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Full Length Research Paper

Study of anti-inflammatory, anti-diabetic, and analgesic activity of *Oscillatoria annae* extract in rats and mice

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The aqueous extract of the cyanobacterium *Oscillatoria annae* was investigated for its anti-inflammatory, anti-diabetic,analgesic and cholesterol regulating properties in different experimental standard animal models. The non-steroidal anti-inflammatory drug, indomethacin (10 mg/kg/body weight) was used as standard in the anti-inflammatory, and analgesic studies, while glibenclamide (600 µg/kg/b.wt.) was used as standard drug in the anti-diabetic study. The results reveal that *O. annae* possesses significant ameliorating effects in the studied animal models, including rats and mice. These effects were comparable to those obtained after treatment with the standard drugs used in this study. The results indicate that the cyanobacterial extract can act as natural remedy and also open a new avenue to identify the active ingredients behind these effects.

Key words: *Oscillatoria annae,* anti-inflammatory, anti-diabetic, analgesic, cholesterol.

INTRODUCTION

Natural products have been isolated from wide variety of taxa and tested for various biological activities. These active principles from plant origin have provided numerous crucial molecules in the search of new drug. The search of natural products has revolutionized the drug discovery programs. Many plant-derived molecules have shown a promising effect in therapeutics. Among these taxa, cyanobacteria are considered as good candidates for applications in agriculture, food industry and in pharmaceuticals. Although, cyanobacteria are still primarily viewed as an environmental nuisance or a source of toxins hazardous to man and aquatic livestock, there are many potential benefits to research on chemicals produced by these organisms. Antibacterial, antiviral, antifungal, algaecides and cytotoxic activities have been reported (Senthilkumar and John, 2008). Research on biological active compounds produced by cyanobacteria has been focused on freshwater species such as *Microcystis, Anabaena, Aphanizomenon* and *Spirulina lonar*. Studies on marine cyanobacterial species are related to filamentous forms in tropical regions. *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 and is mainly blue-green or brown-green. *Oscillatoria* reproduces by fragmentation, forming long cells which can break into fragments called hormogonia. Thehormogonia can grow into a new, longer filament. *Oscillatoria* uses photo-synthesis to survive and reproduce.

The use of plant medicines in the treatment of various ailments, including central nervous system disorders is an age long practice. It is important to note that plant medicines are also gaining popularity in developed

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countries. Herbal medicine is currently enjoying a revival in popularity in the west and in fact it is the primary form of medicine in many parts of the world (Williamson et al., 1996). With the great reliance on this type of medicine, it becomes pertinent to search for potent, effective and relatively safe plant medicines, as well as scientific validation of the success claims about plants already in use by traditional medicine practitioners in order to enhance their safety and efficacy. These are some of the problems making this alternative healthcare system less acceptable, especially by orthodox medicine practitioners. For instance, inflammation is a coordinated response that protects and heals the host after infection or tissue damage and it involves several molecular cues generated from either host or disease agent (Nathan, 2002). The modern drugs, including both steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), are available for the treatment of various inflammatory disorders. These drugs however, offer only temporary relief and often elicit undesirable side effects. Hence, the investigations of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap and have little side effects.

Currently, for the first time we have reported hematological alterations and tissue defense activities of *Oscillatoria annae* on induced rats. Since analgesic and antipyretic properties associated with gastric damage are the most important features of anti-inflammatory drugs, the present study was undertaken to identify the analgesic and anti-inflammatory properties of *O. annae* extract. Cardiovascular diseases and diabetes are major health issues in India; the percentage of deaths is approximately 52% in South East Asia Region (Shah and Mathur, 2010). Diabetes mellitus is a complex endocrine disorder characterized by hyperglycemia and predisposes to affecting the eyes, blood vessels, nerves and kidneys. Several drugs such as biguanides and sulfonylureas are currently existing medicine to reduce hyperglycemia in diabetes mellitus. These drugs, however, have side effects and searching for a novel class of compounds is essential to conquer these problems. Moreover, some cyanobacterial strains have been well characterized with some anti-inflammatory and anti oxidant properties (Nagasathya and Thajuddin, 2003; Rajavel et al., 2009). Hence, this present study aimed to analyze the various pharmacological properties of *O. annae* in rats and mice. Further studies are needed to identify and purify the compound present in the extract of *O. annae*.

MATERIALS AND METHODS

Algae and culture conditions

O. annae culture was collected from the National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, India. The culture was transferred to 500 ml conical flasks containing 300 ml of NPK medium for the production of mass culture. The culture was grown under ambient temperature at pH 7.1 ± 0.2. The culture media (Nitrogen - Phosphorus Potassium (NPK)) contains $NaNO_3$ (1.54 g/L) and $K_2HPO_4.3H_2O$ (0.04 g/L).

