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Screening of Antioxidant Activity of *Oscillatoria annae* In Swiss Albino Mice

Vawhal Pallavi K*[1], R Rajavel[2], Mute Vaishali[1] , Awari Deorao[3]

[1]JSPM'S Jayawantrao Sawant College of Pharmacy& Research, Hadapsar, Pune .(M.S.) India - 411028
[2]Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode - 052, Tamilnadu, India
[3]Sitabai Thite College of Pharmacy, Shirur, Pune (M.S.), India

ABSTRACT

Ethanolic extract of Oscillatoria annae (EEOA) was studied for the antioxidant activity by in vitro and in vivo models. Pretreatment with EEOA (200, 400 mg/kg) enhanced the tissue superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST), glutathione reductase (GSH-R) levels. In addition it showed dose dependent free radical scavenging activity. The amount of total phenolic content were also determined in this study. The results obtained in the present study indicate that the EEOA can be a potential source of natural antioxidant activity.

Keywords: Ethanolic extract of Oscillatoria annae, In vitro activity, In vivo activity.

Corresponding author

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INTRODUCTION

Oxidation reactions are crucial for life, they can also be damaging; eg. glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells.

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms [1]. Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, are common members of the plankton of marine, brackish and freshwaters throughout the world. They also occure on rocks and soil and in symbiosis with plant and fungi. They have a simple structure at subcellular level and lack a nucleus, a characteristic feature defining them, along with bacteria, as prokaryotes [2]. Oscillatoria is a genus of filamentous cyanobacteria which is named for the oscillation in its movement. Filaments in the colonies can slide back and forth against each other until the whole mass is reoriented to its light source. It is commonly found in watering-troughs waters, and is mainly blue-green or brown-green. Oscillatoria is an organism that reproduces by fragmentation. Oscillatoria forms long filaments of cells which can break into fragments called hormogonia. The hormogonia can grow into a new, longer filament. Oscillatoria annae are a morphologically diverse group of oxygenic photosynthetic prokaryotes, which are phylogenetically closed related to each other and to chloroplasts. Oscillatoria annae include unicellular, colonial and filamentous forms some filamentous cyanophytes form differentiated cells called heterocyst, that are speciallized for hydrogen fixation, and resting or spore cells called aconites. Most of the bacteria found in the fresh water, while others are marine occur in damp soil, or even temporarily moistened rocks in deserts [3].

Furthermore, literature survey of Oscillatoria annae revealed that no researcher has yet rreported invivo and invitro antioxidant activity of this alga. Therefore it is worth conducting an investigation on the antioxidant ethanolic extract of O. annae (EEOA).

MATERIALS AND METHODS

MATERIALS

Oscillatoria Annae is an autotropic, filamentous organism. This strain was obtained from national facility for marine cyanobacteria (NFMC), Trichy, Tamilnadu, India.

PREPARATION OF EXTRACT

About 50 gm of powdered Oscillatoria annae was taken in a round bottom flask, 800 ml of ethanol was added and macerated for 7 days. During maceration the whole content was warmed two times a day at intervals. At the end of the 7 day the extract was filtered through muslin cloth while hot the extract was concentrated to a semisolid mass and dried in a desiccators. This extract has been used for various experimental purposes.

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PHYTOCHEMICAL SCREENING

A preliminary phytochemical screening of extract carried out and following constituents have been detected, terpenoid and steroids.

ANIMALS

The male Swiss albino mice weighing 20 – 25 g were used in this study. They were procured from KMCH Pharmacy college, Coimbtore. The animals were housed under 12 hrs day and night conditions for 2 to 3 months. The animals had free access to pellet and tap water ad libitum.

