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In vitro antioxidant activity of the Methanolic Extract of *Oscillatoria terebriformis* C.A. Agardh *ex* Gomont

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Abstract

In this study, the antioxidant activity of methanolic extracts of *Oscillatoria terebriformis* was studied using various *in vitro* assays. The antioxidant activity of MEGA was evaluated by using the free radical scavenging activity assay (DPPH method) Total Antioxidant capacity, Ferric Reducing antioxidant Power Assay, nitric oxide scavenging activity, Thiobarbituric Acid Reactive Assay (TBARS) and H_2O_2 scavenging activity. Total phenolic content was determined by using gallic acid as a standard. The results of the study show that *Oscillatoria terebriformis* possesses significant free radical scavenging properties and a clear correlation exists between the antioxidant activity and phenolic content.

Keywords: Phenolic, *Flavonoids*, Tannin, Free radicals, LPO, DPPH, HRSA, TBARS, MDA, FRAP, *Oscillatoria terebriformis.*

Introduction

With an increasing number of bacteria, fungi and viruses developing resistance to commercial antibiotic and its derivatives, the cyanobacteria hold great promise for novel medicines in modern times as they are found to be rich source of structurally novel and biologically active metabolites (Namikoshi *et al* 1996, Singh *et al* 2005). Secondary or primary metabolites produced by these micro-organisms may even prove to be potential bioactive compounds of interest in the pharmaceutical industry (Tuney *et al* 2006, Febles *et al* 1995, Ely *et al* 2004).

These include antibacterial compounds which in laboratory tests inhibited bacteria that are responsible for many deadly diseases in humans (Kulik *et al* 1995). Apart from the threats imposed by the microorganisms, cancer and cardiovascular as chronic diseases are among the leading causes of death in the world, where oxidative stress induced by reactive oxygen species (ROS) is one of the foci related to these diseases. Oxidative stress is initiated by ROS, which are highly reactive oxidant molecules that seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Hazra.*et al* 2008). These free radicals are endogenously generated through regular metabolic activities, lifestyle, and diet. Hence, there is strong evidence that this damage may contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Hazra *et al* 2009)

The presence of certain substances acting as antioxidant or free radical scavenger may protect the body from the consequences of oxidative stress.

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage-free radicals might otherwise cause .Antioxidant acts as cell protectors. Oxygen, an essential element for life, can create damaging by, by-products during normal cellular metabolism. Antioxidants counteract these cellular by-products during normal cellular metabolism. Antioxidants counteract these cellular by-products called free radicals, and blind with them before they can cause damage. If left unchecked, free radicals may cause heart damage, cancer, cataracts, and a weak immunes system. Antioxidants come in a variety of forms include vitamin C, vitamin E, Carotenoids. Thus, antioxidants play an important role in the protection of cells against oxidative damage caused by ROS (Khan *et al*.2005). The occurrence of many compounds

possessing antioxidant activity in biological systems in higher plants is well documented, while in micro algae little information is available (Colla, et a1 .2007)
An attempt has been made to screen the cyanobacterial extracts for their bioactive potential as they appear to be largely unexplored and represent a rich opportunity in the search of novel compound of pharmacological.

The aim of the present study was to evaluate antioxidant properties of methanolic extract of *Oscillatoria terebriformis*

Materials and Methods

Culturing and growth of algal organisms

Oscillatoria terebriformis, a thermophilic cyanobacterium were obtained from the culture collection of Phycospectrum Environmental Research Centre (PERC), Chennai. Biomass was obtained by growing algal cultures in 20L of water and 0.25g / L of NPK fertilizer was added with a facility to pump the culture with aeration pump. The algae was grown for 20 days and harvested.

Preparation of Algal extract:

0.5g of dried algal material was extracted in 20ml of methanol kept in an orbital shaker for overnight as described by **(**Sivasubramanian et.al.2011). The obtained extracts were filtered with Whatman no.1 filter paper and the filtrate was collected. The solvents were removed under reduced pressure at 50°C to yield a concentrated extract (15%).

