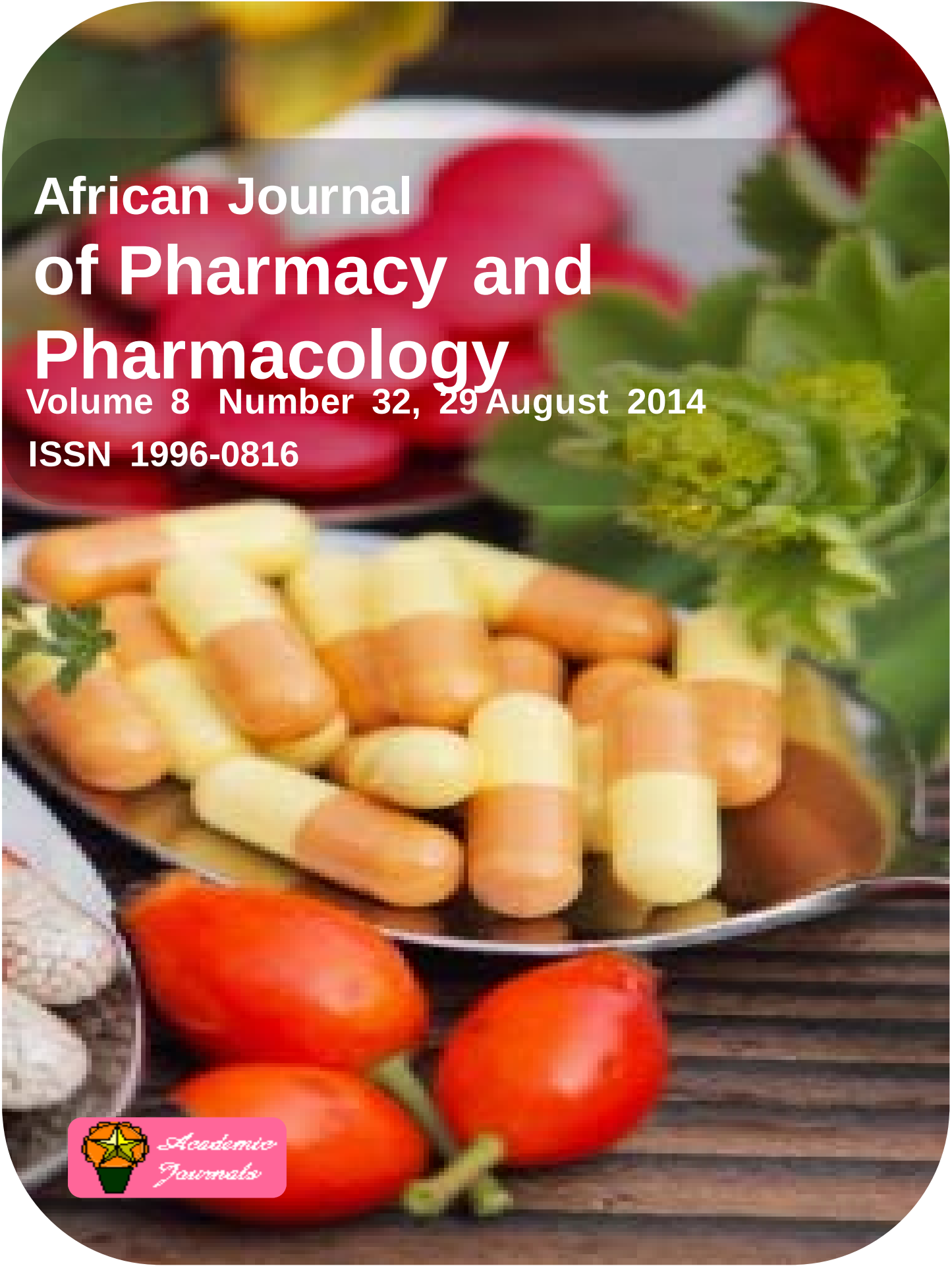

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Full Length Research Paper

Coal fly ash nanoparticles induced cytotoxicity and oxidative DNA damage and apoptosis in Chang liver cells

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Coal is the main source widely used for electric generation and industrial applications due to its low cost and abundance of this fuel. Exposure to coal fly ash particulate matter (CFA-PM) is a major health concern in developing countries. The *in vitro* cytotoxicity, oxidative DNA damage and apoptosis of coal fly ash nanoparticles (CFA-NPs) were determined by lactate dehydrogenase (LDH) enzyme, reactive oxygen species (ROS), 4'-6-diamidino-2-phenylindole (DAPI) and expression of apoptosis associated proteins in cultured Chang liver cell lines. The release of LDH was increased based on dose-dependent and time dependent manner in CFA-NPS treated cells. CFA-NPs induced reactive oxygen species (ROS) with increase in dose concentration. The CFA-NPs treated cells showed severe DNA damage and inhibits the cell viability and leads to apoptosis. The apoptotic proteins showed significant changes, with increase in the level of BAX and decrease in the level of B-cell lymphoma 2 (Bcl-2).The studies showed significant amount of toxicity in CFA-NPs treated Chang liver cell lines.

Key words: Coal fly ash nanoparticles, lactate dehydrogenase (LDH), reactive oxygen species, 4'-6-diamidino-2-phenylindole (DAPI), BAX, Bcl-2

INTRODUCTION

Coal is the major source widely used for electricity generation and industrial applications; due to its low cost and abundance of this fuel. Combustion of coal generates massive amount of ultra fine particles (UFP) formed by mineral transformation during high temperature combustion process. Exposure to coal fly ash particulate matter (CFA-PM) is a major health concern in developing countries like India and china; it is because coal is the main power source used widely. In United States and Europe; the annual production of coal fly ash (CFA) is about 67.7 million tons and 40.4 thou-sand metric tons (Dwivedi et al., 2012). In India, currently there are 82 coal-fired power plants, which consume about 260 million tonnes of coal to produce 100 million tons of fly ash per year (Dwivedi et al., 2012; Manerikar et al., 2008). The CFA emitted from the thermal power plants are controlled by several approaches; such as electrostatic precipitators, particles scrubbers, fabric filters, and mechanical

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collectors (Helble et al., 2000; Gilmour et al., 2004). The collection efficiency of these technologies is not effective for particles with aero diameter in the range of 0.1 to 1 µm (Helble et al., 2000; Zhuang et al., 2000; Dwivedi et al., 2012). These UFPs are formed as a result of shoot formation, vaporization of material matter and inorganic ash present in the coals. These vaporized particles nucleate to form large number of nano sized particles, which then grows through condensation and coagulation to form accumulation mode of aerosol (Gilmour et al., 2004). The UFP, once generated, are almost totally dispersed in the atmosphere. Due to its extremely small size, these fractions remains airborne for long period of time; which may be inhaled by the coal miners and highly impacted communities and deposited deep into the lungs epithelial cells by diffusion and entering the body through layers of cells lining alveoli of the lung and causing diseases (Costa and Dreher, 1997; Carbone et al., 2009) such as pneumoconiosis. These nanosized particles are smaller than the size of the cells and the cellular organelles, which allows them to penetrate through the basic biological organs and disturbs their normal functions, causing inflammation of tissues and alters the redox balanced towards oxidation and leads to cell death (Buzea et al., 2007).

The ultrafine particles fraction (D < 100 nm) of CFA has highest impact on human health. The greater the surface area of UFP compared with larger particles with the same chemical compositions makes them more environmentally active with respect to the bio uptake and associated health risks (Gilmour et al., 2004; Oberdoerster et al., 2005; Xia et al., 2006). The emission of particulate matter (PM) from CFA is much higher when compared to any other combustion derived nanoparticles. This emission of CFA-PM into the atmosphere causes serious environmental pollution and serious health hazards; it is due to presence of surface bound hydrocarbons and toxic heavy metals (Kalra et al., 1998; Zhou et al., 2005). The toxic heavy metals present in the CFA are Pb, Cr, Hg, Ni, V, Ba, As, Cd, Mo, Se, Zn (Aitken et al., 1984; Dalmau et al., 1990; Okeson et al., 2003).

Several toxicological studies with airborne PM, CFA and oil fly ash (OFA) has been performed. The nanosized particles provide larger surface area for binding of chemicals including transition metals which enters easily into the cells (Donaldson et al., 2004; Gwinn and Vallyathan, 2006; Duffin et al., 2007; Saquib et al., 2012). The *in vitro* CFA treatment on rat epithelial cells showed acellular generation of hydroxyl radicals and the iron in the CFA induced cytotoxicity and oxidative DNA damage (Van Maanen et al., 1999). Recent studies demonstrated that the transition elements adsorbed to OFA cause damages to DNA, mitochondrial dysfunction and lipid peroxidation in cultured human epithelial cells (Di Pietro et al., 2009; Di Pietro et al., 2011a). The transition metals present in fine PM; generated as a result of combustion induce oxidative DNA damage and inflammation (Di Pietro et al., 2011b). Cellular exposure to PM may result in pulmonary inflammation, epithelial cell damage, increased epithelial permeability and hyperreactivity (Fabbri et al., 1984).

Earlier studies have not considered the CFA particle characteristics and the toxicity of nanosized coal fly ash particles, in terms of cytotoxicity, ROS production and oxidative DNA damage in Chang liver cells. There was only limited information regarding the toxicity of CFA-NPs (Sambandam et al., 2014; Diawedi et al., 2012). In our previous work, the collection, characterization techniques such as scanning electron microscopy-energy dispersive X-ray (SEM-EDAX) analysis, particle size analyzer (PSA) and transmission electron microscope (TEM) analysis of CFA-NPs were performed (Sambandam et al., 2014). The present study elucidates the role of CFA-NPs induced cellular toxicity, generation of ROS, oxidative DNA damage and changes the ratio of apoptotic proteins in cultured human liver cell line (Chang liver cells). Previous studies have used Chang liver cells for assessing the toxicity (Viluksela et al., 1996; Ahamed et al., 2011). Since Chang liver cells were derived from human liver tissue the results can be extrapolated to mammalian models and humans. Thus, Chang liver cells were chosen for assessing the toxicity of CFA-NPs.

