International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 196-200
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Antimicrobial Activities of Some Selected Marine Cyanobacteria Isolated from Bay of Bengal of Odisha Coast

Satyabrata Dash[1], Sabyasachy Parida[2] and Biswajit Rath[3*]
Department of Biotechnology, North Orissa University, Sriram Chandra Vihar, Takatpur, Baripada, Odisha, India – 757003.

Received: 20 Mar 2019 / Accepted: 19 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** brath_2000@yahoo.com

Abstract
Bioactive compounds were found to be extracted from marine cyanobacteria. Many cyanobacteria are known to produce intracellular and extracellular secondary metabolites with diverse biological activity. The characteristics of such metabolites in nature are not yet completely understood. The present study was carried out to observe the antibacterial effect of benzene, acetone and methanol extracts of four marine cyanobacteria (*Phormdium ambiguum, Planktolyngbya limnetica, Lyngbya. martentiana, Oscillatoria pseudogeminata*) isolated from Odisha coast was tested against few pathogenic bacteria and fungus. The methods adopted is based on agar well diffusion principle using spread plate technique. Antibacterial effects were observed as visible zone of inhibition. The highest zone of inhibition exhibited against *E.coli* (20±2mm) followed by 18±1mm against *V. Cholerae* in benzene extract of *P. limnetica,* whereas least antibacterial activity was observed in benzene extract of *L.martensiana* against *E. coli* (11±1mm). The acetone extract of L*yngbya martensiana* exhibited highest antifungal zone of inhibition against *A. niger* (21±1mm) followed by *V. Cholera* (17±2mm) whereas the acetone extract of *L.martensiana* exhibited least activity against *C.albicans* (12±1mm). Further phytochemical analysis exhibited presence of alkaloid, glycoside, tannin, flavanoid, steroid, saponin, resin, quinine, anthocyanin and phenols in all the species. Results indicates the presence of promising antibacterial compounds.

Keywords
Antimicrobial, Bioactivity, Cyanobacteria, Extract, Phytochemical, Solvent, Zone

*****

INTRODUCTION
The marine environment is an exceptional reservoir of bioactive natural compounds, which exhibit structural / chemical features not found in terrestrial natural products [1]**.** Cyanobacteria is considered as being one of the potential organisms, which constitute a versatile group of microorganisms, occur in diverse habitats ranging from hot springs to snowfields in the poles and freshwater to marine habitat, which can be useful to mankind in various ways [2]. Recently, much attention has been focused on the marine microalgae and cyanobacteria as sources of structurally novel and biologically active metabolites [3]. Cyanobacteria have been identified as the most promising group of organisms capable of producing bioactive compounds [4,5,6].

They produce a wide range of bioactive substances with antimicrobial, enzyme inhibiting, immunostimulant, cytotoxic and antioxidant activities [7,8,9] which offer rich pharmacological potential [10]. A number of significant advances have occurred in cyanobacterial biotechnology in the recent years
[11]. The use of cyanobacteria in the field of medical industries for its secondary metabolites such as vitamins, toxins, enzymes, pharmaceuticals and pharmacological probes is become popular worldwide and also used in food, fuel, fertilizer, pharmaceuticals and other industries. Several authors have studied the antimicrobial activities of marine cyanobacteria in different parts of India [12,13,14,15,16,17] and explored several bioactive compounds and affirmed promising applications encompassing antibacterial and antifungal activities. Further the bioactive compounds derived from cyanobacteria will prove to have beneficial and much more effective role as compared with traditional treatment methods. Antimicrobial activity depends on both algal species and the solvents used for their extraction [18].
Since not much work has been reported from this region in this aspect hence attempt has been taken to evaluate the antimicrobial activity of four selected marine cyanobacteria isolated from (Odisha coast, India) extracted with three solvents of decreasing polarity extraction (methanol, acetone, benzene) against four pathogenic bacteria and two pathogenic fungus in order to find out the antimicrobial compounds.

MATERIALS AND METHODS

Sample collection, isolation and culture

Four cyanobacterial species (*Phormdium ambigum, Planktolyngbya limnetica, Lyngbya. Martentiana, Oscillatoria pseudogeminata)* were collected from Odisha coast, India. Samples were stored in plastic bag and transported to the laboratory. The cyanobacterial samples were cleaned and necrotic parts were removed. Then the samples rinsed with sterile distilled water to remove any associated debris. The identification of cyanobacterial strains was performed by special key[19,20,21]. The isolated cyanobacterial species were cultured and maintained in ASN III media at 25±1 ^{0}C temperature and 2000-3000 lux light intensity.