Quantitative analysis of antioxidative compounds

Estimation of Total phenolic compounds: Total phenolic content was determined with Folin & Ciocalteau reagent according to the method described by Malick and Singh, *et al* (1980).

Estimation of Total Flavonoids:

Total Flavonoids content was determined by the method described by Jia *et. al* (1999).

Estimation of Tannin:

Tannin content was determined by the method described by Sadasivam and Manickam et. *al. (*1992).

Antioxidant activities assays

DPPH Radical Scavenging Assay: Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Shimada *et al* (1992).various concentrations of methanol extract of the sample (4.0 ml) was mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM.The Mixture was shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517 nm. BHA was used as control .The Percentage of DPPH decolorization of the sample was calculated according to the equation:

Scavenging activity (%) = [Abs (control) – Abs (standard)] ×100.

Where, Abs (control): Absorbance of DPPH radical + methanol
Abs (standard): Absorbance of DPPH radical +extract/standard.

Total Antioxidant capacity (TAC)

To 0.1ml of varying concentration of extract, 1ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and incubated at 95 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.(Prieto *et.al* 1999*).*

Ferric Reducing antioxidant Power Assay: Ferric Reducing antioxidant Power Assay was determined by the method described by Benzene and strain, (1996).

Hydrogen peroxide scavenging assay:

Hydrogen peroxide scavenging strengths of extracts were determined the method described by Ruch *et al*. (1984). A solution of H2O2 (10mM) was prepared in phosphate buffer (pH 7.4). Reaction mixtures contained 10mM of H2O2 and different concentrations of test samples, and absorbance values were measured at 10 min and after 60 min using wavelength of 240nm. Ascorbic acid was used as the standard.

Thiobarbituric Acid Reactive Assay (TBARS) The assay was performed as described by Halliwell and Gutteridge *et al* (1999) in which the extent of LPO was estimated from the concentration of Malondialdehyde,, a thiobarbituric acid reacting substance which is produced due to lipid peroxidation. Various concentrations of the algal extract were taken in a test tubes and were evaporated to dryness at 80°C .1 ml of 0.15M potassium chloride was added to the tubes and followed by 0.5ml of goat liver homogenate (10% W/V in Phosphate buffered saline; calcium magnesium free). Peroxidation was initiated by the addition of 100 µl of 2mM ferric chloride. After incubating the tubes for 30min at 37°C, the peroxidation reaction was stopped by adding 2ml of ice-cold HCL (0.25N) containing 15% TCA & 0.38%

ISSN: 2229- 6905

TBA. The tubes were kept at 80°C for 1 hr, cooled and centrifuged at 7500rpm. The absorbance of the supernatant, containing TBA-MDA complex was read at 532nm. The anti-lipid peroxidation activity (ALP %) was calculated using the formula: **ALP% = [Abs (control) - Abs (sample)] x 100 Abs (control).**

Nitric oxide radical scavenging activity: Scavenging activity of the extracts was tested by Nitric oxide radical scavenging activity as described by Govindarajan *et al* (2003). Various concentrations of the extract were mixed with 1.0 ml of 1mM sodium nitrite. Then the mixture was added to 8ml of 0.2 M citrate buffer, pH 4.2. The mixture was incubated for 1 hour at 37 *C.1.0 ml of the solution was withdrawn and added to 2.0 ml of 2% acetic acid and 0.4 ml of Griess reagent. The mixture was incubated at room temperature for 15 minutes and the absorbance was measured at 520 nm. Nitric oxide scavenged (%) = [Abs (control) – Abs (standard) / Abs (control)] × 100.

Results and Discussion

Phenolic Compounds:

Phenolic compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. Phenolic compounds may contribute directly to antioxidative action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans.