RESEARCH PROTOCOL APPROVAL

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (Registration No. NCP/IAEC/PG/2009) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

METHODS

In-vitro Activity:

Determination of total Phenolic Content

1ml of extract solution in a volumetric flask was diluted glass- distilled water (46ml). Folin- Ciocaltau reagent (1ml) was added and the contents of the flask were mixed thoroughly. After 3 min. 3ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the ethanolic extract, determined as microgrammes of pyrocatechol equivalents, by using an equation that was obtained from the standard pyrocatechol graph, is given as:

$$\text{Absorbance} = 0.00246\ \mu g \text{ pyrocatechol} + 0.00325\ X\ R^2\ (R^2 = 0.9996)$$

DPPH Assay

The hydrogen atom or electron donating abilities of the resultant compounds and some untainted compounds was measured from the bleaching of the purple – colored methanol solution of 2,2 –diphenyl- 1- picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical, DPPH as a reagent. One thousand microlitre of diverse concentrations of the extracts in ethanol were added to 4ml of 0.004% methanol solution of DPPH. After a 30 min. incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (1%) was calculated in following way:

$$1\% = (A_{blank} - A_{sample} / A_{blank}) \times 100,$$

Where, A_{blank} is the absorbance of control reaction and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of extract.

Determination of nitric oxide (NO) radical scavenging activity

This procedure is based on the principle that sodium nitropruside in aqueous solution at physiological pH instinctively generates nitric oxide which interacts with oxygen to produce nitric ions that can be anticipated using Greiss reagent. Scavengers of nitric oxide contend with oxygen leading to rediced production of nitrite ions. For the experimentation sodium nitroprusside (10mM) in phosphate buffered saline was diverse with different concentrations of extract dissolved in suitable solvent and incubated at room temperature for 150 min. The same reaction mixture without the methanolic extract of sample but with equivalent amount of methanol served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 1% N –(1 - naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Curcumin was used as positive control. The NO radical scavenging activity is calculated as

$$1\% = (A_{blank} - A_{sample} / A_{blank}) \times 100,$$

Where, A_{blank} is the absorbance of control and A_{sample} is the absorbance of the test compound.

Determination of hydroxyl radical (OH) scavenging activity

Stock solution of EDTA (1mM), $FeCl_3$ (10mM), ascorbic acid (1mM), H_2O_2 (10 mM) and deoxyribose (10 mM) was prepared by adding de- ionized water. The attempt was performed by adding up 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml deoxyribose, 1 ml of sample extract (10- 100 g / ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid added. The mixture was incubated at 37^{o}C for 1 hr. A 1 ml of incubated mixture was mixed with 1 ml of 10% trichloro acetic acid and 1 ml of 0.5 % thiobarbituric acid (in 0.025M NaOH containing 0.025% BHA) to urbanized the pink color measured at 532 nm. The hydroxyl radical scavenging activity is reported as percent inhibition of deoxyribose sugar dilapidation and is calculated as

$$1\% = (A_{blank} - A_{sample} / A_{blank}) \times 100,$$

Where, A_{blank} is the absorbance of control and A_{sample} is the absorbance of the test compound.

STATISTICAL ANALYSIS

The results were subjected to the statistical analysis by using ANOVA followed by Dunnett's test. $P< 0.01$ was considered significant.

***In-Vivo* Activity:**

The adult male Swiss albino mice (20-25 g) were divided into five groups of five animals each. Group I and group II were received only vehicle orally for seven days. Group III and Group IV animals were treated with ethanolic extract of *Oscollatoria Annae* at a dose of 200mg/kg/day, 400mg/kg/day, respectively, orally for seven days and Group V animals were treated with silymarin (25mg/kg per day) orally for seven days. On the seventh day to the animals of Group II to Group V, single dose of equal mixture of carbon tetrachloride and olive oil (50% v/v, 5 ml/kg) was administered intraperitoneally. All animals were sacrificed by cervical decapitation under light ether anesthesia on the eighth day. Immediately after sacrifice, the liver were dissect out, wash in the ice-cold saline, and the homogenate was prepared in 0.1M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of various enzymes. The superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were assayed by the method of Rotruck et al. Glutathione-S-transferase (GST), glutathione reductase (GSH-R) were measured by using the method of James et al [4-13].