Test microorganisms

The bacterial strains used for the experiment were two Gram-positive (*Staphylococcus aureus,* MTCC-96*, Bacillus subtilis* MTCC-441) and two Gram-negative (*Vibrio Cholerae,* MTCC-3906 *Eschercichia coli,* MTCC-443) and two strains of fungus (*Candida albicans,* MTCC 183 *Aspergillus niger,* MTCC-1344). These microorganisms were obtained from IMTECH Chandigarh and maintained at Post Graduate Department of Biotechnology, North Orissa University, Baripada, Odisha, India.

Extract Preparation

The cyanobacterial biomass was harvested by centrifugation at 5000rpm for 10 min. at room temperature and the cell pellet was air-dried. The dried biomass was ground well using a sterile mortar and pestle and soaked for 48hrs with 95% methanol, acetone and benzene (0.2g/10ml). The extracts were then filtered through Whatmann No.1 filter paper. The filtrate was evaporated under reduced pressure. The extracts were then tested for their antimicrobial activity against selected pathogens.

Antimicrobial activity

Antimicrobial activity of cyanobacterial extracts was assayed by Agar well diffusion method [22]. The pathogenic organisms were grown in the sterile nutrient broth for 24h at 37°C (bacteria) and for 72h 28°C (fungi). For antimicrobial assay, Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) was prepared for bacteria and fungi respectively. About 20ml of sterilized media were poured aseptically into Petri plates and allowed for solidification. After solidification, the test microbial suspension was spread uniformly on the plates using sterile cotton swab. A well punch machine was used to create wells of 8 mm at equal distances. Then 50µl of the extract was loaded in the wells. The antibiotic such as Ampicillin and Clotrimazole (5µg/well) were used as a positive control for bacteria and fungi respectively. Then the plates were incubated at 37°C for 24h. After incubation period the zone of inhibition was observed and the diameter was measured in millimeters.

Phytochemical analysis

Cyanobacterial extracts were prepared using solvents (methanol, acetone and benzene). The extracts were analyzed for the presence of alkaloids, glycosides, phenolic compounds, flavonoids, saponins, Tannins, Steroids, Anthrocyanin, Quinones, and Resins following the method of Dhanalakshmi et al., 23as maintioned below.

Phenolic compounds:
In extract (1 mL) few drops of Hydrochloric acid was added. Yellowish brown color indicated the presence of Phenolic compounds.

Flavonoid (Ferric chloride test): Few drops of Ferric chloride were added into the extracts and formation of brown precipitate, indicates presence of flavonoid.

Saponins: Each extract (3 mL) was taken in a tube. The suspension was vigorously shaken. The formation of stable foam was taken as an indication for the presence of saponins.

Alkaloids (Wagner's test): Few drops of Wagner's reagent was added at the side of the test tube. The formation of reddish-brown precipitate showed the presence of alkaloids

Glycoside test: 1 mL of concentrated sulphuric acid was added to extract and formation of reddish brown colour indicate a positive test for glycosides.

Tannins (Ferric chloride test): Few drops of ferric chloride solution was added to the extract. Brackish precipitate showed the presence of Tannins.

Steroids: To small amount of extact, few drops of acetic acid was added and subsequently few drops of conc. sulphuric acid was added. Appearance of reddish brown colour showed the presence of steroids.

Anthocyanin: A small amount of extract was treated with 2 ml of NaOH and observed. Blue green colour was formed which indicate the presence of anthocyanin.

Quinones: To 1 ml of the extract, Alcoholic KOH solution was added. Colour termed from
red to blue showed the presence of quinones.
Resins: To small amount of extract, 2-3 ml of copper sulphate solution was added, and the contents were mixed for 2 minute and then solution was allowed to separate. Formation of green colour precipitate showed the presence of resin.

Statistical analysis

The results of the data were statistically analysed by using standard error. The values are mean **±** standard error **(**S.E**)** of three measurements (n=3).