Total phenolic Flavonoid, Tannin contents in the methanolic extract were expressed as mg/g and were presented in **Figure 1**

FIGURE. 1. TOTAL PHENOLIC FLAVONOID AND TANNIN CONTENTS OF METHANOLIC EXTRACTS OF OSCILLATORIA TEREBRIFORMIS

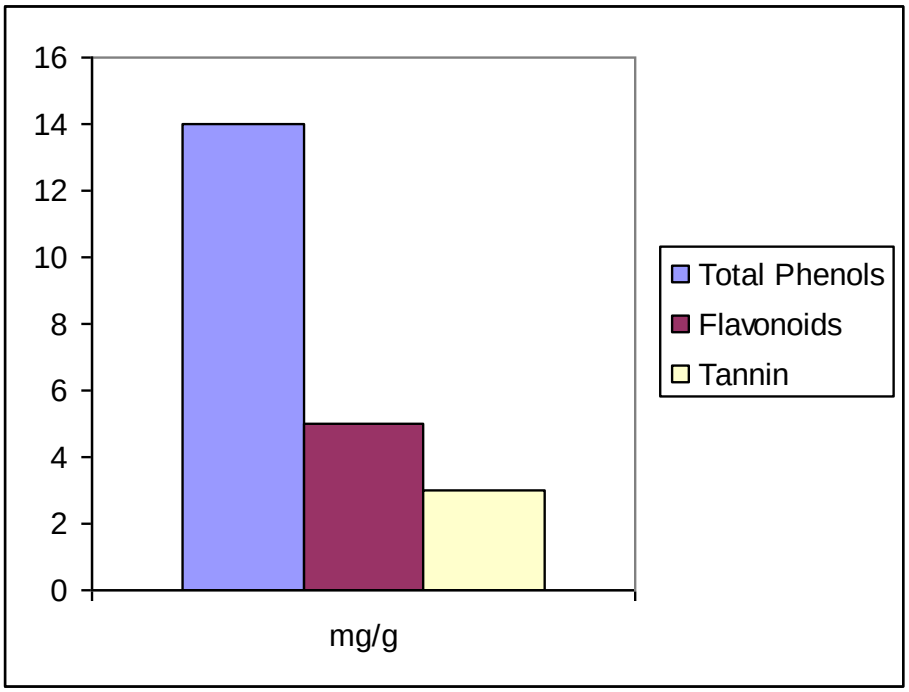

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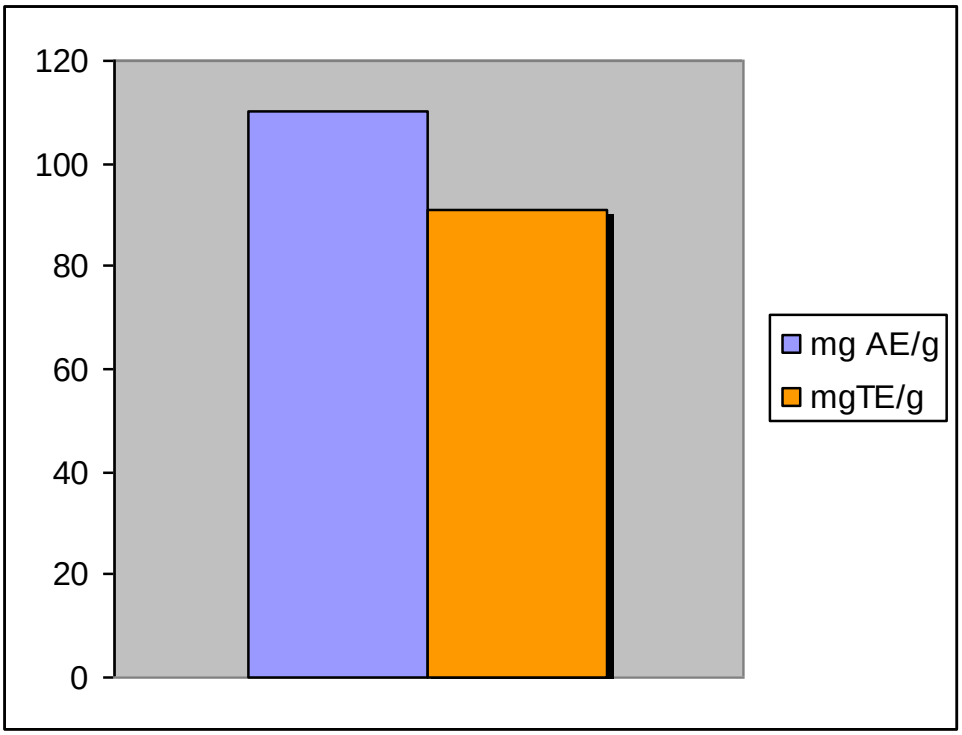
Figure 2: TOTAL ANTIOXIDANT CAPACITY BY PHOSPHOMOLYBDENUM ASSAY OF OSCILLATORIA TEREBRIFORMIS