Preparation of ethanolic (80%) extract from cyanobacteria

The cyanobacterium *O. annae* was harvested after 15 days using clean nylon cloth filter and washed thoroughly with tap water quickly to remove salts and other adhering substances, followed by washing with distilled water. The biomass was placed in a filter paper to remove excess moisture and weighed. The wet biomass was ground with 80% ethanol and the slurry was kept at 4°C for 12 h. Next, the supernatant was collected after centrifugation at 10000 rpm for 10 min. The process was repeated till the biomass become grey in color. The pooled supernatant was dried. The dried extract of exactly 200 mg was re-suspended with 0.2 ml of Tween 80 (1%) and then ground well with 5 ml distilled water. This extract was used for the animal studies.

Experimental animals

Experimental animals included healthy female Swiss albino mice having weight around 20 to 40 g and healthy female rats of Wistar strain with weight around 100 to 200 g. They were maintained under appropriate laboratory conditions. The animals were maintained on a standard feed and water *ad libitum* throughout the experiment. The experiments were carried out between 08:00 and 16:00 h in a quiet laboratory with an ambient temperature. This study was conducted at the Department of Biotechnology and Arulmigu Kalasalingam College of Pharmacy, Kalasalingam University, Tamil Nadu, India during January to April 2009.

Drugs and chemicals

Indomethacin was used in the analgesic and anti-inflammatory studies. Carrageenan was used in the anti-inflammatory study which is the standard anti-inflammatory drug. Diazepam was used for the study of muscle relaxation property and central nervous system (CNS) depressant/stimulant activity study. Simvastatin was used as the cholesterol-regulating drug, while alloxan monohydrate, glibenclamide were used for the anti-diabetic study. The chemicals used are Tween 80 (1%), ethanol, ether, chloroform, acetic anhydride, sodium bisulphate and concentrated sulfuric acid (H_2SO_4).

Assessment of analgesic activity by hot-plate test

In this method, heat was used as a source to induce the pain. Animals were individually placed on a hot plate maintained at a constant temperature of 55°C. The reaction of animals to the heat such as paw licking or the jump response is taken as the end point as described previously (Eddy and Leimback, 1953; Williamson et al., 1996). Analgesic was used to increase the reaction time. To study the effect of the extracts, animals were tested before the administration of the control and conventional drugs. Then they were tested again after 15, 30, 45, 60 and 90 min of the intraperitoneal administration of saline (as negative control), conventional drug (as positive control) and the tested extract. Animals were placed individually in 21 glass beakers placed on a

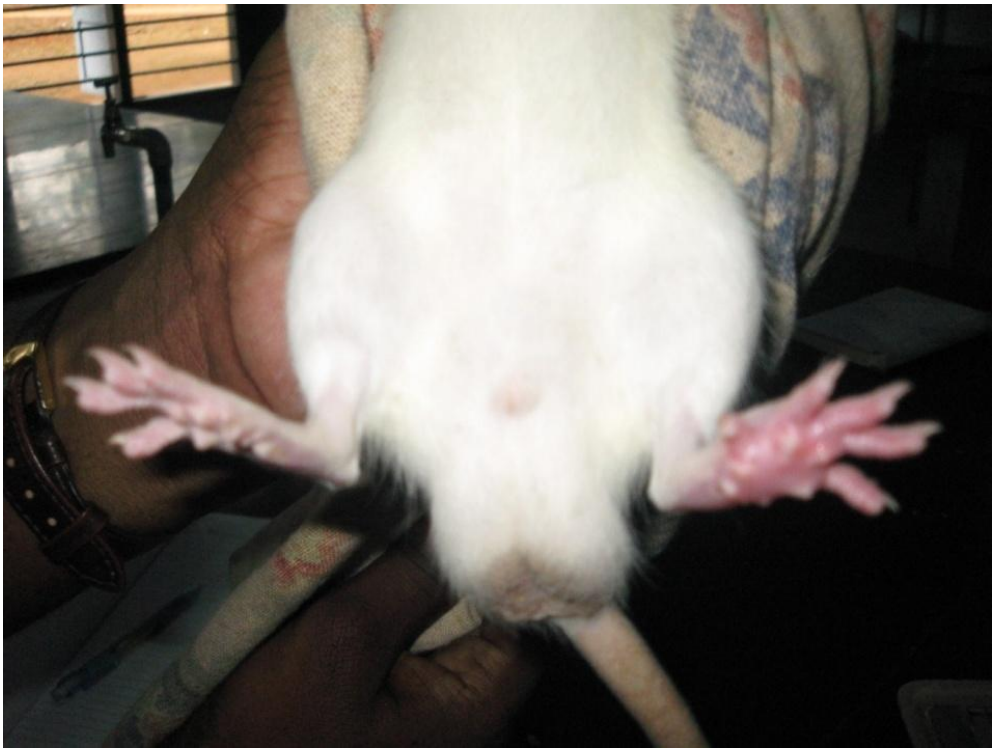


Figure 1. Carrageenan-induced paw edema.

thermostatically controlled hot plate, model HC 500 (Bibby Sterlin Ltd, England), maintained at 50 to 55°C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The time taken for the mice to exhibit these characteristics (time reaction) was noted by means of a stopwatch. The animals were first tested for the paw-licking or jump response and only those that reacted after 4 s were used for the experiment. The ability of the plant extract to delay the reaction time was taken as the analgesic response.