MATERIALS AND METHODS

Collection and characterization of CFA-NPs

CFA particles were collected from the electrostatic precipitator (ESP) of coal fired boilers located in the Thermal Power station. The collected CFA-NPs were characterized using SEM-EDAX, PSA and TEM analysis.

Cell culture

Chang liver (human liver) cell lines were purchased from National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), D-Glucose and antibiotics at 37°C under the humidified atmosphere containing 5% CO_2 in SLIM CELL incubator.

Determination of lactate dehydrogenase (LDH)

The release of LDH, an indicator of plasma membrane integrity, was measured using LDH release quantification (Fischer scientific, India) Cytotoxicity Assay Kit, in accordance with manufacturer's instructions. The percent of LDH released from the cells was determined by units/mg of protein. Release of cytosolic enzyme, lactate dehydrogenase (LDH), in the cell culture medium is indicative of cell membrane damage. Briefly, Chang liver cells (2×10^4 cells/well) were treated with various concentrations of (12.5 to 1000 µg/ml) CFA-NPs and incubated for 24 and 72 h at 37°C. Upon completion of the incubation, 50 µl of the upper medium were collected from each well. The untreated cells were then lysed with a cell lysis solution (10% Triton X 100) for 40 min at room temperature and the lysate collected. LDH activity was measured in a 96-well plate with triplicates of each group at an absorbance of 490 nm and with the reference wavelength at 630 nm. The total protein was estimated using the method reported by Lowry et al. (1951).

Determination of reactive oxygen species (ROS)

Intracellular ROS production was quantified by using fluorescent probe DCFH-DA according to previously described method (Cheng et al., 2004). For deduction of intracellular ROS, Chang liver cells were seeded into 96-well plates and incubated for 24 h at 37°C in CO_2 incubator. DMEM was replaced by PBS with glucose (5.5 mM) and the cells were treated with 1 µM of 2, 7-dichlorofluorescindiacetate (CM-H2 DCFH-DA) for 30 min at 37°C in dark. The fluorescence was recorded at 495 nm excitation and 530 nm emissions by a fluorescence microscope (Labomed, India). The generation of ROS was quantified using image J software (Version.2.1).

Nuclear staining with DAPI fluorescent dye

Apoptosis of nuclei was detected by 4'-6-diamidino-2-phenylindole (DAPI) staining assay. DAPI staining was performed as described earlier by Lou et al. (2013). Briefly, the Chang liver cell lines were seeded onto glass slides and treated with various concentrations of CFA-NPs (control, 50, 100, 250, 500 µg/ml) for 24 h. Untreated and treated cells were rinsed with phosphate buffered saline (PBS), fixed with ice-cold 10% trichloroacetic acid, and further washed with cold 70, 80, 90% and absolute ethanol. The cells were permeabilized with Triton-X (10% v/v) and stained with 1 µg/ml DAPI for 3 min. To reduce the background, the stained cells were washed with PBS, cover-slipped with 90% glycerol and observed under a fluorescence microscope (Labomed- Carl zeiss Lens with blue filter Olympus India). The image was quantified using image J software (Version.2.1) for scoring the DNA damage.

RNA extraction and cDNA synthesis

The RNA extraction and cDNA synthesis was performed by following the procedure described by Amirkhiz et al. (2013). The Chang liver cells were cultured and mRNA was synthesized for gene expression studies. Total RNA was extracted using RNX-plus solutions according to the manufacturer's protocol. Purified RNAs were dried and dissolved in 50 µl DEPC-water. Then the cDNAs was transcribed from mRNAs as decribed further. Prior to cDNA synthesis, the extracted mRNA was quantified using nanodrop UV spectrophotometer. The 20 µl reaction mixture contains 3 µg of mRNA and 15 µl of AcccuPower RT PreMix tubes. The mixture tube were loaded in thermal cycler apparatus under the following conditions: cDNA synthesis at 42°C for 60 min and 94°C for 5 min to inactivate RT-ase and terminate the reaction.

Semi-quantitative RT-PCR PCR.

The volume of each reaction was considered to be 20 µl. The Ampliqon master mix that is 2× was used to make reaction mixture as follows: 2 µl of each cDNA sample was added to 10 µl of master mix then 1 µl of each forward and reverse primers (10 mM) of Bcl-2 forward 5'-CTCGTCGCTACCGTCGTGACTTCG-3'; reverse 5'-CAGATGCC GGTTCAGGTACTCAGTC-3'; BAX forward Sense primer: 5'-CATGTTTTCT GACGGCAACTTC-3' reverse primer: 5'-AGGGCCTTGAGCACCAGTTT-3' and GAPDH forward 5'-TTACTCCTTGGAGGC CATGTGGGCC-3' reverse 5'-ACTGCCACCCAGAAGACTGTGGATGG-3' gene for internal control and 6 µl of distilled, deionised, sterile water were added to make the final volume of 20 µl and $MgCl_2$ concentrationsof 1.5 mM. In the end, one drop of mineral oil was added to the top of each sample to avoid evaporation of samples. Then microtubes were put in the thermal cycler apparatus under the following conditions: initial denaturation at 94°C for 4 min, followed by 35 amplification cycles, each consisting of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, with an additional extension step at the end of the procedure at 72°C for 5 min. All RT-PCR products were visualized by electrophoresis through 2% agarose gel followed by ethidium bromide staining.

RESULTS

Characterization of CFA-NPs

The CFA-NPs characterization and its effects were studied briefly in our previous work (Sambandam et al., 2014). The composition of CFA varies with the origin and the quality of the coal. The morphological analysis of CFA-NPs showed spherical shaped particles. The EDAX analysis revealed the presence of several major elemental constituents such as A1, Fe, Ca, C, S, Si, Mg, Na, Mo and other trace elements such as Zn, Rh, Ar, Cd, K, Ba, Ti, V, Nd, Cr, Mn, Co, Ti, Pb, Se, Sr were also associated with the particle surfaces. The particle size analysis of CFA-NPs showed the uniform distribution of CFA-NPs with an average size about 50 nm. The trans-mission electron microscopy (TEM) image of CFA-NPs shows the presence of spherical shaped particles with the size range varying from 9 to 24 nm. The average size of the collected CFA particles ranges below the PM 0.1 which was confirmed by PSA and TEM analysis (results not shown).

CFA-NPs effects on LDH

The cytotoxic effect and cell lysis was determined by LDH quantification was shown in Figure 1. The CFA-NPs treated Chang liver cells showed an increase in the level of LDH release in cell supernatant. It reached up to 15.13 and 16.23 U/mg during 24 and 48 h incubation at higher dose concentration. It was observed that, there is a gradual release in the level of LDH in all the CFA-NPs treated concentrations. Thus the release of LDH was observed in time- and dose dependent process. The in-creased LDH levels and LDH mediated cell death are due to CFA-NPs toxicity. The increase in the concentration of CFA-NPs treated cells showed increase in the release of LDH levels. The cytoplasmic enzyme release was expo-nential at higher dose treated CFA-NPs concentrations and the release of these enzymes starts even at initial concentration of CFA-NPs treated cells. Diabate et al. (2011) reported that the release of LDH was increased when treated with MAF98 fly ash and decreased against CBI4 NPs. The toxic effect differs, due to chemical nature and place of origin. The release of LDH from the cells were measured in mice; after intratracheal instillation of 25 and 100 µg of ultrafine coal fly ash showed significant increase after 4 h exposure when compared with the control (Gilmour et al., 2004).

CFA-NPs effects on ROS production

Intracellular ROS generation induced by CFA-NPs was

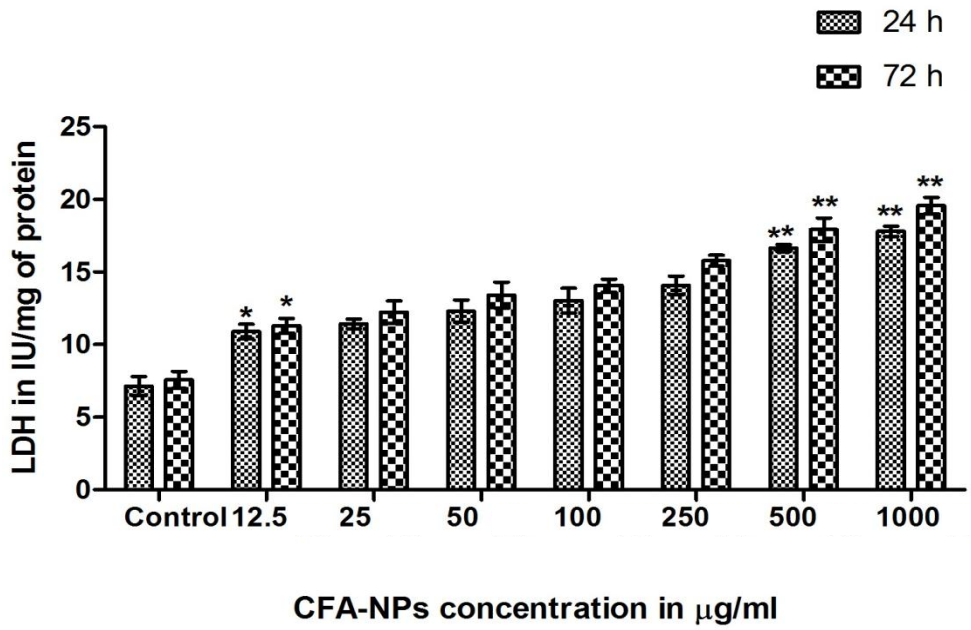

Figure 1. Cytotoxicity effects of CFA-NPs on the LDH enzyme. Chang liver cells treated with various concentrations (12.5, 25, 50, 100, 250, 500 and 1000 µg/ml) of CFA-NPs showed concentration and time dependent increase in the relase of LDH enzyme.

