidant activity of T.conoides can be the result of their high concentration of phenolic compounds.

## CONCLUSION

On the basis of results in this study, it can be concluded that the methanolic extracts of *T.conoides* is capable of scavenging a wide range of synthetic and naturally occurring free radicals. Our study also demonstrated the appriciable invitro anti oxidant efficaxy of t. conides, which coud be used as a natural antioxidant in food supliments and pharmaceutical industry.

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