Assessment of anti-inflammatory activity by carrageenan induced paw edema

Anti-inflammatory activity of *O. annae* on carrageenan-induced paw edema (Figure 1) was evaluated as previously described by Winter et al. (1962). The rats were divided into three groups, each group consisting of three animals as follows: Group 1: rats (treated with saline); Group 2: rats treated with *O. annae* extract (200 mg/kg) as a single dose intraperitoneally; Group 3: rats treated with the standard drug indomethacin (10 mg/kg). Carrageenan (0.1 ml of a 1% solution in saline) was injected into the left rear plantar region of the left hind paw of the experimental rats using a glass syringe (2 ml). The measurement of foot volume was carried out initially, that is before carrageenan injection, and then again the foot volume was measured at 30, 60, 90, 120 and 180 min after carrageenan injection by using the screw gauge.

Assessment of CNS depressant activity by spontaneous locomotor activity

The photocell activity cage was utilized to determine the degree of depression. The actions of cyanobacterial extract on spontaneous locomotor activity were measured automatically by using Actophotometer (Medicraft Actophotometer, Mode No. 600-40, India). The units of the activity counts were arbitrary and based on the beam breaks by movement of the mice. Selected animals were divided into three groups: the first group was with saline as control; the second group was injected with the standard drug diazepam (5 mg/kg), and the third group was treated with the extract from *O. annae* (200 mg/kg) as single dose intraperitoneally. The spontaneous locomotor activity was measured at 0 and 30 min interval by placing animals in a novel cage. The treatments were randomized throughout the day, between 09:00 and 17:00 h,to control for diurnal variations in activity.

Assessment of muscle relaxation activity by rota rod test

This test is useful for analyzing the muscle relaxation activity by measuring the loss of muscle grip as indication to the muscle relaxation. This effect can thus be easily studied in animals using inclined plane or rotating rods. The difference in the fall-off time from the rotating rods between the control and diazepam treated animal is taken as an index of muscle relaxation. Fresh mice were placed on a horizontal rod of 32 mm diameter, rotating at a speed of 20 rpm. The mice capable of remaining on the top for 3 to 5 min in three successive trials were selected for the study. The selected animals were divided into three groups: the first group was with saline as the control; the second group was injected with the standard drug diazepam (5 mg/kg), and the third group was treated with the extract from *O. annae* (200 mg/kg) as single dose intraperitoneally. Then the capability of the animals to remain on top of the rod without falling (falling time) was recorded as previously described (Dunham and Miya, 1957).

Table 1. Analgesic activity of *Oscillatoria annae* on mice.

Treatment	Basal reaction time (s)					
	Before drug administration	After drug administration				
		15 min	30 min	45 min	60 min	90 min
Control (Untreated)	6±0.58	6.34 ± 0.33	5.34 ± 0.33	6.34 ± 0.33	6.34 ± 0.66	5.67 ± 0.66
Standard (Indomethacin)	7.67 ± 0.33	11.67 ± 0.88** (52.6%)	10.34 ± 0.88** (35.08%)	9.67 ± 0.33** (26.07%)	9.67 ± 0.33* (26.07%)	9.34 ± 0.33** (21.77%)
Test (*Oscillatoria annae*)	7 ± 0.58	11 ± 0.99* (57.14%)	11.67 ± 0.33*** (66.71%)	11 ± 0.58** (57.14%)	9.67 ± 1.20 (38.14%)	9.34 ± 0.33** (33.42%)
F	2.4	12.5	28.28	25.75	5.23	15.66
Probability (P)	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

N = 3 in each group; df= 2, 6. The values are expressed in terms of Mean ± SEM. Percentage protection by the Oscillatoria annae Extract and reference drug (Indomethacin) are indicated as (%). P value was calculated from the comparison of Control versus Standard and Control versus Test.*P<0.05, **P<0.01, ***P<0.001 compared to control.

Assessment of anti-diabetic activity

Diabetes was induced in female albino rats of Wistar strain by treating them with intraperitoneal injection of 150 mg/kg body weight alloxan after fasting for 24 h (Ajabno and Tilmisany, 1988). Blood glucose was assessed in all the animal groups by using a drop of the blood from paw. The blood glucose level was estimated after 1 day and those rats having blood glucose level 240 to 280 mg/100 ml of blood were included in the study. The rats were randomly divided into three groups (n = 3): group 1 was injected with saline as the diabetic control; group 2 was treated daily with oral administration of 5 mg/kg body weight *O. annae* ethanolic extract orally until the experimental period; group 3 served as a standard group. The blood glucose level was analyzed using glucometer (One touch horizon, Johnson & Johnson, U.S).