Total Antioxidant capacity

Figure.2 shows the total antioxidant capacity, which was reported as ascorbic acid and trolox equivalents. It was found to be 111(mg AE /g) and 91.0 (mg TE/g) with 0.3 ml of the methanolic extract of cyanobacterial sample of Oscillatoria terebriformis by phosphomolybdate method. The method is based on the reduction of Mo (VI) – Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. Oscillatoria terebriformis extract showed strong total antioxidant capacity (Akter, Hasan Raquibul *et al*.2008).

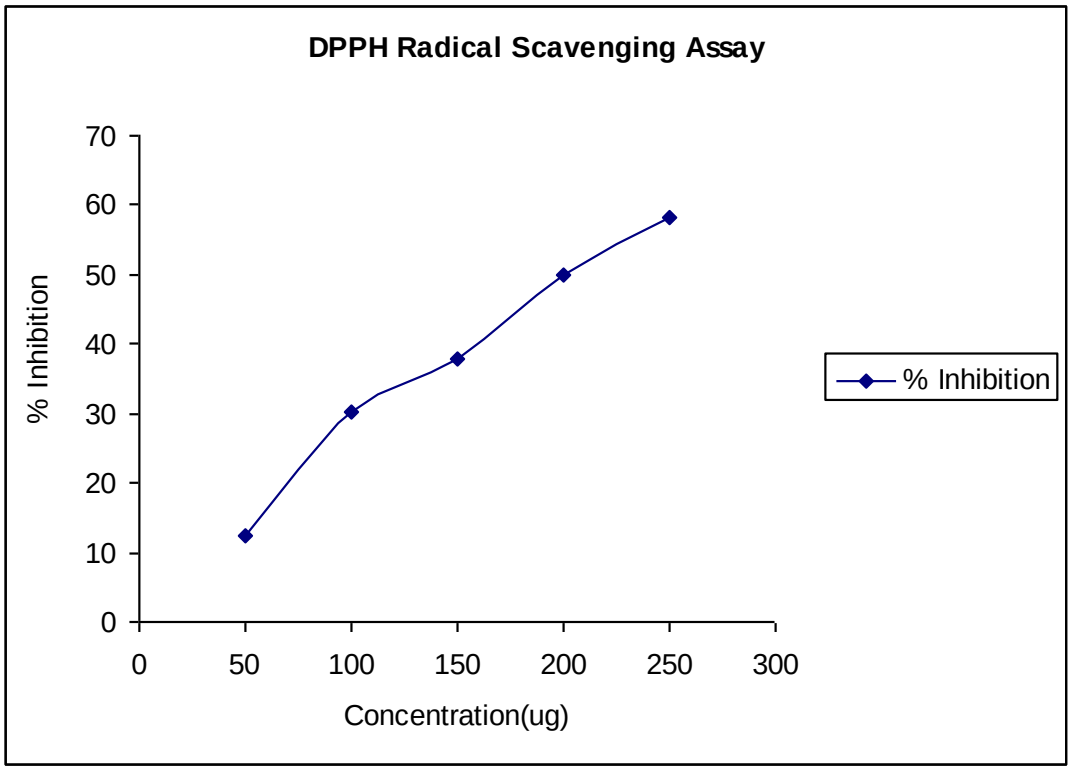
DPPH Radical scavenging assay:

Figure 3 shows the dose-response curve of DPPH radical scavenging activity of the Oscillatoria Terebriformis .At a concentration of 250 µg, the scavenging activity of methanol extract of *Oscillatoria terebriformis* was 58.1%.

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Shirwaikar, Prabhu.et*.al*.,2006). Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic acid at 100 µg/ ml, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

ISSN: 2229- 6905

FIGURE 3 .*DPPH RADICAL SCAVENGING ASSAY OF OSCILLATORIA TEREBRIFOMIS*



Hydrogen peroxide scavenging assay (HRSA):

Figure 4 shows the dose-response curve of *Hydrogen peroxide scavenging assay* activity of the Oscillatoria Terebriformis .At a concentration of 250 µg, the scavenging activity of methanol extract of *Oscillatoria terebriformis* was 60.1%. Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Shirwaikar et.al., 1987). Scavenging of H2O2 by extracts may be attributed to their phenolics, which can donate electrons to H2O2, thus neutralizing it to water. The extract was capable of scavenging hydrogen peroxide in a concentration-dependent manner.

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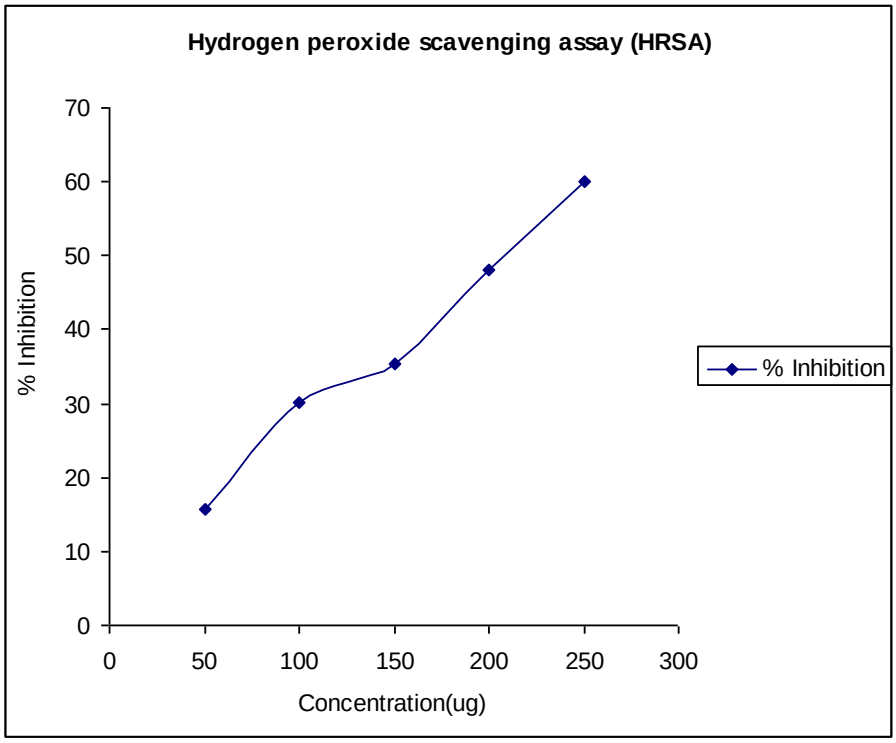
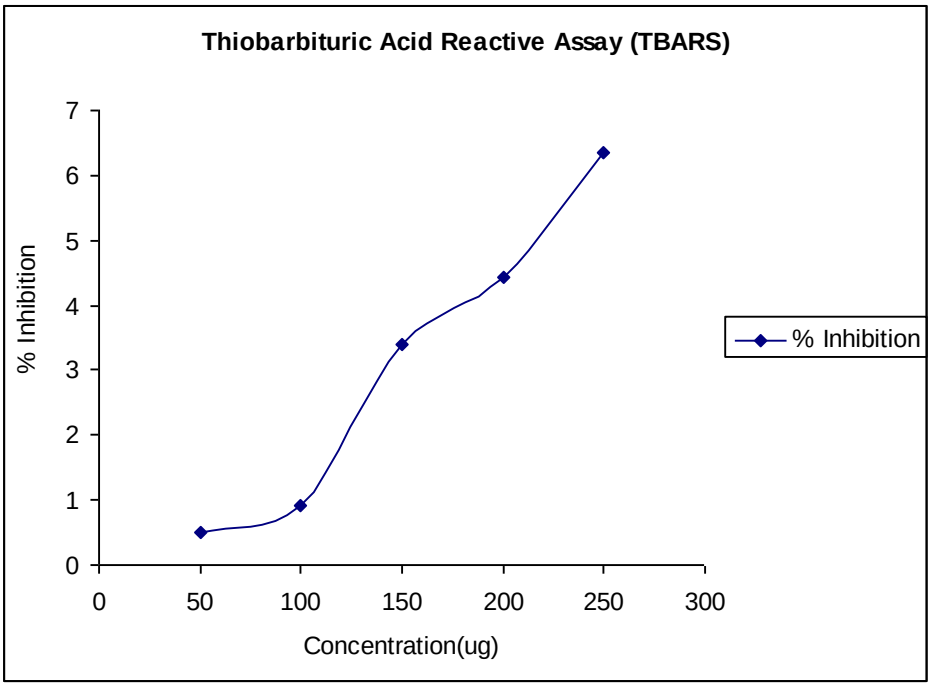
ISSN: 2229- 6905