RESULTS

The methanol, acetone and benzene extracts of four marine cyanobacteria were tested for antibacterial activity against four human bacterial pathogens and two fungal pathogens. The degree of activity varied with reference to concentration of cyanobacterial extracts. The extracts of *Phormidium ambiguum* with all the three solvents exhibited better antifungal property against both the test fungal pathogens (*C. albicans* and *A. niger*) as compared to bacterial pathogens (both gram + ve and -ve). The acetone extract of *P. ambiguum* exhibited highest zone of inhibition i.e. 21±1mm against *S. aureus* followed by 18±2mm and 17±1mm for benzene and methanol extract against *B. subtilis* and *S.aureus* respectively (fig.1). All the three solvents extracted with *P. ambiguum* showed least antibacterial property against *V. cholerae* and *E. coli* showing least zone of inhibition (11±1mm). On the contrary benzene extract of *P. limnetica* exhibited highest zone of inhibition against *V. cholerae* and *E. coli* i.e.18±1mm and 20±1mm respectively followed by *A. niger* (17±1mm) and *C. albicans* (16±2mm). In *Planktolyngbya limnetica* among the three solvents used for extraction, benzene showed better zone of inhibition against all the test pathogen followed by methanol and acetone extract (fig.1). The highest zone of inhibition i.e. 20±2mm against *E.coli* was displayed followed by 18±1mm against *V.Cholerae* in benzene extract of *P. limnetica.*

The acetone extract of L*yngbya martensiana* 21±1mm showed a zone of inhibition against *A. niger* followed by *V. Cholerae* i.e 17±2mm. On the other hand, the methanolic extract of *P. limnetica* exhibited highest zone of inhibition which was observed against *B. subtilis* (17±1mm) and *A. niger* (16±2mm). The benzene extract of *L. martensiana* displayed zone of inhibition as 19±1mm and 16±2mm against *A. niger* and *S. aurues* respectively (fig.1). In *L. martensiana* the antifungal property is more prominent than the antibacterial property as evidenced by display of zone of inhibition in all the three solvent extracts (fig.2). In *Oscillatoria pseudogeminata* the highest zone of inhibition was 20±1mm in both methanol and acetone extract against *B. subtilis* and *E. coli*. Further in *O. pseudogeminata* acetone extract showed better zone of inhibition against all the test pathogens (bacteria and fungi) followed by methanol and benzene extracts (Fig.1 and 2).

E.coli (11±1mm) whereas highest antibacterial activity was observed in acetone extract of *P. ambiguum* against *S. aureus* and *A.niger* (21±1mm) and subsequently by methanolic extract of *O.pseudogeminata* against *E.coli* (20±2mm)(Fig.1 and 2).

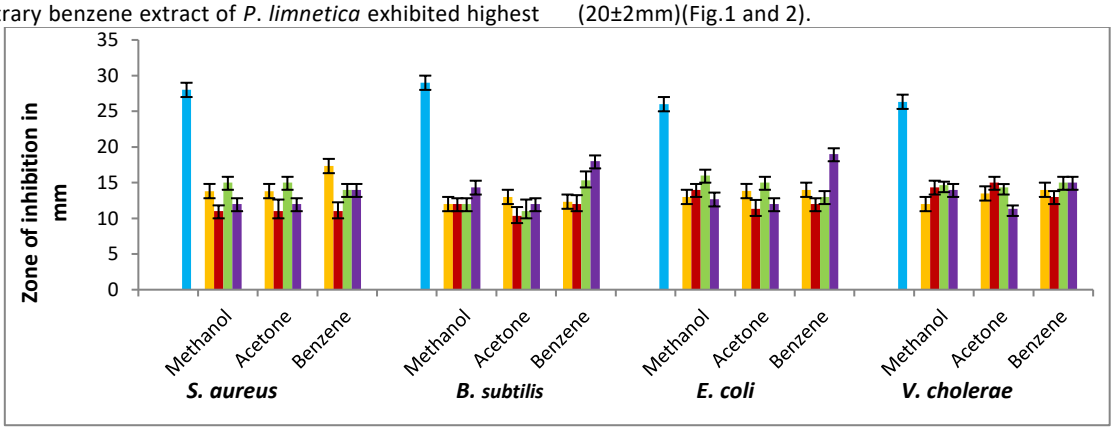

Figure 1: Antibacterial activity of four cyanobacterial species against four test pathogenic bacteria