Anti-bacterial screening assay

Four bacterial cultures were used, including *Staphylococcus* sp., *Proteus* sp., *Pseudomonas* sp., and *Bacillus* species. These cultures were grown in nutrient broth overnight. This culture was used for well diffusion antibacterial assay. Fresh culture was spread over the surface of Mueller-Hinton (Merck) agar plates. Four sterilized paper disks impregnated with the ethanolic extract of *O. annae* were placed on the surface of the inoculated medium. Plates were incubated for 24 h at the correspondent appropriate growth temperature. As controls, four sterilized blank paper disks impregnated only with solvent (ethanol) used for extractions were tested for each microorganism. The disks were kept overnight in a laminar flow bench sterilized by ultraviolet (UV) light, to evaporate the solvent. After incubation, each plate was examined and the diameter of the zones with complete inhibition of growth was measured to the nearest millimeter using a ruler and expressed in millimeter (mm). The minimum inhibitory concentration (MIC) values were determined with those bacterial strains that showed significant inhibitory zones. The bacterial strains selected for this study were *Staphylococcus* sp*., Proteus* sp*., Pseudomonas* sp. and *Bacillus* sp. The concentrations of 5, 10, 15 and 20 mg/L were prepared and the MIC values were determined as the lowest concentration of the constituent that completely inhibited the growth (Lokhande et al., 2007).

Statistical analysis

All the data obtained were analyzed using the “Unpaired student’s t-test”. The analyzed data were expressed as mean (± S.E.M).

RESULTS AND DISCUSSION

Analgesic activity of *O. annae* on mice

The treatment of animals with *O. annae* (200 mg/kg) produced significant ($p<0.05$ to $p<0.001$) analgesic effects against thermally induced pain in the plate (Table 1). Experimental evidence obtained in the present study indicates that *O. annae* extract can significantly induce analgesic effect. The hot plate test has been found to be suitable for evaluation of centrally acting analgesics (Ojewole, 2006). In the hot plate test, *O. annae* extract showed a significant analgesic action after 15 min of its administration and showed a maximum of 66.71% analgesic activity at 30 min. Similar effects in *Spirulina* extract with Swiss albino mice was also recorded.

Anti-inflammatory activity of *O. annae* extract on rat

Treatment with the *O. annae* extract induced anti-inflammatory effect with the highest effect at 200 mg/kg as compared to the effect of the conventional drug indomethacin. The extract showed maximum inhibition of 28.69% at dose of 200 mg/kg body weight after 180 min of drug treatment in carrageenan induced paw edema as compared to 23.69% inhibition after 180 min of

Table 2. Anti-inflammatory activity of *Oscillatoria annae* extract on rat.

Treatment	Diameter of right paw (mm)	Diameter of left paw (mm)				
		0 min	30 min	60 min	120 min	180 min
Control (Untreated)	25.33 ± 0.877	39 ± 1	37 ± 0.578	36.67 ± 0.334	36.33 ± 0.334	35 ± 0.57
Standard (Indomethacin)	23.67 ± 0.334	37.33 ± 0.663	34.67 ± 0.878 (7.12%)	33.67 ± 1.483 (9.80%)	31.3 ± 1.76* (16.04%)	28.67 ± 1.327* (23.19%)
Test (*Oscillatoria annae*)	23.67 ± 0.334	38.33 ± 2.725	34.67 ± 3.377 (9.54%)	31.67 ± 3.52 (17.37%)	29.34 ± 2.332* (23.45%)	27.33 ± 1.76* (28.69%)
F	2.15	0.22	0.43	1.247	4.36	1.838

The values are expressed in terms of Mean ± SEM. Percentage inhibition of the carrageenan -induced inflammation by the Oscillatoria annae extract and the reference drug used [Indomethacin] are indicated as (%).P value is calculated from the comparison of Control versus Standard and Control versus Test. *P<0.05 compared to control. N = 3 in each group; df=2, 6.

indomethacin treatment (Table 2). The results indicate also that the ethanolic extract of *O. annae* had a significant anti-inflammatory effect against carrageenan-induced paw edema. Interestingly, the anti-inflammatory effect of *O. annae* extract was more potent than those induced by the conventional drug indomethacin. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) is commonly used for the treatment of inflammation. Indomethacin reduces inflammation and swelling by inhibiting prostaglandin synthesis or production (Ojewole, 2006). The extract caused a marked inhibition (28.69%) at a dose of 200 mg/kg after 180 min of drug treatment in the carrageenan induced paw edema model. The results of the present study suggest that the *O. annae* used probably produced its anti-inflammatory effect by inhibiting the release, synthesis and/or production of inflammatory mediators, including polypeptide (kinins), prostaglandin and so forth, like indomethacin.