STATISTICAL ANALYSIS

The results were subjected to the statistical analysis by using ANOVA followed by Dunnett's test. $P< 0.05$ was considered significant.

RESULTS

Preliminary phytochemical studies revealed the presence of carbohydrates, proteins, steroids, terpenoids and phenolic compound.

The antioxidant activity of EEOA in five in vivo models was studied. EEOA demonstrated antioxidant property in all models. However, EEOA showed better reducing property than the standard i.e. silymarin. Pretreatment with EEOA (200, 400 mg/kg) enhanced the tissue SOD, CAT, GSH, GPx, GST levels (see Table 1 and fig.1 to fig.5)

The antioxidant activity of EEOA in four in vitro models was studied. EEOA demonstrated antioxidant property in all the models. However, EEOA showed better reducing power than the standard. (see table 2)

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Table1: In vivo antioxidant effect of Ethanolic extract of *Oscillatoria annae*.

Group	SOD	CAT	GSH	GPx	GST
Ctrl Gp.	8.36± 0.88	42.46± 3.30	3.60± 0.74	9.51± 1.80	5.07± 1.09
Toxin Cntl. Gp.	4.55± 1.06	22.02± 1.56	2.013± 0.61	5.03± 1.46	2.75± 1.48
Std Gp.	7.90± 1.22	38.81± 1.26	3.095± 0.77	8.9± 1.00	4.85± 1.54
EEOA (200 mg/kg)	8.11± 1.11	36.53± 3.43	2.58± 0.56	7.94± 0.93	4.31± 1.44
EEOA (400 mg/kg)	11.35± 0.87	44.95± 3.39	3.48± 0.53	9.09± 0.16	5.01± 0.95

Values are expressed as Mean ± SEM, of 5 observations, statistical comparison as follows: Significant at*P < 0.05 ,compared to control group

Fig. 1:- Effect of Ethanolic extract of *Oscillatoria annea* on Superoxide Dismutase:

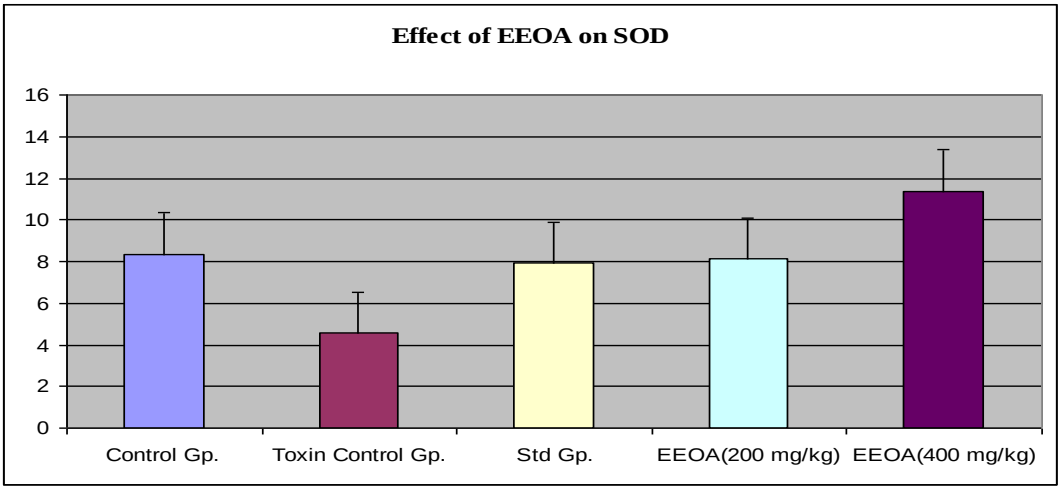

Fig. 2:- Effect of Ethanolic extract of *Oscillatoria annea* on Catalase:

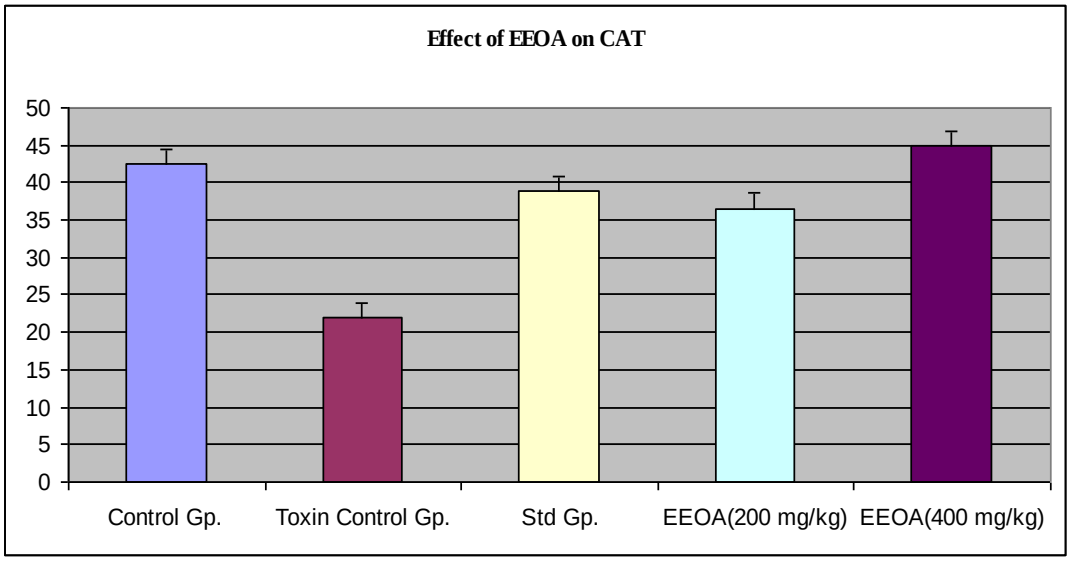

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Fig. 3:- Effect of Ethanolic extract of *Oscillatoria annea* on Glutathione Reductase:

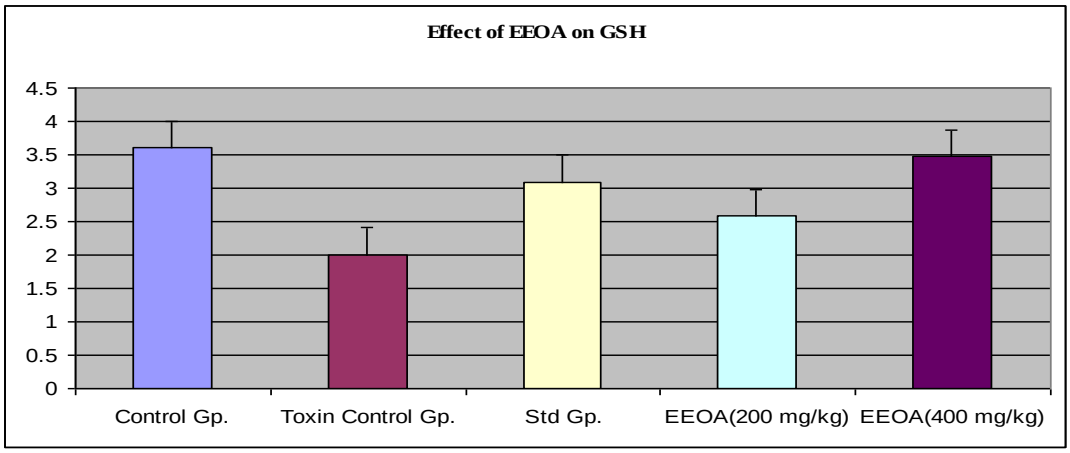

Fig. 4:- Effect of Ethanolic extract of *Oscillatoria annea* on Glutathione Peroxidase:

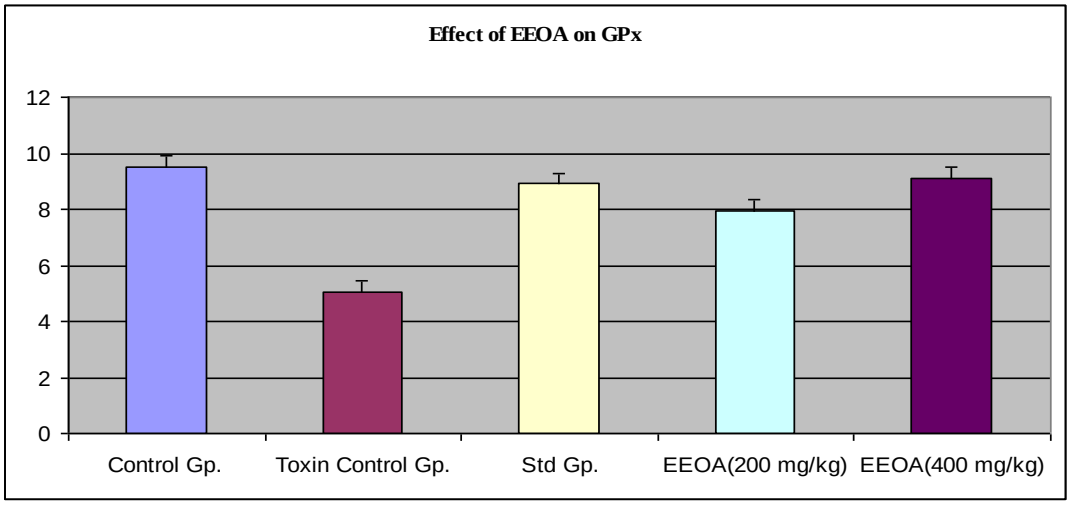

Fig. 5:- Effect of Ethanolic extract of *Oscillatoria annea* on Glutathione-S-Transferase:

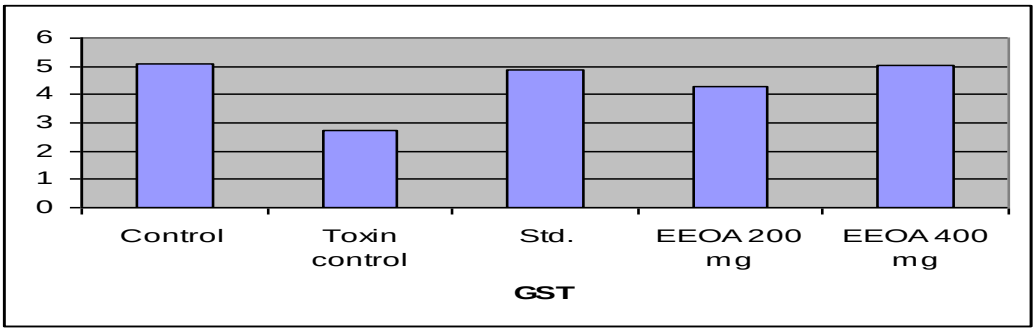

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Table 2: *In- vitro* antioxidant effect of Ethanolic extract of *Oscillatoria annae.*

Group	Phenolic content	DPPH scavenging	Nitric Oxide scavenging	Hydroxyl radical scavenging
Control	-	0.84±0.09	0.29 ± 0.02	0.31± 0.08
Curcumin	-	-	0.074 ± 0.001	0.071 ± 0.001
EEOA (5µg/kg)	6.9 ± 1.71	0.74 ± 0.059	0.24 ± 0.05	0.24 ± 0.05
EEOA (10µg/kg)	7.4 ± 1.68	0.65 ± 0.07	0.22 ± 0.03	0.21 ± 0.02

Values are expressed in mean ± SD, P < 0.01, One way ANOVA followed by Dunnett's test.

DISCUSSION

Antioxidants are compounds that protects cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injuries and inflammation and neurodegenerative diseases. Many terpenoids and steroidal compounds may help to provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body.

In this connection the phytochemical results also indicate the predominant active constituent present in the EEOA. The result obtained from the in vivo and in vitro activities reveals that the potent antioxidant activity of EEOA in comparison with corresponding standards.

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