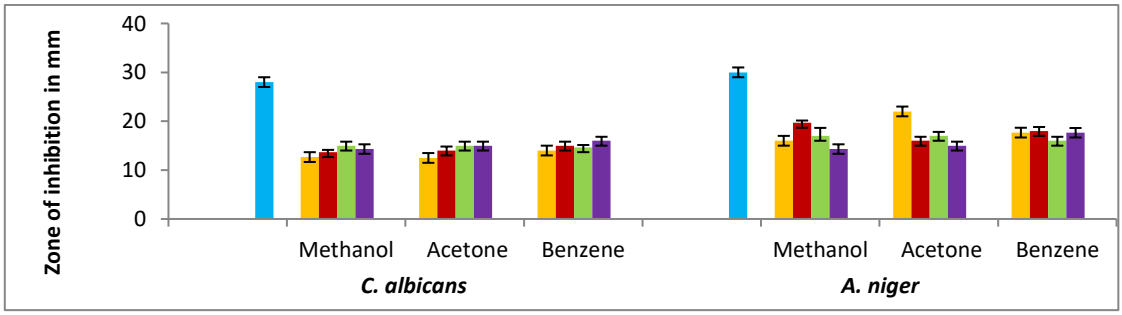

Figure 2: Antifungal activity of four cyanobacterial species against two test pathogenic fungus
Positive control, *Phormidium ambiguum,* *Planktolyngbya limnetica,* *Lyngbya martensiana* *Oscillatoria pseudogeminata*

Phytochemical analysis

The extract four marine cyanobacterial species were prepared using three solvents (methanol, acetone and benzene). The phytochemical present in cyanobacterial species were identified as alkaloid, glycoside, tannin, flavanoid, steroid, saponin, resin, quinone, anthocyanin and phenols (table.1). *Phormidium ambiguum* extracted with acetone showed presence of all the ten phytochemicls for which test has been conducted but benzene extract did not show presence of glycoside methanol extract alkaloid and tannin was not found. In *Planktolyngbya limnetica* all the three solvent extracts did not show presence of quinine and anthocyanin but other seven phytochemicals were present. Similarly, in *Lyngbya martentiana* except tannin and quinones, all other phytochemicls were present in all the three solvents used for extraction. In *Oscillatoria pseuogeminata* methanol and acetone extract showed presence of all the phytochemicals except steroid and quinones whereas benzene extract did not show the presence of tannin, flavanoid and saponin. Phenol and resin was found to be present in all the extracts of four species of cyanobacteria and was the predominant phyochemical. Quinon and anthocyanin was found to be least present in all the four test cyanobacterial extracts. (table.1).

Table 1: Phytochemical analysis of four marine cyanobacteria

Species	Solvent	Alkaloid	Glycoside	Tannin	Flavonoid	Steroid	Saponin	Resin	Quinones	Anthrocyanin	Phenol
Phormidium ambiguum	**Methanol**	-	+	-	+	+	+	+	-	-	+
	Acetone	+	+	+	+	+	+	+	+	+	+
	Benzene	+	-	+	+	+	+	+	+	+	+
Planktolyngbya limnetica	**Methanol**	+	-	+	+	+	+	+	-	-	+
	Acetone	+	+	+	-	+	+	+	-	-	+
	Benzene	+	+	+	+	-	+	+	-	-	+
Lyngbya martensiana	**Methanol**	+	+	-	+	+	+	+	-	-	+
	Acetone	+	+	-	+	+	+	+	-	+	+
	Benzene	+	+	-	+	+	+	+	-	+	+
Oscillatoria pseudogeminata	**Methanol**	+	+	+	+	-	+	+	-	+	+
	Acetone	+	+	+	+	-	+	+	-	+	+
	Benzene	+	+	-	-	+	-	+	+	+	+

(+) Found (-) Not found

DISCUSSION

In the present investigation it was observed that all the test marine cyanobacterial species exhibited differential antimicrobial activity against human pathogens. Among the test species *Phormidium ambigumuum* and *Lyngbya martentian* exhibited better antimicrobial activity. The present results are in agreement with those studied using the strains of *Oscillatoria sp*. [24] and *phormidium sp*. [4]. The acetone extract of *Spirulina subsalsa* showed high inhibitory activity on both Gram (+) and Gram (-) bacteria. Similarly, Prasantkumar *et al.,* [25] studied the antimicrobial activity in various organic extracts of six species of marine algae against different bacterial pathogens. Salem et al., [26]indicated that *Microcystis sp*.the acetone extract showed antifungal activity against *A. niger*. Shrivastava [27] found that *M. aeruginosa* methanolic extract significantly inhibited the mycelial growth of *Aspergillus fumagatus*, *Candida albicans* and *Rhizoctonia solani.* Antimicrobial activity depends on both cyanobacterial species and the solvents used for their extraction [18].