CNS depressant activity of *O. annae* extract on mice

The effect of extract on the loco motor activity was measured after 1 h of drug administration. The extract significantly decreased the spontaneous loco motor activity in mice as compared to the effect of the conventional drug diazepam (4 mg/kg) treated rats as shown in the (Table 3). The effect of the ethanolic extract of *O. annae* on the CNS showed a significant increase in the hypnotic effect induced by the diazepam, indicating its sedative activity. The method employed for this assay is considered as a very sensitive way and denote agent with depressor activity on the CNS. The reduction of awareness and depressant action may be due to the action of the extract on CNS. The reduction in the exploratory behavior in animals treated with ethanol extract of *O. annae* is similar to those of the action of other CNS depressant agents. The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS (Sundaram et al., 2008).

Muscle relaxant activity of *O. annae* extract on mice

The results obtained from the rota-rod test showed that treatment with the ethanolic extract of *O. annae* at 200 mg/kg significantly reduced the motor coordination of the tested animals as compared to the effect of conventional drug diazepam (4 mg/kg) treated rats (Table 4). The result obtained from rota-rod test showed that ethanolic extract of *O. annae* can significantly reduce the motor coordination as indicated by the increase in the number of falls and the decrease in the time on the bar. The rota-rod method (Dunham and Miya, 1957) was used to determine the forced coordinated motor ability of the animals. The intensity of reduction in exploratory behaviors in treated animal rats resembled those obtained after treatment with the standard reference drug diazepam. These results indicated that the extract can be used to relax the basal tone of the muscle. Our current data showed that extract is also capable of inhibiting response to a wide range of contractile stimuli, such as neurotransmitters acetyl choline and histamine (Rosa martha et al., 2009).

Anti-diabetic activity of *O. annae* extract

The diabetic rats showed a significant increase in the blood glucose level. Oral administration of *O. annae* extract to diabetic rats at a dose of 5 mg/kg showed a significant decrease in the blood glucose level as compared to treatment with the conventional drug glibenclamide (600 µg/kg) treated diabetic rats (Table 5).

Table 3. CNS depressant Activity of *Oscillatoria annae* extract on mice.

Treatment	Locomotor activity (scores) in 1 min		
	Before drug administration	After drug administration	Percentage change in activity (%)
Control (Untreated)	98.67 ± 2.332	98.33 ± 1.449	0.34
Standard (Diazepam)	94.33 ± 1.853	23.33 ± 0.883*	75.26
Test (*Oscillatoria annae*)	101 ± 5.565	25.67 ± 1.2*	74.58
Probability (P)	>0.05	<0.05	-

The values are expressed in terms of Mean ± SEM. P value was calculated from the comparison of Control versus Standard and Control versus Test. *P<0.001 compared to control.

Table 4. Muscle relaxant activity of *Oscillatoria annae* extract on mice.

Treatment	Fall off time (s)		
	Before drug administration	After drug administration	
		120 min	240 min
Control (Untreated)	220.33 ± 5.77	211.67 ± 6.11	205.67 ± 7.881
Standard (Diazepam)	210.33 ± 10.64	24 ± 1.15* (88.58%)	22.67 ± 0.877* (89.22%)
Test (*Oscillatoria annae*)	234 ± 13.45	56.57 ± 2.40* (75.78%)	36.33 ± 1.454* (84.47%)
Probability (P)	> 0.05	< 0.05	< 0.05

The values are expressed in terms of Mean ± SEM. P value was calculated from the comparison of Control versus Standard and Control versus Test. *P<0.001 compared to control.

Table 5. Anti-diabetic activity of *Oscillatoria annae* extract on rat.