FIGURE 4. HYDROGEN PEROXIDE SCAVENGING ASSAY OF OSCILLATORIA TEREBRIFOMIS



Thiobarbituric Acid Reactive Assay (TBARS)

FIGURE 5. THIOBARBITURIC ACID REACTIVE ASSAY OF OSCILLATORIA TEREBRIFORMIS



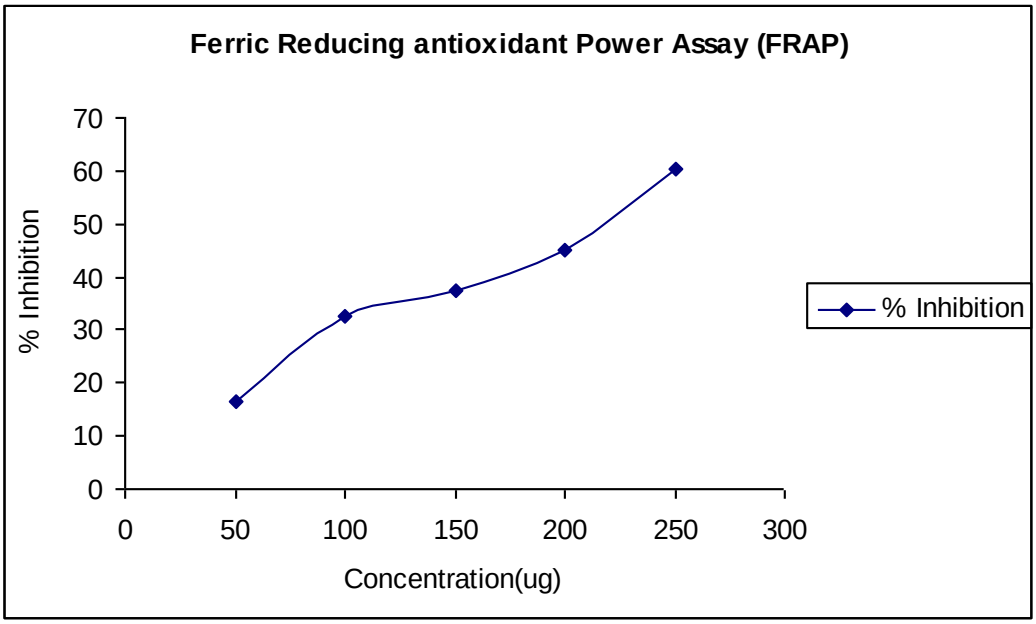
ISSN: 2229- 6905

Methanolic extract of Oscillatoria Terebriformis was capable of preventing the formation of MDA in a dose dependent manner. The extract is observed to be significantly better inhibitor of Lipid peroxidation. Figure 5 shows the ALP % potential of oscillatoria terebriformis.

Ferric Reducing antioxidant Power Assay
FRAP ASSAY

Methanolic extract of *Oscillatoria terebriformis* showed good ferric reducing antioxidant power (Figure 6) between 50 and 250 µg/ml. At a concentration of 250 µg, the scavenging activity of Oscillatoria *terebriformis* was 60.2%.

FIGURE 6. ***FERRIC REDUCING ANTIOXIDANT POWER ASSAY OF OSCILLATORIA TEREBRIFOMIS***



Nitric Oxide Scavenging Assay