Further the phytochemical analysis was performed to know the phytoconstituents and present in test species and their effect on antibacterial activity. The results of the present study reveal presence of phenols in all the solvent extracts of four species which is thought to be responsible for phenolic toxicity to microorganisms by enzyme inhibition [28] and by the oxidizing compounds possibly due to reactions with sulphahydryl groups through nonspecific interaction with the proteins. Similarly, flavonoids exhibit effective antimicrobial activity against wide range of microorganisms due to their ability to complex with extra cellular and soluble protein and to complex with bacterial cell wall [29]. The disparities reported by different workers on antimicrobial activity of marine cyanobacteria may be due to geographical variations, wide variation of habitats in the marine environments, different culture and preservation methods before extraction, different solvents of extraction and to the different susceptibilities among bacterial strains.

CONCLUSION

In conclusion the results of the present study indicate that the antibacterial property of the four species of cyanobacteria against the selected strains of human pathogenic bacteria varies depending upon the solvent medium used for extraction. The result of the present investigation reveals that *S. aureus* was most sensitive bacteria which displayed higher zone of inhibition to benzene and methanol extract of all the four marine cyanobacteria. On the contrary *E. coli* and *A. niger* were more resistant bacteria and fungi in the experimental result. These results give an indication of the presence of promising antibacterial compounds in the marine cyanbacteria under investigation. Further phytochemical studies are carried out to elucidate the components responsible for antibacterial activity of these extracts

against bacteria. Further studies have to be made on fractionation and separation of extracts in order to find out the principal antimicrobial compound which might be useful for therapeutic purpose.

ACKNOWLEDGEMENTS

The authors express gratitude to the Department of Science and Technology, Govt. of Odisha for financial assistance under major research project (27552800232014/20/2799, ST Bhubaneswar) to carry out the present investigation. We are also thankful to the Head, Department of Biotechnology, North Orissa University for providing necessary infrastructure facility.