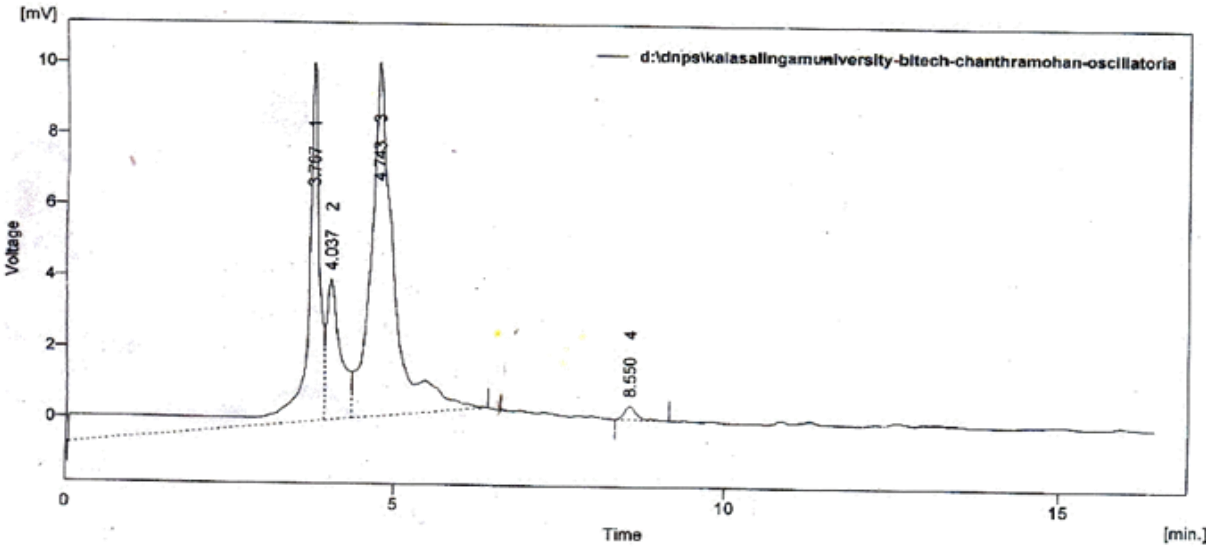
Treatment	Blood glucose level (mg/dl)			
	Normal rat	Diabetes induced rat		
		0 day	3rd day	6th day
Control (Untreated)	88 ± 1.15	277.33 ± 4.37	304.67 ± 3.17	307.33 ± 2.96
Standard (Glibenclamide)	90 ± 2.07	278.67 ± 6.87	72 ± 1.73* (74.16%)	69 ± 1.52* (75.23%)
Test (Oscillatoria annae)	88.67 ± 2.33	274.33 ± 7.88	105.33 ± 2.96* (61.60%)	109.67 ± 3.75* (60.02%)
Probability (P)	> 0.05	> 0.05	< 0.05	< 0.05

The values are expressed in terms of Mean ± SEM. Percentage inhibition of the blood glucose level for reference drug (glibenclamide) and test (*Oscillatoria annae*) were indicated as %. P value was calculated from the comparison of Control versus Standard and Control versus Test. *P<0.001 compared to control.

Treatment of diabetic rats with ethanolic extract of *O. annae* or glibenclamide reduced the blood glucose level significantly as compared to the untreated diabetic rats. The effect of the extract was less significant than that of the standard drug glibenclamide. In the prolonged study (six days), the extract produced a sustained significant reduction in the blood glucose levels of the diabetic rats, indicating to the long-lasting anti-diabetic effect of the extract. Similar results were reported for the extract derived from *Spirullina plantensis* (Layam and Reddy, 2006). The possible mechanism by which *Oscillatoria* extract brought about its antihyperglycemic action may be

Table 6. Anti-bacterial activity of *Oscillatoria annae* extract on some common pathogens.

Bacterial strains	Absorbance at 600 nm				
	Control	5 mg/L	10 mg/L	15 mg/L	20 mg/L
Bacillus sp.	1.152	0.901	0.819	0.673	0.510
Proteus sp.	1.361	1.340	1.348	1.352	1.354
Pseudomonas sp.	0.831	0.825	0.822	0.814	0.811
Staphylococcus sp.	0.675	0.659	0.660	0.668	0.666



Result Table (Uncal-d:\dnps\kalasalingamuniversity-bitech-chanthramohan-oscillatoria)

S/N	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05
1	3.767	197.818	10.080	39.7	41.3	0.13
2	4.037	58.913	3.968	11.8	16.3	0.23
3	4.743	236.737	9.967	47.5	40.9	0.30
4	8.550	5.213	0.385	1.0	1.6	0.18
Total		498.681	24.400	100.0	100.1	

Figure 2. HPLC chromatogram of *Oscillatoria annae* extract.