examined using a DCFH-DA fluorescence probe. The fluorescence intensity of oxidative DCF in reaction with various concentrations of CFA-NPs (12.5, 250 and 1000 µg/ml) was determined in Chang liver cells was shown in Figure 2. The ROS was generated significantly with increase in concentrations of CFA-NPs. The CFA-NPs treated Chang liver cells showed 1.5 fold increases in the generation of ROS when compared to the control cells. The generation of ROS represents an early and sensitive cellular response to PM (Valko et al., 2006). Nel et al. (2006) reported that production of ROS is one of the primary mechanisms of nanoparticles toxicity; it results in oxidative stress, inflammation and damaged cell membranes and DNA. Rapid generation of ROS to an extent may overwhelm the antioxidant defence system and results in oxidation, and therefore destruction of cellular biomolecules, such as DNA, leading to heritable mutations (Risom et al., 2005; Peters et al., 2006). ROS generation and induction of oxidative stress may be due to particle surface or metals associated on the surface (Diabate et al., 2011). Dwivedi et al. (2012) showed that the Fe (III) associated CFA-NPs showed a dose dependent increase in the level of generation of ROS in PBMN cells. Fly ash treatment on rat lung epithelial cells showed increase in the generation of ROS in dose dependent manner; which may be due to insoluble compounds present in the fly ash (Diabate et al., 2011).

DAPI assay

DNA morphological changes in cells undergoing apoptosis (condensation and fragmentation of DNA) can be monitored via DAPI-staining method. DAPI displays enhanced blue fluorescence upon selective binding to double stranded DNA. The nuclear staining assay was used to determine the morphological changes of apoptosis in Chang liver cells. The DAPI staining of CFA-NPs treated cells showed significant induction of nuclear rounding, chromosomal alterations and nuclear membrane shrinkage. In liver cells at concentration 500 µg/ml of CFA-NPs treated cells it increased up to 3.2 fold in the level of nuclear modification, and damage compared to control cells was shown in Figure 3. The DAPI dye stains the nuclear DNA of cells and indicates the morphological changes in the nuclei of treated cells undergoing apoptosis. The changes in the nuclei and DNA damage was observed in liver cells of all tested concentrations. At higher concentration of CFA-NPs treated cells showed up to 3.5 folds of DNA damage when compared to control cells. There was significant damage of DNA observed with increase in concentration of CFA-NPs treated cells. Upadhyay et al. (2003) showed that the PM induced the dose-dependent DNA damage and apoptosis in Alveolar epithelial cells. The PM [benzo[a]pyrine adsorbed on carbon black (CB+BaP)] treatment on cultured macrophages cells showed a time dependent expression and release of TNF-α and also induced cell death by DNA damage (Upadhyay et al., 2003). The blastocytes isolated from rats showed concentration dependent increase in the rate of apoptosis when compared to the control groups on treatment with various increasing concentrations of diesel exhaust particles (Januario et al., 2010).

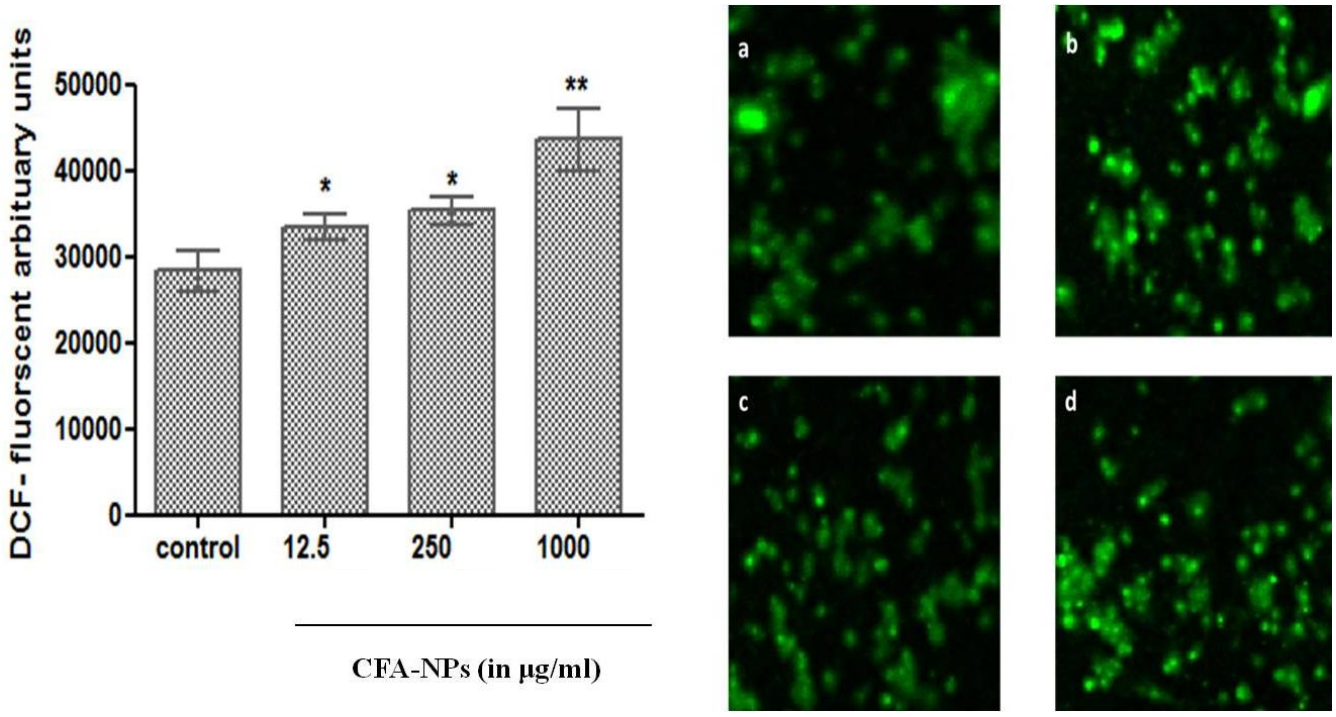

Figure 2. The level of intracellular ROS in CFA-NPS treated Chang liver cells showed concentration dependent increase in the level of ROS (a - Control, b, c, d - 12.5, 250 and 1000 µ/ml of CFA-NPs treated cells).

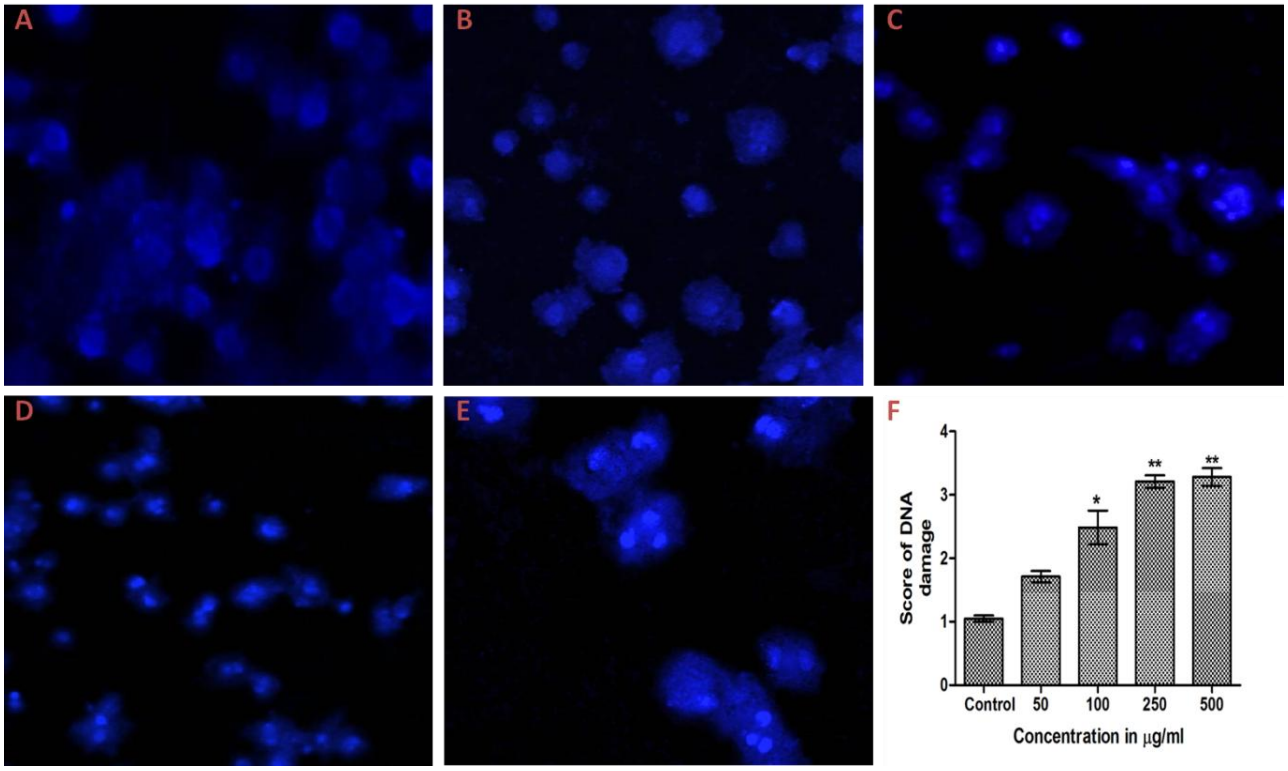

Figure 3. The various concentrations of CFA-NPs treated DAPI stained cells showed morphological changes and nuclear condensation in Chang liver cells (A - control, B, C, D, E - fluorescent microscopy images of various concentration of CFA-NPs treated DAPI stained Chang liver cells (50,100, 250 and 500 µg/ml), F- graphical representation of various concentrations of CFA-NPs treated DAPI stained Chang liver cells).