REFERENCES

1. Carter BK, Biomedical potential of marine natural products. Bioscience*;* 46, 271-86. (1996)
2. Giorgio A, Carraturo F, Aliberti F, Salvatore DB, Libralato G, Morra M and Guida M. Characterization of microflora composition and antimicrobial activity of agal extracts from Italian thermal muds**.** Journal of Natural Science, Biology and Medicin; 9, 150-158. (2019)
3. Saleh B and Al-Mariri A. Antimicrobial Activity of the Marine Algal Extracts against Selected Pathogens. Journal of Agricultural Science and Technology, 19, 1067-1077. (2017)
4. Fish SA and Codd GA. Bioactive compound production by thermophilic and thermotolerant cyanobacteria (blue-green algae). World Journal of Microbial Biotechnology; 10, 338-347. (1994)
5. Schlegel I, Doan NT, De Chazol N and Smith GD. Antibiotic activity of new Cyanobacterial isolates from Australia and Asia against green and Cyanobacteria. Journal of Applied. Phycology; 10, 471-479. (1999).
6. Asthana RK , Tripathi MK Srivastava, A Singh AP, Singh SP and Nath G. Solation and identification of a new antibacterial entity from the Antarctic Cyanobacterium Nostoc CCC 537.Journal of Applied Phycology; 21(1), 81-88. (2009)
7. Ghasemi YM, Yazidi T, Shafiee A, Amini A, Shokravi S. and Zarrini G. A novel antimicrobial substance from *Fischerella ambigua*. Pharmaceutical Biology; 2*,* 318-322. (2004)
8. Katircioglu H, Akin BS and Atici T. Microalgal toxin(s): Characterization and importance, African Journal of Biotechnology*, 3,* 667-674. (2004)
9. Venkatesan R, Karthikayen R, Periyanayagi R, Sasikala V and Balasubramanian T. Antibacterial activity of the marine diatom, *Rhizosolenia alata* (Brightwell, 1858) against human pathogens. Research Journal of Microbiology, 2*,* 98-100. (2007)
10. Crasta PJ, Rabi raja NS and Sridhar KR. Antimicrobial activity of some marine algae of south east coast of india. Indian journal of marine Science; 26, pp-201-205. **(**1997)
11. Caires TA, Dasilva AMS, Vasconcelos VM, Affe AMJ, de Souza Neta LC, Boness HVM, Sant'Anna CL and Nunes JMC. Biotechnological potential of Neolyngbya (Cyanobacteria), a new marine benthic filamentous genus from Brazil. Algal Research; 36*,* 1-9. (2018)
12. Battu G R, Ethadi S, Prayaga MP, Praneeth VS and Rao M. In-vitro antibacterial activity and preliminary phytochemical screening of three algae from Visakhapatnam Coast, Andhra Pradesh, India. International Journal of Pharmacy and Pharmaceutical Sciences; 3(4), 399-401. (2011)
13. Selvi MR, Selvaraj and Anandhi C. Screening for antibacterial activity of macroalgae. Seaweed Research Utilization; 23(1&2), 59-63. (2001)
14. Tuney I, Cadirci BH, Unal D and Sukatar A. Antimicrobial activities of the extracts of marine algae from the coast of Ural (Izmir Turkey) Brittany France. Turkish Journal of Biology; 30*,* 171 -175. (2006)
15. Veeragurunathan Vand Geetha T. Screening for antimicrobial activity of marine algae from Gulf of Mannar. Tamil Nadu. Seaweed Research Utilization; 31(1&2), 151-155. (2009)
16. Veeragurunathan V, Vellaiyan R, Siva Subramanian S. Sadannantham G and Bahkyaraj R. Studies on the antibacterial activity of selected seaweeds from Gulf of Mannar. Seaweed Research Utilization, 30(1 &2), 147-152. (2008)
17. Sanmukh S, Bruno B, Ramakrishnan U, Khairnar K and Swaminathan S. Bioactive compounds derived from microalgae showing antimicrobial activities. Journal of Aquaculture Research and Development; 5(3), 224. (2014)
18. Radhika D, Veerabahu C and Priya R. Antibacterial activity of some selected seaweeds from the Gulf of Mannar Coast, South India. Asian Journal of Pharmaceutical and Clinical Research;5(4), 89-90. (2012)
19. Desikachary TV, Cyanophyta. Indian Council of Agricultural Research, New Delhi; 686. (1959)
20. Anand N-Handbook of blue-green algae of rice fields of South India, Bishan Singh Mahendra Pal Singh, Dehradun. (1989)
21. Santra SC. Biology of Rice fields blue green algae, Daya Publishing House, Delhi. (1993)
22. Abedin R M A, Taha, H M, Antibacterial and Antifungal Activity of Cyanobacteria and Green Microalgae. Global Journal of Biotechnology and Biochemistry Research; 3, 22-31. (2008)
23. Dhanalakshmi M and Angayarkanni J. Phytochemistry and antibacterial activity of Chlorosarcinopsis species. International Journal of Scientific and Technology Research; 4(10), 315-321. (2013)
24. Issa AA. Antibotic production by the cyanobacteria *Oscillatoria angustissma* and *Calothrix parietina*. Environmental Toxicology and Pharmacology; 70, 61-63. **(**1999)
25. Prashantkumar P, Angadi S and Vidyasagar G. Antimicrobial activity of blue green algae. International Journal of Pharmaceutical Sciences and research; 68(5), 647-648. (2006)
26. Salem O, Hoballah E, Ghazi and Hanna S. Antimicrobial activity of microalgal extracts with special emphasize on *Nostoc* sp. Life Science Journal; 11(12)*,* 52-758. (2014)
27. Shrivastava D. Toxicity of cyanobacteria against pathogenic bacteria and fungi. Indian Journal of Science & Research, 4(1)*,* 1-7. (2014)
28. Mason T L and Wasserman BP. Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. Phytochemistry*;* 26, 2197–2202. (1987)
29. Tsuchiya H, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T and Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of Ethno pharmacology; 50, 27–34. (1996)