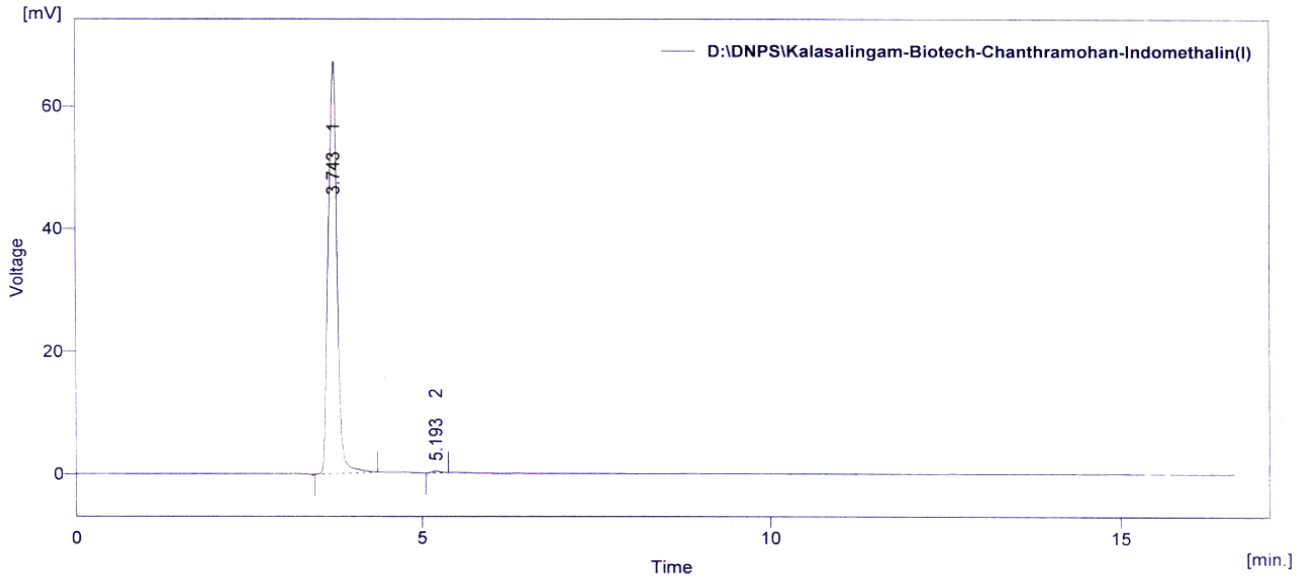
through potentiating the pancreatic secretion of insulin from islet β-cell or the enhanced transport of blood glucose to the peripheral tissue, or due to the down-regulation of NADPH and NADH, a cofactor in fat metabolism (Layam et al., 2006).

Anti-bacterial activity of *O. annae* extract on some common pathogens

In vitro treatment with the ethanolic extract of *O. annae* showed significant antibacterial activity against *Bacillus* sp. (Gram positive), slight activity against *Pseudomonas* sp. and poor anti-bacterial effect against the other bacterial strains (Table 6). The lack of activity against cyanobacteria is consistent with the fact that most Gram-negative bacteria such as cyanobacteria are resistant to toxic agents in the environment due to the barrier of lipopolysaccharides of their outer membrane (Priya et al., 2007).

Identification of compounds by high performance liquid chromatography (HPLC)

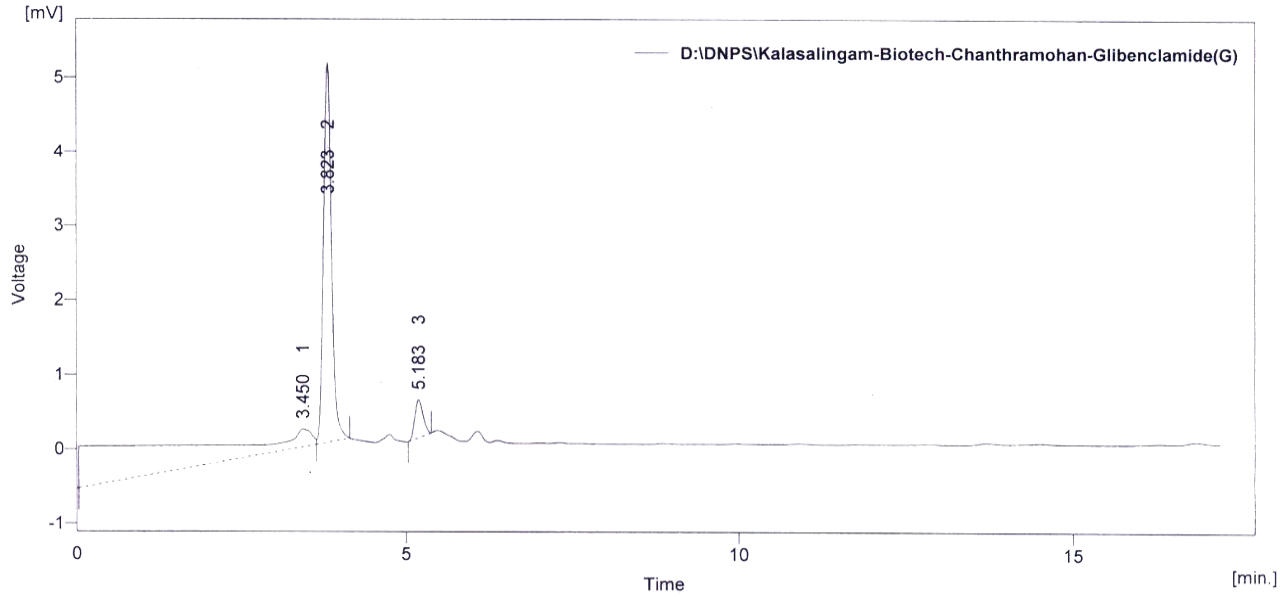
High performance liquid chromatography (HPLC), Column: C-18, mobile phase was injected with 0.02 ml of acetonitrile and the ethanolic extract. The *O. annae* extract showed comparable retention times to the standard drug, indicating to the presence of bioactive compounds that might be responsible for the pharmacological properties of *O. annae* extract (Figures 2 to 4).