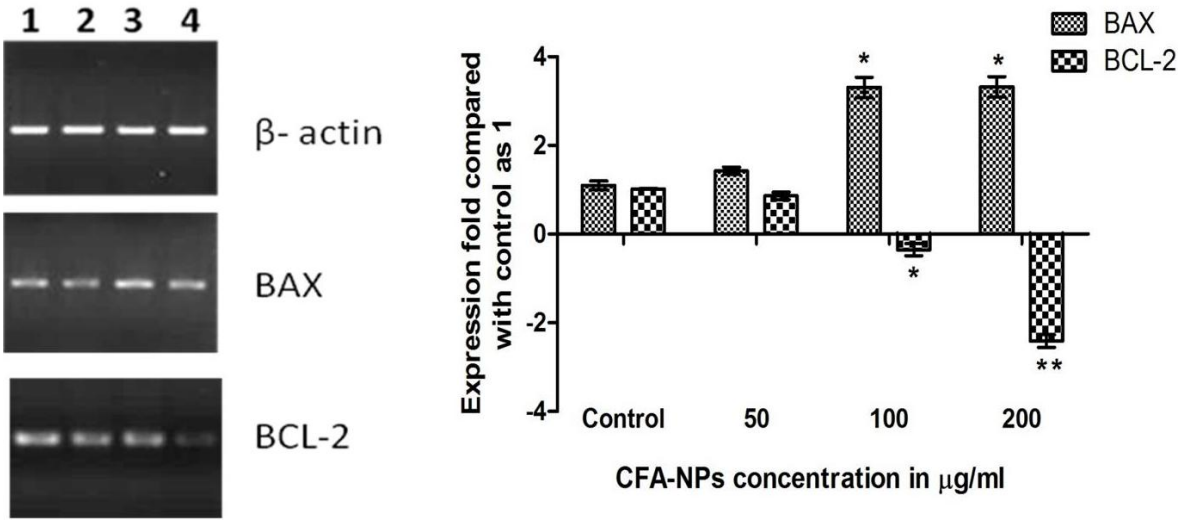

Figure 4. The various concentrations of CFA-NPs induced alteretion in expression of BAX and Bcl-2 protein (Lane 1 - control, Lane 2, 3, 4 - various concentrations (50, 100 and 200 µg/ml) of CFA-NPS treated changliver cells expressed alteration in level of BAX and Bcl-2. The expression were compared with the β-actin served as a control.

The effect of CFA-NPs on BAX/Bcl-2 ratio in chang liver cells

The ratio of BAX-to- Bcl-2 protein can determine the susceptibility of the cell apoptosis. Changes in the regulation of mRNA on treatment with CFA-NPs were examined by reverse transcriptase PCR in order to determine cell death by altering the ratio between Bcl-2 and BAX. The Figure 4 showed treatment on Chang liver cells with various concentrations of CFA-NPs showed a significant dose-dependent decrease in the level of expression of Bcl-2 and increase in the level of BAX when compared to the control. A densitometric analysis of the bands showed that CFA-NPs resulted in a dose dependent increase in the BAX/Bcl-2 ratio.The alteration in the expression of BAX and Bcl-2 leads to high changes in the regulation of BAX/Bcl2 which is an important factor in determining the cell's vulnerablity to apoptosis. These two proteins play a prominent role in the regulation of apoptosis. The Bcl-2 mRNA levels were down regulated at 100 and 200 µg/ml of CFA-NPs treated concentrations. The significant upregulation of pro-apototic gene (BAX) expression may result in altering mitochondria per-meability. It is a key regulator of mitrochondirial damage in lung cells (Lee et al., 2012). All the tested mRNA gene expressions were compared with internal control gene expression (β-actin). Ahamed et al. (2011) showed that cells exposed to ZnO nanorods showed significant decrease in the level of apoptotic mRNA of Bcl-2 and increase in the level of pro-apoptotic protein; suggesting that these two proteins could be the excellent biomarker to apoptotic response to NPs (Zhu et al., 2007). Previous reports findings demonstrated that metal oxide NPs have greater potential to induce apoptotic cell death (Ahamed et al., 2010; Nel et al., 2006; Gojova et al., 2007).

Conclusion

We demonstrated that CFA-NPs induced cytotoxicity, oxidative stress, DNA damage and apoptosis in Chang liver cells. The toxicity of CFA-NPs depends on size of the particles and chemical composition. CFA-NPs with surface adsorbed heavy metals can act as a cellular and DNA toxicant, capable of inducing oxidative stress, DNA damage and cell death. The expression of pro-apoptotic protein BAX were upregulated, and the antiapototic protein Bcl-2 were down regulated by CFA-NPs. These suggest that the CFA-NPs induced apoptosis in Chang liver cells, which acts as an indicating event in triggering process for carcinogenesis and mutagenesis. Considering the level of toxicty at cellular levels, the population exposed to CFA-NPs are being subjected to major health effects. Primary exposure to CFA-NPs induces oxidative damage in the cells; on prolonged exposure to this CFA-NPs may cause deadly diseases. It is necessary to investigate the potential risk of CFA-NPs to human health by further implementing *in vivo* toxicity studies to divulge the general mechanisms of organ toxicity. The population affected by these CFA-NPs should be treated with appropriate drugs. The future studies should be focussed on further evaulating the toxicty of CFA-NPs using different challenge studies in cell models. The flavonoids can be suggested as a drug for its protective and curative effects against CFA-NPs toxicity.

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REFERENCES

Aitken RL, Campbell DJ, Bell LC (1984). Properties of Australian flyashes relevant to their agronomic utilization. Austr. J. Soil Res. 22(4):443-453.

Ahamed M, Siddiqui MA, Akhtar MJ, Ahmad I, Pant AB, Alhadlaq HA (2010). Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. Biochem. Biophys. Res. Commun. 396:578-583.

Ahamed MJ, Akhtar MA, Siddiqui MA, Ahmad J, Musarrat J, Al-Khedhairy AA, Alsalhi MS, Alrokayan SA (2011). Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. Toxicology 283(2-3):101-108.

Amirkhiz MB, Rashtchizadeh, N, Nazemiyeh, H, Abdolalizadeh, J, Mohammadnejad L, Baradaran B (2013). Investigating apoptotic effects of methanolic extract of *Dorema glabrum* seed on WEHI-164 cells. ISRN Pharmacology 949871:1-9.

Buzea C, Pacheco Blandino II, Robbie K (2007). Nanomaterials and nanoparticles: sources of toxicity. Biointerphases 2(4):17-71.

Costa DL, Dreher KL (1997). Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. Environ. Health Perspect. 105(5):1053-1060.

Cheng YW, Lee WW, Li CH, Lee CC, Kang JJ (2004). Genotoxicity of motercycle exhaust particles In vivo and In vitro. Toxicol. Sci. 81(1):103-111.

Carbone F, Pagliara R, Barone AC, Beretta F, D'Anna A (2009). Characterization of Nano-Ashes Generated during Pulverized Coal Combustion. National Agency for New Technologies, Energy and Environment Program Agreement Ministry of Economic Development – ENEA. Report RSE/2009/109. Italy. P 6.

Dalmau JL, Garau MA, Felipo MT (1990). Laboratory prediction of soluble compounds before soil recycling of wastes. Int. J. Environ. Analyt. Chem. 39(2):141-146.

Donaldson K, Stone V, Tran CL, Kreyling W, Borm PJ (2004). Nanotoxicology: a new frontier in particle toxicology relevant to both the workplace and general environment and to consumer safety. Occup. Environ. Med. 61:727–728.

Duffin R, Mills NL, Donaldson K (2007). Nanoparticles, a thoracic toxicology perspective. Yonsei Med. J. 48(4):561–572.

Di Pietro A, Visalli G, Munaò F, Baluce B, La Maestra S, Primerano P, Corigliano F, De Flora S (2009). Oxidative damage in human epithelial alveolar cells exposed in vitro to oil fly ash transition metals. Int. J. Hyg. Environ. Health 212(2):196–208.

Di Pietro A, Visalli G, Baluce B, Micale R, Maestra SL, Spataro P, De Flora S (2011a). Multigenerational mitochondrial alterations in pneumocytes exposed to oil fly ash metals. Int. J. Hyg. Environ. Health 214(2):138–144.

Di Pietro A, Baluce B, Visalli G, Maestrab SL, Micale R, Izzoti A (2011b). Ex vivo study for the assessment of behavioural factor and gene polymorphisms in individual susceptibility to oxidative DNA damage metals-induced. Int. J. Hyg. Environ. Health 214(3):210–218.

Diabate S, Bergfeldt B, Plaumann D, Ubel C, Weiss C (2011). Anti-oxidative and inflammatory responses induced by fly ash particles and carbon black in lung epithelial cells. Analyt. Bioanalyt. Chem. 401(10):3197-3212.

Dwivedi S, Saquib Q, Al-Khedhairy AA, Ali AS, Musarrat J (2012). Characterization of coal fly ash nanoparticles and induced oxidative DNA damage in human peripheral blood mononuclear cells. Sci. Total Environ. 437:331-338.

Fabbri LM, Aizawa H, Alpert SE (1984). Airway hyper-responsiveness and changes in cell counts in bronchoalveolar lavage after ozone exposure in dogs. Am. Rev. Respir. 129(2):288-291.

Gilmour MI, O'Connor S, Dick CAJ, Miller CA, Linak WP (2004). Differential pulmonary inflammation and in vitro cytotoxicity of size-fractionated fly ash particles from pulverized coal combustion. J. Air Waste Manag. Assoc. 54(3):286-295.