From Figure 7, Methanolic extract of *Oscillatoria terebriformis* showed moderately good nitric oxide scavenging activity between 50 and 250 µg/ml. At a concentration of 250 µg, the scavenging activity of Oscillatoria *terebriformis* was 81.4 %.The percentages of inhibitions were increased with increasing concentration of the extracts. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological condition

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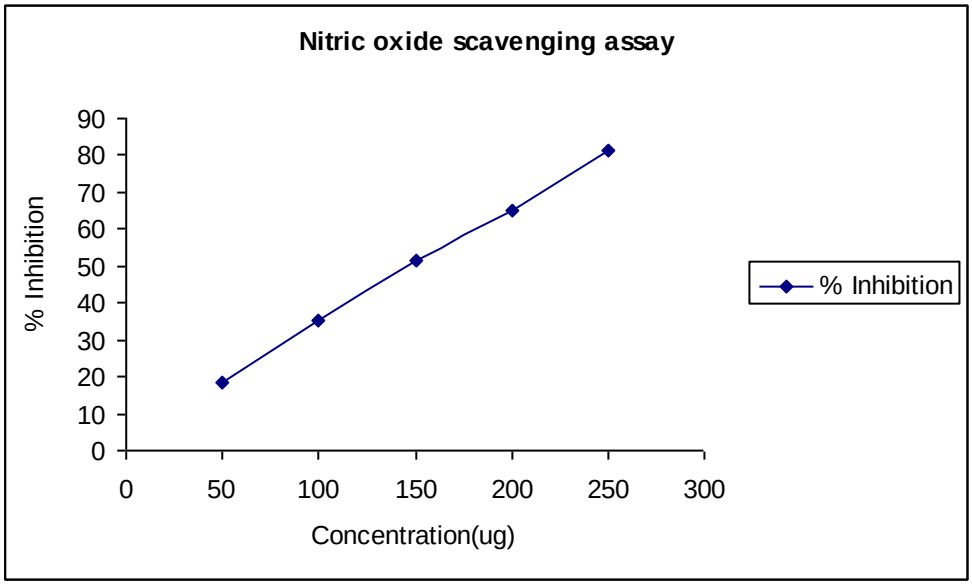
ISSN: 2229- 6905

FIGURE 7. NITRIC OXIDE SCAVENGING ASSAY OF OSCILLATORIA TEREBRIFOMIS



Conclusion

On the basis of the results obtained in the present study, it is concluded that a methanolic extract of *Oscillatoria terebriformis*, which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These in vitro assays indicate that this cyanobacterial extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activities are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the cyanobacterial extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

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