Result Table (Uncal-d:\dnps\kalasalingamuniversity-bitech-chanthramohan-oscillatoria)

S/N	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05
1	3.743	588.089	67.333	99.6	99.5	0.14
2	5.193	2.658	0.324	0.4	0.5	0.14
Total		590.747	67.657	100.0	100.0	

Figure 3. HPLC chromatogram of the standard drug, indomethacin.



Result Table (Uncal-d:\dnps\kalasalingamuniversity-bitech-chanthramohan-oscillatoria)

S/N	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05
1	3.450	64.341	0.236	57.7	4.0	0.29
2	3.823	42.756	5.107	38.4	87.3	0.13
3	5.183	4.354	0.508	3.9	8.7	0.14
Total		111.451	5.851	100.0	100.0	

Figure 4. HPLC chromatogram of the standard drug, glibenclamide.

Conclusion

Taken together, our results suggest that the crude extract from *O. annae* has anti-inflammatory, anti-diabetic, anti-pyretic, and analgesic activities indicating its beneficial pharmacological effects in different diseases settings. Further investigation is underway to determine the exact phytoconstituents that are responsible for the above reported pharmacological effects of the ethanolic extract of *O. annae*.

REFERENCES

Dunham NW, Miya TS (1957). A note on a simple apparatus for detecting neurological deficit in rats & mice, J. Am. Pharmaceut. Assoc 46: 208-209.

Gutierrez RMP, Diaz SL, Reyes IC, Gonzalez AMN (2010). Anti-glycation effect of spices and chilies uses in traditional Mexican cuisine. J. Natural Prod. 3: 95-102.

Layam A, Reddy CLK. (2006). Anti diabetic property of *Spirulina*, Diabetologia Croatica, 35(2): 29-33.

Lokhande PD, Gawai KR, Kodam KM, Kuchekar BS, Chabukswar AR, Jagdale SC. (2007) Antibacterial activity of extracts of *Piper longum*. J. Pharmacol. Toxicol. 2: 574-579.

Nagasathya A, Thajuddin. (2003). Antioxidant property of hypersaline cyanobacteria, *Phormidium tnue* (KMD 33). Int. J. Pharmacol. pp. 1-5.

Nathan C *(*2002*)*. Points of control in inflammation. Nature (Lond), 420: 846-852.

Ojewole JAO. (2006). Analgesic anti inflammatory and hypo glycaemic effects of Ethanol extracts of *Zingiber officinale* (Roscoe) Rhizomes (Zingiberaceae) in Mice and Rats, Phytother. Res. 20: 764-772.

Priya K, Ganjewala D. (2007). Anti bacterial activities and phytochemical analysis of different plant part of *Nyctanthes arbour-tristis* (Linn.). Res. J. Phytochem. 1(2): 61-67.

Rajavel R, Sivakumar T, Jagadeeswaran M, Malliga P. (2009). Evaluation of analgesic and anti-inflammatory activities of *Oscillatoria willei* in experimental animal models. J. Medicinal Plants Res. 3: 533-537.

Senthilkumar R, Ahmed John S (2008). Hypoglycemic activity of marine cyanobacteria in alloxan-induced diabetic rats. Pharmacol. Online, 2: 704-714.

Shah B, Mathur P (2010). Surveillance of cardiovascular disease risk factors in India: The need & scope. Indian J. Med. Res. 132: 634-642.

Sharma VK, Kumar S, Patel HJ (2010). Hypoglycemic activity of Ficus *Glomerata* in alloxan induced diabetic rats. Int. J. Pharmaceut. Sci. Rev. Res. 1: 18-22.

Sundaram SR, Sampathumar R, Sivakumar P, Nethaji R, Senthil V, Murthy VN, Kanagasabi R. (2008). CNS activity of the methanol extracts of *Careya arborea* in experimental animal model, J. Bangladesh Pharmacol. Soc. (BDPS), 3: 36-43.

Williamson EM, Okpako DT, Evans FJ. (1996). Pharmacological methods in phytotherapy research: selection, preparation and pharmacological evaluation of plant material, Wiley, Chichester, pp. 131-154.

Winter CA, Risley EA, Nuss GW (1962). Carrageenan-induced oedema in the hind paw of rat as an assay or anti-inflammatory activity. Proc. Soc. Exp. Biol. Ther. 111: 544-547.