Gwinn MR, Vallyathan V (2006). Nanoparticles: health effects-pros and cons. Environ. Health Perspect. 114:1818–1825.

Gojova A, Guo B, Kota RS, Rutledge JC, Kennedy IM, Barakat AI (2007). Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environ. Health Perspect. 115:403-409.

Helble JJ (2000). A model for the air emissions of trace metallic elements from coal combustors equipped with electrostatic precipitators. Fuel Process. Technol. 63:125-147.

Januario DANF, Perin PM, Maluf M, Lichtenfels AJ, Saldiva PHN (2010). Biological Effects and Dose–response Assessment of Diesel Exhaust Particles on In Vitro Early Embryo Development in Mice. Toxicol. Sci. 117(1):200–208.

Kalra N, Jain MC, Joshi HC, Choudhary R, Harit RC, Vatsa BK, Sharma SK, Kumar V (1998). Fly ash as a soil conditioner and fertilizer. Bioresour. Technol. 64(3):163–167.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265-275.

Lee JS, Jung WK, Jeong MH, Yoon TR, Kim HK (2012). Sanguinarine Induces Apoptosis of HT-29 Human Colon Cancer Cells via the Regulation of Bax/Bcl-2 Ratio and Caspase-9-Dependent Pathway. Int. J. Toxicol. 31(1):70-77.

Lou S, Zhong L, Yang X, Xue T, Gai R, Zhu D, Zhao Y, Yang B, Ying M, HE Q (2013). Efficacy of all-trans retinoid acid in preventing nickel induced cardiotoxicity in myocardial cells of rats. Food Chem. Toxicol. 51:251-258.

Manerikar RS, Apte AA, Ghole VS (2008). In vitro and in vivo genotoxicity assessment of Cr(VI) using comet assay in earthworm coelomocytes. Environ. Toxicol. Pharmacol. 25:63–68.

Nel A, Xia T, Madler L, Li N (2006). Toxic potential of materials at the nano level. Science 311(5761):622-627.

Oberdoerster G, Oberdoerster E, Oberdoerster, J (2005). Nanotoxicology: An emerging discipline evolving studies of ultrafine particles. Environ. Health Persp. 113:823–839.

Okeson CD, Riley MR, Fernandez A, Wendt JL (2003). Impact of combustion generated fine particles on epithelial cell toxicity: influences of metals on metabolism. Chemosphere 51(10):1121-1128.

Peters A, Veronesi B, Calderon-Garciduenas L, Gehr P, Chen LC, Geiser M, Reed W, Rothen Rutishauer B, Schurch S, Schultz H (2006). Translocation and potential neurological effects of fine and ultrafine particles. A critical update Part. Fibre Toxicol. 3:1-13.

Risom L, Moller P, Loft S (2005). Oxidative stress-induced DNA damage by particulate air pollution. Mutat. Res. 592:119-137.

Saquib Q, Al-Khedhairy AA, Siddiqui MA, Abou-Tarboush FM, Azam A, Musarrat J (2012). Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells. Toxicol. In vitro 26(2):351–361.

Sambandam B, Palanisami E, Abbugounder R, Prakhya B, Thiyagarajan D (2014). Characterizations of coal fly ash nanoparticles and induced in vitro toxicity in cell lines. Nanopart. Res. 16:2217-2226.

Upadhyay D, Panduri V, Chio A, Kamp W (2003). Particulate matter induces alveolar epithelial cell damage and apoptosis. Am. J. Respir. Cell Mol. Biol. 29(2):180-187.

Viluksela M, Vainio PJ, Tuominen RK (1996). Cytotoxicity of macrolide antibiotics in a cultured human liver cell line. J. Antimicrob. Chemother. 38:465-473.

Van Maanen JMS, Borm PJA, Knaapen A, van Herwijnen M, Schilderman PAEL, Smith KR, Aust AE, Tomatis M, Fubini B (1999). In vitro effects of coal fly ashes: hydroxyl radical generation, iron release, and DNA damage and toxicity in rat lung epithelial cells. Inhalation Toxicol. 11(12):1123-1141.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160:1-40.

Xia T, Kovochick M, Brant J, Hotze M, Sempf J, Oberley T, Sioutas C, Yeh JI, Wiesner MR, Nel AE (2006). Comparisons of the ability of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett. 6(8):1794–1897.

Zhuang Y, Kim YJ, Lee TG, Biswas P (2000). Experimental and theoretical studies of ultra-fine particle behaviour in electrostatic precipitators. J. Electrostatics 48(3-4)**:**245-260.

Zhu L, Chang DW, Dai L, Hong Y (2007). DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. Nano Lett. 7(12):3592-3597.

Zhou JB, Wang TG, Huang YB, Mao T, Zhong NN (2005). Size distribution of polycyclic aromatic hydrocarbons in urban and suburban sites of Beijing, China. Chemosphere 61(6):792–799.

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Review

Antibacterial activity of freshwater microalgae: A review

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The photoautotrophic microorganisms collectively termed 'micro-algae' (including micro-eukaryotes and cyanobacteria) are known to produce a wide range of secondary metabolites with various biological actions. They are known as well for their richness in bioactive compounds, with promising applications in pharmaceutical formulations. Their cell-free extracts have accordingly been tested as additives for food and feed formulation, in attempts to circumvent use of antimicrobial compounds of synthetic origin, or subtherapeutical doses of regular antibiotics. The increased use of antibiotics and chemotherapeutants for disease treatment leads to emergence of drug resistant forms. It also adversely affects the ecosystem. Microalgae are rich source of antimicrobial agents and provide a safer and cost effective way of treating bacterial infections. This article describes the antibacterial properties of some freshwater microalgae like, *Euglena*, *Microcystis*, *Chlorella*, *Chroococcus*, *Anabaena*, *Oscillatoria* and *Spirulina*.

Key words: Antibacterial, *Aeromonas hydrophila*, bioactive compounds, extraction, *Euglena viridis*.

INTRODUCTION

Microbial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world. There is a widespread belief that green medicines are healthier and harmless or safer than synthetic ones because of their limited side effects. Algae are now drawing a greater interest following the increase in demand for biodiversity in the screening programs seeking therapeutic drugs from natural products. Microalgae exhibit a notable biodiversity; they can in fact be found as individual cells, colonies or extended filaments. These microorganisms account for the basis of the food chain in aquatic ecosystems; they possess the intrinsic ability to take up H_2O and CO_2 that, with the aid of solar energy, are used to synthesize complex organic compounds, which are subsequently accumulated and/or secreted as primary or secondary metabolites. They are ubiquitously distributed throughout the biosphere, where they have adapted to survival under a large spectrum of environmental stresses for example, heat, cold, drought, salinity, photo-oxidation, anaerobiosis, osmotic pressure and UV

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exposure (Tandeau-de-Marsac, 1993); hence, they may grow essentially under all environmental conditions available, ranging from freshwater to extreme salinity, and can survive in moist, black earth and even desert sands and they have as well been found in clouds, being in addition essential components of coral reefs. This wide spectrum of ecosystems contributes to the myriad of chemical compounds that they are able to synthesize, thus accounting for their unique potential as stakeholders in blue biotechnology. As many as 30,000 distinct micro-algal species might inhabit the earth and over 15,000 novel compounds have been chemically obtained from them (Cardozo et al., 2007; Rodr´ıguez-Meizoso et al., 2010). In addition, their importance as a source of novel compounds is growing rapidly, and researches have indicated that these compounds exhibit diverse biological activities (Wijesekara et al., 2010).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The majority of clinically used antimicrobial drugs have drawbacks like toxicity, lack of efficacy, inhibiting cost and their frequent use leading to the emergence of resistant strains. Thus, there is an urgent need to develop alternative biodegradable agents, which should have minimal side effects. It is generally considered that natural compounds are biodegradable and so more environmentally acceptable. Commercial applications of microalgae derived compounds have, as yet, received little attention in the area of pharmaceuticals, antibiotics and other biologically active compounds. Some researchers have envisioned the enormous possibilities of algae and microalgae as potential source of bioactive compounds; particularly, some microalgae have been studied as a potential natural source of different functional compounds (Herrero et al., 2006; Rodrı´guez-Meizoso et al., 2008). They are able to produce a wide range of biologically active substances with antibacterial, antiviral, antifungal, enzyme inhibiting, immunostimulant, cytotoxic and antiplasmodial activities (Ghasemi et al., 2004).

Freshwater microalgae as source of antibacterial agents

Microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to be produced by chemical synthesis (Borowitzka and Borowitzka, 1989; Goud et al., 2007; Kaushik and Chauhan, 2008). Most of those compounds are accumulated in the microalgal biomass; others are excreted during growth into the environment (Jaki et al., 2000, 2001). This review article describes some freshwater microalgae, which contains potential bioactive compounds for antimicrobial activity.

Euglena viridis

Euglena viridis is a unicellular flagellate algal protist, which occur both in freshwater and marine forms. The taxonomic position of this alga is Phylum Euglenozoa, Class Euglenida, Order *Euglenales* and Family *Euglenaceae* (Ehrenberg, 1830). It is usually a free swimming, fusiform, elongate, lanceolate, spindle-shaped, flexible unicellular mobile animal. It has one or rarely two flexible flagella issuing out of an anterior notch at the base of which is an oval aperture and distinctive red pigment spot known as eye-spot. *E. viridis* is characterised by a single stellate group of band shaped chloroplast and finely striated delicate periplast, varying from 40 to 150 µm in length. It exhibits positive phototaxis, determined by a photoreceptive spot in the wall of the contractile vacuole. All members contain chlorophyll a, b, β-carotene and xanthophylls (Cunningham and Schiff, 1986; Fiksdahla and Liaaen-Jensena, 1988). There is no cell wall and the reserve food material is paramylon and oil. *Euglena* forms red blooms in all type of water bodies when density is very high, characterised by formation of haematochrome during bright sunny days. The coloration is green in cloudy days (Biswas, 1949).

Microcystis aeruginosa

Microcystis is a type of blue green alga (also referred to as cyanobacteria) and are the dominant phytoplankton group in eutrophic freshwater bodies (Davidson, 1959; Negri et al., 1995). The unicellular, planktonic freshwater cyanobacterium belongs to Phylum Cyanobacteria; Class Cyanophyceae; Order Chroococcales and Family Microcystaceae (Kützing, 1846). Among all the species reported worldwide, *Microcystis aeruginosa* is one of the important cyanobacteria characterized by mucilage covering with a cell size of 3 to 4 µm and with varying colonies ranging from few to hundreds of cells (Biswas, 1949). It is one of more than 700 species of algae that may be found in water samples collected and usually blooms in mid to late summer. The extracellular covering of *M. aeruginosa* is divided into several layers: the cytoplasmic membrane or plasmalemma, the peptidoglycan layer, and the multilayered structure of the cell wall (Kim et al., 1997). These common bloom-forming algae are especially abundant in shallow, warm, nutrient enriched fresh waters and lower salinity estuaries. These are also found in polluted water low in oxygen and can grow to form thick scums that could colour the water (Stotts et al., 1993). *M. aeruginosa* produces vast number of peptides for example, aeruginosins, microginins, microviridins, aeruginoguanidins, aeruginosamides, kasumigamide, some of which are highly toxic (Ishida and Murakami, 2000; Ishida et al., 2002). They are producing two kinds of toxin, the cyclic peptide hepatotoxin and the alkaloid neurotoxin.

Chlorella vulgaris

The simple and common fresh water, single-celled green algae of the genus *Chlorella* (Beijernick, 1890), are placed under the order Chlorococcales and family Chlorellaceae (Hoek et al., 1995). Cells are spherical in shape tending to aggregate into colony; yellowish green, 4 to 8 µm in diameter, and is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast often with one pyrinoid, more or less situated in the middle. Cell wall is smooth and thin hyaline. *Chlorella vulgaris* (*C. vulgaris*) is a genus of unicellular green algae containing high level of protein (50 to 70% of dry matter), lipid, vitamins and minerals (Phang, 1992). *Chlorella* is a nutrient-dense super food that contains 60% protein, 18 amino acids (including all the essential amino acids), and various vitamins and minerals. When dried, it is about 45% protein, 20% fat, 20% carbohydrate, 5% fiber, and 10% minerals and vitamins (Phang, 1992; Khatun et al., 1994). The nucleus of *Chlorella* cell contains a unique nucleotide-peptide complex known as *Chlorella* growth factor (CGF) which actually promotes cell growth in the body, stimulating tissue repair and healing to an extent. It is also a reliable source of essential fatty acids that are required for many important biochemical functions, including hormone balance. *Chlorella* also contains high levels (ranging from 3 to 5%) of chlorophyll, beta-carotene and RNA/DNA (Gouveia et al., 1996). Chlorophyll is one of the greatest food substances for cleansing the bowel and other elimination systems, such as the liver and the blood. More than 20 vitamins and minerals are found in *Chlorella*, including iron, calcium, potassium, magnesium, phosphorous, pro-vitamin A, vitamins C, B1, B2, B5, B12, E and K, inositol, folic acid (Milner, 1953).

Spirulina platensis

Spirulina is a photosynthetic, filamentous, spiral-shaped, multicellular blue green microalga, generally found in fresh water. The two most important species are *Spirulina maxima* and *Spirulina platensis*. It contains carotenoid, chlorophyll, and major phycocyanin pigment. It belongs to Cyanophyceae class, Oscillatoriaceae family; this cyanobacteria is characterized by spiral chains of the cells enclosed in a thin sheath. It is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates and γ-linolenic acid (James et al., 2006). It consists of 60 to 70% protein in dry weight. The protein elements consist of 18 types of amino acids, several vitamins, such as vitamins A, B, E, and K, minerals, and fatty acids necessary for the body. It is gaining more and more attention, not only for the food aspects but also for the development of potential pharmaceuticals (Quoc and Pascaud, 1996). *Spirulina* contains a whole spectrum of natural mixed carotene and xanthophyll phytopigments which, together with phycocyanin, seem to be related to its antioxidant activity (Pineiro et al., 2001). Basic constituents of different freshwater microalgae were represented in Figure 1.

Antibacterial compounds from freshwater microalgae

Microalgae constitute one of the commercially important living and renewable resources. They contain more than sixty trace elements including minerals, proteins, iodine, bromine and many bioactive substances. To date, many chemically unique compounds of fresh water origin with various biological activities have been isolated, and some of them are under investigation and some are being used to develop new pharmaceuticals. Algae are a very interesting natural source of new compounds and many of them possess antioxidant, antimicrobial, and antiviral activities (Plaza et al., 2010; Rodr´ıguez-Meizoso et al., 2010). These organisms live in habitats exposed to extreme conditions, and therefore they must adapt rapidly and efficiently, and as a consequence, produce a great variety of biologically active secondary metabolites that participate in the natural defense mechanisms (Rodr´ıguez-Meizoso et al., 2010). These defense strategies can result in a high level of structural and chemical diversity of compounds, originating from different metabolic pathways. Microalgae can biosynthesize, metabolize, accumulate and secrete a great diversity of primary and secondary metabolites, many of which are valuable substances with potential applications in the food, pharmaceutical and cosmetics industries (Yamaguchi, 1997). A large number of algal extracts have been found to have antimicrobial activity (Mao and Guo, 2010; Plaza et al., 2010). Major group of antimicrobial agents found in freshwater microalgae are fatty acids, lipids, pigments, polyphenols, carbohydrates, simple hydrocarbons and some other derivatives (Table 1).

Much attention has been focused on the microalgae as sources of novel, biologically active compounds such as phycobiline, phenols, phenolic glycosides, saponins and phytoalexins terpenoids, steroids and polysaccharide (Li et al., 2007). The important compounds also identified as antimicrobial are fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols (Kannan et al., 2010). Many of the structures identified as fatty acids and hydroxyl unsaturated fatty acids, glycolipids, steroids, phenolics and terpenoids. lauric acid, palmitic acid, linolenic acid, oleic acid, stearic acids are known to be potential antibiotics (MacMillan et al., 2002; Shanab, 2007; Tan, 2007). Algal lipids are composed of glycerol, sugars or bases esterified to saturated or unsaturated fatty acids (12 to 22 carbon atoms). Among all the fatty acids in microalgae, some fatty acids of the ω3 and ω6

Table 1. Antibacterial compounds from different algae and their target bacterial pathogens.

Antibacterial compound	Microalgae	Target bacterial pathogens	References
Pigments	*Anabaena cylindrical Chlorococcum humicola, Spirulina platensis, Nostoc*	*E. coli, S. typhimurium*, *K. pneumoniae*, *V. cholerae*, *S. aureus*, *B. subtilis, Streptococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *E. coli, Enterobacteria aerogens*	Jaya Prakash et al. (2007), Bhagavathy et al. (2011), Muthulakshmi et al. (2012) and Fan et al. (2013)
Fatty acids and Lipids	*Dunaliella salina*, *Haematococcus pluvialis*, *Phaeodactylum tricornutum, Chaetoceros muelleri, Spirulina platensis*	*Escherichia coli, Staphylococcus aureus,* MRSA, *Listonella anguillarum, Lactococcus garvieae, Vibrio* spp	Xue et al. (2002), Herrero et al. (2006) and Santoyo et al. (2009)
Carbohydrates	*Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica, S. platensis, Porphyridium cruentum*	*E. coli, S. typhimurium, S. faecalis*	O'Doherty et al. (2010) and Abdo et al. (2012)
Polyphenols	*Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica* and *Spirulina platensis*	*Salmonella typhi, Streptococcus*, *E. coli* and *Staphylococcus aureus*	Gao and Zhang (2010), Klejdus et al. (2010), Shu et al. (2011), Abdo et al. (2012) and Hetta et al. (2014)

families are of particular interest (Spolaore et al., 2006). Many authors have found that antibacterial activities of micro algae are due to fatty acids (Kellam et al., 1988). Antibacterial activity of unsaturated and saturated long chain fatty acids of chain length more than 10 carbon atoms induced lysis of bacterial protoplasts.

Naviner et al. (1999) reported inhibition of various marine bacteria using fatty acids of the alga, *Skeletonema costatum*. *Chlamydomonas reinhardtii* has unsaturated fatty acids like linolenic, linoleic, oleic and palmitoleic acid and saturated fatty acids such as palmitic, stearic, myristic acid. Crude fatty acid extracts of *C. reinhardtii* showed antibacterial activity (Sudalayandi et al., 2012). *Spirulina* lipids were investigated as a natural source of functional bioactives because of its usefulness for human health (Ramadan et al., 2008). Specifically, the antimicrobial activity of the methanolic extract of *S. platensis* was explained by the presence of γ-linolenic acid (Demule et al., 1996), an antibiotically active fatty acid present in a high concentration in this alga (Xue et al., 2002). A bioactive modified peptide, aeruginosamide has been isolated from the cyanobacterium *Microcystis aeruginosa* (Lawton et al., 1999).

Carbohydrates in microlagae can be found in the form of starch, glucose, sugars and other polysaccharides. Most microalgae produce polysaccharides and some of them could have industrial and commercial applications, considering the fast growth rates and the possibility to control the environmental conditions regulating its growth. Aqueous extract of five freshwater algal species, *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica*, *Spirulina platensis* and *Cosmarium leave* contains polysaccharides which showed antibacterial activity when tested against *Escherichia coli, Salmonella typhimurium* and *Streptococcus faecalis* (Abdo et al., 2012). The major carbohydrate of *Euglena*, the glucose polymer paramylon, and its role in the biogenesis of chloroplasts have been investigated widely during the past 15 years (Dwyer and Smillie, 1970; Manners and Sturgeon, 1982). The most promising microalga for commercial purposes is the unicelular red alga *Porphyridium cruentum*, which produces a sulphated galactan exopolysaccharide that can replace carrageenans in many applications (Gouveia et al., 2008). Certain highly sulphated algal polysaccharides also present pharmacological properties acting on the stimulation of the human immune system (Pulz and Gross, 2004). Among many different algal polysaccharides, the most important are galactans, fucoidan, laminarin and alginates (Ferreira et al.,

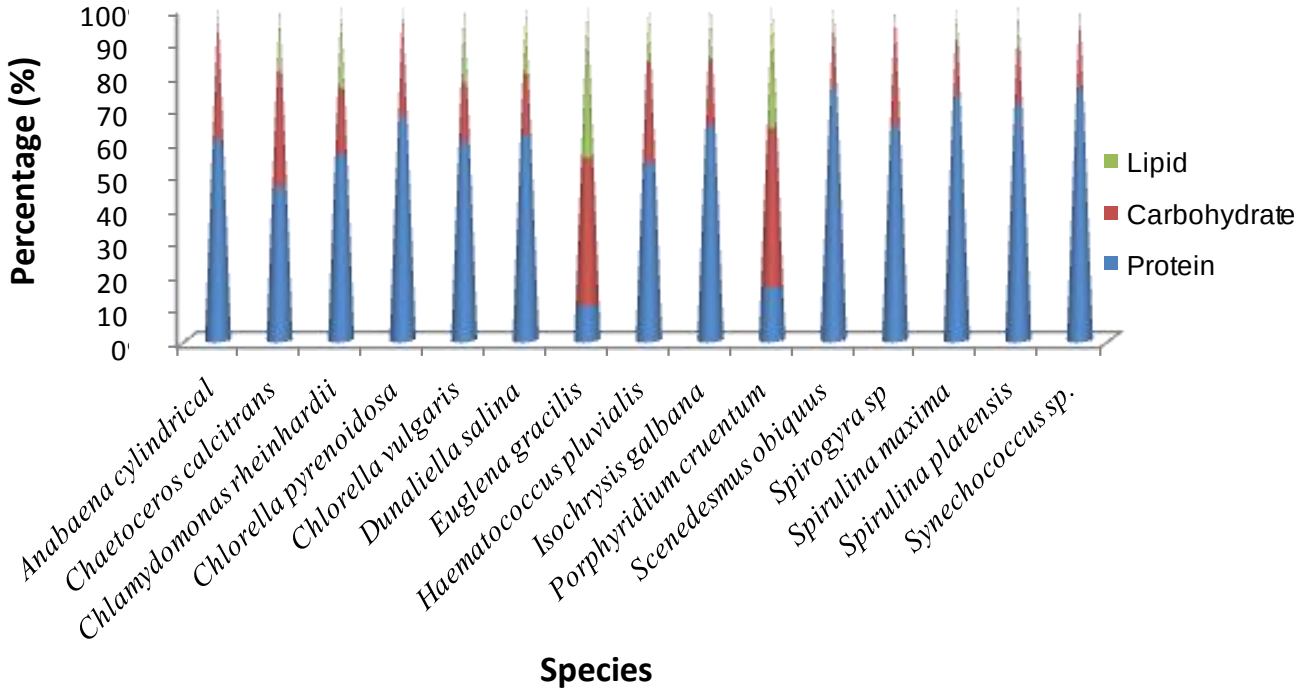


Figure 1. Basic constituents of different freshwater microalgae in percentage.
Source: Gouveia et al. (2008).

2012). Laminarin is one of the major polysaccharides found in brown algae with antiviral and antibacterial properties (O'Doherty et al., 2010). Fucoidan is a sulfated polysaccharide present in the cell wall matrix of brown algae that has been demonstrated to inhibit certain enveloped viruses with low toxicity (Elizondo-Gonzalez et al., 2012).

In some instances it appears that water soluble chlorophyll derivatives have antibacterial activity (Mowbray, 1957). Smith states that "chlorophyll is not strictly bactericidal but that it does exert a definite bacteriostatic and even a bactericidal effect under suitable environmental conditions." Nevin and Bibby (1954) showed that chlorophyll inhibited the growth of staphylococci, streptococci and lactobacilli. Chlorophyll inhibited the growth of the tested oral bacteria, particularly *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (Saeki et al., 1993). Jaya et al. (2007) evaluated the antibacterial activity of chlorophyll a and b from certain fresh water micro-algae from the sriver Godavari (India).

The main carotenoids produced by microalgae are β-carotene from *Dunaliella salina* and astaxanthin from *Haematococcus pluvialis*. Carotenoids were reported to have antibacterial activity (Mahanom et al., 1990). The protective effect of β-carotene from green algae, *Chlorococcum humicol* has also been reported (Bhagavathy and Sumathi, 2010). The main natural resources of phycobiliproteins are the cyanobacterium *Spirulina* (*Arthrospira*) for phycocyanin (blue) and the rhodophyte *Porphyridium* for phycoerythrin (red) chromophoric prosthetic groups, named phycobilins. Phycobiliproteins are water soluble pigments produced by cyanobacteria (blue-green algae), red algae and crypto-monads (Mihov et al., 1996). Cyanobacteria, Rhodophyta and Cryptomonads algae contain phycobiliproteins, deep colored water-soluble fluorescent pigments, which are major components of a complex assemblage of photosynthetic light-harvesting antenna pigments, the phycobilisomes (Glazer, 1994). In *Spirulina*, phycocyanin is a phycobiliproteins; it is used against many bacterial infections and has anti-inflammatory, antioxidant and antiviral properties. It is effectively active against human pathogens such as *Streptococcus* sp., *Staphylococcus* sp., *E. coli*, *Bacillus* spp., and *Pseudomonas* spp. (Muthulakshmi et al., 2012; Murugan, 2012). Large quantities phycocyanin was isolated and partially purified from *Anabaena cylindrical* and filamentous fresh water cyanobacterium *Westiellopsis* spp., which was tested against Gram positive and Gram negative bacteria (Sabarinathan et al., 2008; Abdo et al., 2012).

It was reported that five freshwater microalgae, *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica*, *Spirulina platensis* and *Cosmarium leave* from an Egyptian water station contain quercetin with antibacterial activity (Rattanachaikunsopon and Phumkhachorn,

2010; Abdo et al., 2012). Three isoflavone compounds were found in freshwater algae and cyanobacteria (Klejdus, et al., 2010). It was also reported that the phenolic content are active as antibacterial against different types of microorganisms like *Salmonella typhi* (Ouattara et al., 2011) and the flavonoids are active against several strains like *Streptococcus* (Shu et al., 2011); *E. coli* and *Staphylococcus aureus* (Gao and Zhang, 2010).

Many phytochemicals not mentioned have been found to exert antimicrobial properties. This review has attempted to focus on reports of chemicals which are found in multiple instances to be active. It should be mentioned, however, that there are reports of antimicrobial properties associated with microalgal-derived oxylipins, the antibacterial activities of polyunsaturated aldehydes deserve a special mention. Such compounds are synthesized by diatoms, for example *S. costatum* and *Thalassiosira rotula*. One illustrative example is decadienal – probably derived from (the polyunsaturated) arachidonic acid (C20:4 n-3), which exhibits a strong activity against such important human pathogens as multi resistance *S. aureus* (MRSA) and *Haemophilus influenza* as well as against *E. coli* and *Pseudomonas aeruginosa*, and *S. aureus and S. epidermidis* (Smith et al., 2010). Some volatile components and various extracts of *Spirulina* also showed antibacterial activities (Ozdemir et al., 2004; El-Sheekh et al., 2014).

Antibacterial activity

Microalgae have for long been used with therapeutic purposes; their systematic screening for biologically active principles began in the 1950s. The first investigation on the antibiotic activity of algae was carried out by Pratt et al. (1944). However, in the last decade microalgae have become the focus of extensive research efforts, aimed at finding novel compounds that might lead to therapeutically useful agents (Mendes et al., 2003; Cardozo et al., 2007). Pratt et al. (1944) isolated the first antibacterial compound from a microalga, *Chlorella*; a mixture of fatty acids, viz. chlorellin, was found to be responsible for that inhibitory activity against both Gram+ and Gram- bacteria. The methanolic extract of cyanobacteria has been investigated for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, E. coli, P. aeruginosa, Aspergillus niger* and *A. flavus* using agar cup plate method (Prashantkumar et al., 2006). Although microalgae can synthesize a few useful products, search for novel antibiotics is still incipient; illustrative examples are presented in Table 2.

Antibiotics are typically less effective against Gram-negative bacteria because of their complex and multilayered cell wall, which makes it more difficult for the active compound to penetrate (Ördög et al., 2004); this justifies why the antibacterial activity of the supernatant (and methanolic extracts) is more potent against Gram-positive than Gram-negative bacteria (Ghasemi et al., 2004, 2007). Hexadecatrienoic acid isolated from *P. tricornutum* displays activity against (the Gram-positive pathogen) *S. aureus*. Pressurized (liquid) ethanol extracts from *Haematococcus pluvialis* in its red stage possess antimicrobial activity against a Gram-negative bacterium, *E. coli*, and a Gram-positive bacterium, *S. aureus;* this was once again associated with the presence of short-chain fatty acids, namely butanoic and methyl lactic acids (Santoyo et al., 2009). The antimicrobial potential of fresh water microalgae viz., *Oscillatoria sancta* (*O. sancta*) (Kuetz), *Lyngbya birgei*, *Oedogonium echinospermum*, *Spirogyra decimina* (Muller), *Spirogyra grantiana*, *Spirogyra crassa*, *Spirogyra biformis* and *Spirogyra condensata* (Vaucher) against human bacterial pathogens were screened. Antimicrobial study was carried out by disc diffusion method against the pathogens viz., *E. coli* (ATCC 35218), *S. aureus* (ATCC 6538), *S. typhi* (MTCC 733), *Proteus vulgaris*, *Proteus mirabilis* and *Streptococcus pyogenes* (Prakash et al., 2011).

Mechanism of action of antibacterial agents

Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. Broadly, antibacterial agents may be described as either bacteriostatic or bactericidal (Salvador et al., 2007). However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. The modes of action of different antibacterial agents are represented in Table 3.

The exact mechanism of action of fatty acids remains unknown: they may act upon multiple cellular targets, even though cell membranes are the most probable ones as membrane damage will likely lead to cell leakage and reduction of nutrient uptake, besides inhibiting cellular respiration; conversely, Desbois et al. (2009) claimed a peroxidative process. Furthermore, compounds synthesized by *Scenedesmus costatum*, and partially purified from its organic extract, exhibited activity against aquaculture bacteria because their fatty acids are longer than 10 carbon atoms in chain length which apparently induce lysis of bacterial protoplasts. Since the fatty acid analysis indicated the presence of other fatty acids that had been also reported to have some antimicrobial activity, specifically palmitoleic and oleic acids, it was hypothesized that lipids kill microorganisms by leading to disruption of the cellular membrane (Lampe et al., 1998).

Table 2. Antibacterial features of selected compounds from microalgae.

Microalgae	Target bacterial pathogens		References
	Gram +ve	Gram -ve	
Euglena viridis	-	*Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp., *E. coli*, *Edwardsiella tarda*	Das and Pradhan (2010)
Spirulina platensis	*Streptococcus* sp., *Bacillus* sp., *Staphylococcus* sp.	*Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp., *E. coli*, *E. tarda*	Mar et al. (2008), Kaushik and Chauhan (2008), Ranga Rao et al. (2010), Muthulakshmi et al. (2012), Pradhan et al. (2012) and Helen et al. (2014)
Chlorella vulgaris	*S. aureus*, *S. epidermidis*, *B. subtilis*	*E. coli*, *Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp., *E. tarda. Salmonella typhi*, *Klebsiella pneumoniae*	Pratt et al. (1944), Matusiak et al. (1965), Ghasemi et al. (2007) and Vishnu and Sumathi (2014)
Microcystis aeruginosa	*B. subtilis, S. aureus, S. mutans*	*Pseudomonas putida, P. aeruginosa, P. fluorescens, E. coli, Aeromonas hydrophila,, Vibrio* sp., *Edwardsiella tarda*	Ishida et al. (1997), Madhumathi et al. (2011) and Silva-Stenico et al. (2014)
Haematococcus pluvialis	*Staphylococcus aureus*	*Escherichia coli,*	Jaime et al. (2010)
Nostoc	*Staphylococcus aureus*, *Bacillus subtilis*	*P. aeruginosa*, *E. coli*, *Salmonella*	Zornitza et al. (2000), Goud et al. (2007), Jaya Prakash et al. (2007) and Silva-Stenico et al. (2014)
Phaeodactylum tricornutum	*Listonella anguillarum, Lactococcus garvieae*	*Vibrio* spp.	-
Chroococcus turgidus	*Streptococcus faecalis, Salmonella*	*E. coli*	Abdo et al. (2012)
Anabaena sphaerica	*Streptococcus faecalis, Salmonella*	*E. coli, Salmonella*	Goud et al. (2007) and Abdo et al. (2012)
Oscillatoria limnetica	*Streptococcus faecalis, S.mutants, Staphylococcus aureus, B. subtilis*	*E. coli, K. pneumonia, P. aeruginosa*	Goud et al. (2007), Abdo et al. (2012) and Helen et al. (2014)

The susceptibility of Gram-negative bacteria to killing by lipids was notable (Bergsson et al., 2002) and is probably due to the differences in the outer membrane or the cell wall of bacteria.

Conclusion

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The majority of clinically used antimicrobial drugs have drawbacks like toxicity, lack of efficacy, inhibiting cost and their frequent use leading to the emergence of resistant strains. Thus, there is an urgent need to develop alternative biodegradable agents, which should have limited side effects. It is generally considered that natural compounds are biodegradable and so more environmentally acceptable. Commercial applications of microalgae-derived compounds have, as yet, received little attention in the area of pharmaceuticals, antibiotics and other biologically active compounds. So this search prompted the exploration of natural algal products.

Scientists from divergent fields are investigating algae anew with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories around the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro*. More of these compounds should be subjected

Table 3. Mechanism of action of some antimicrobial agents.

Antimicrobial agent	Mechanism of action	References
Carotenoids	Digestion of cell wall by lysozyme enzymes.	Cucco et al. (2007)
Flavonoids	Increase in permeability of the inner bacterial membrane and a dissipation of the membrane potential	Mirzoeva et al. (1997)
Polyphenols	Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption	Amaro et al. (2011)
Polysaccharide	Inhibition of hyaluronidase.	Amaro et al. (2011)
Fatty acids and Lipids	Disruption of the cellular membrane	Lampe et al. (1998) and Desbois et al. (2009)

to animal and human studies to determine their effectiveness in whole-organism systems, including particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results would be facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings.

REFERENCES

Beijernick MW (1890). Cultureversuche mit Zoochlorellen, Lichenengoniden und anderen. Algen Bot. Ztg. 45:726-739, 741-753, 757-767,781-784.

Bergsson G, Steingrímsson O, Thormar H (1999) In vitro susceptibilities of *Neisseria gonorrhoeae* to fatty acids and monoglycerides. Antimicrob. Agents Chemother. 43(11):2790-2792.

Bhagavathy S, Sumathi P (2010). Protective role of β-carotene from *Chlorococcum humicola* against reactive oxygen species and lipid peroxidation in Benzo(a)Pyrene induced toxicity. J. Pharmacol. Res. 1(2):21-35

Biswas K (1949). Common fresh and brackish water algal flora of India and Burma, Part I. Records Botanical Survey of India, p. 15

Borowitzka MA, Borowitzka LJ (1989). Vitamins and fine chemicals from micro algae. In: Microalgal Biotechnology. Press syndicate of the University of Cambridge, New York, USA.

Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E (2007). Metabolites from algae with economical impact. Comp. Biochem. Phys. Part C Toxicol. Pharmacol. 146:60-78

Cucco MB, Guasco G, Malacarne Oltonelli R (2007). Effects of beta-carotene on adult immune condition and antibacterial activity in the eggs of Grey partridge, *Perdix perdix*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 147:1038-1046

Cunningham FX, Schiff JA (1986). Chlorophyll-Protein Complexes from *Euglena gracilis* and Mutants Deficient in Chlorophyll b: I. Pigment Composition. Plant Physiol. 80:223-230.

Davidson FF (1959). Poisoning of wild and domestic animals by a toxic water bloom of *Nostoc rivulare* Kuetz. J Am. Water works Assoc. 51:1277-1287.

Demule MCZ, Decaire GZ, Decano MS (1996). Bioactive substances from *Spirulina platensis* (cyanobacteria). Int. J. Exp. Bot. 58:93-96

Desbois AP, Mearns-Spragg A, Smith VJ (2009). A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA). Mar. Biotechnol. 11:45-52

Dwyer MR, Smillie RM (1970). A light-induced β-l,3-glucan breakdown associated with the differentiation of chloroplasts in *Euglena gracilis*. Biochim. Biophys. Acta 216:392-401

Ehrenberg CG (1830). Neue Beobachtungen über blutartige Erscheinungen in Aegypten, Arabien und Sibirien, nebst einer Uebersicht und Kritik der früher bekannten. Ann. Phys.
und Chem. Ser 2(8):477-514.

El-Sheekh MM, Daboor SM, Swelim MA, Mohamed S (2014). Production and characterization of antimicrobial active substance from *Spirulina platensis*. Iranian J. Microbiol. 6:112-119

Fan M, Liao Z, Wang R, Xu N (2013). Isolation and antibacterial activity of anabaena phycocyanin. Afr. J. Biotechnol. 12:1869-1873.

Ferreira LG, Noseda MD, Gonçalves AG, Ducatti DR, Fujii MT, Duarte ME (2012). Chemical structure of the complex pyruvylated and sulfated agaran from the red seaweed *Palisada flagellifera* (Ceramiales, Rhodophyta). Carbohydr. Res. 347(1):83-94

Fiksdahla, Liaaen-Jensena S (1988). Diacetylenic carotenoids from *Euglena viridis*. Phytochemistry 27:1447-1450.

Gao D, Zhang Y (2010). Comparative antibacterial activities of crude polysaccharides and flavonoids from *Zingiber officinale* and its extraction. Asian J. Trad. Med. 5:235-238

Ghasemi Y, Yazdi MT, Shafiee A, Amini M, Shokravi S, Zarrini G (2004). Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*. Pharm. Biol. 42:318-322

Ghasemi Y, Moradian A, Mohagheghzadeh A, Shokravi S, Morowvat MH (2007). Antifungal and antibacterial activity of the microalgae collected from paddy fields of Iran: characterization of antimicrobial activity of *Chlorococcus dispersus*. J. Biol. Sci. 7:904-910.

Glazer AN (1994). Phycobiliproteins - a family of valuable widely used fluorophores. J. Appl. Phycol. 6:105-112

Goud MJP, Seshikala D, Charya MAS (2007). Antibacterial activity and biomolecular composition of certain fresh water microalgae from River Godavari (India). Sci. World J. 2(3):19-23

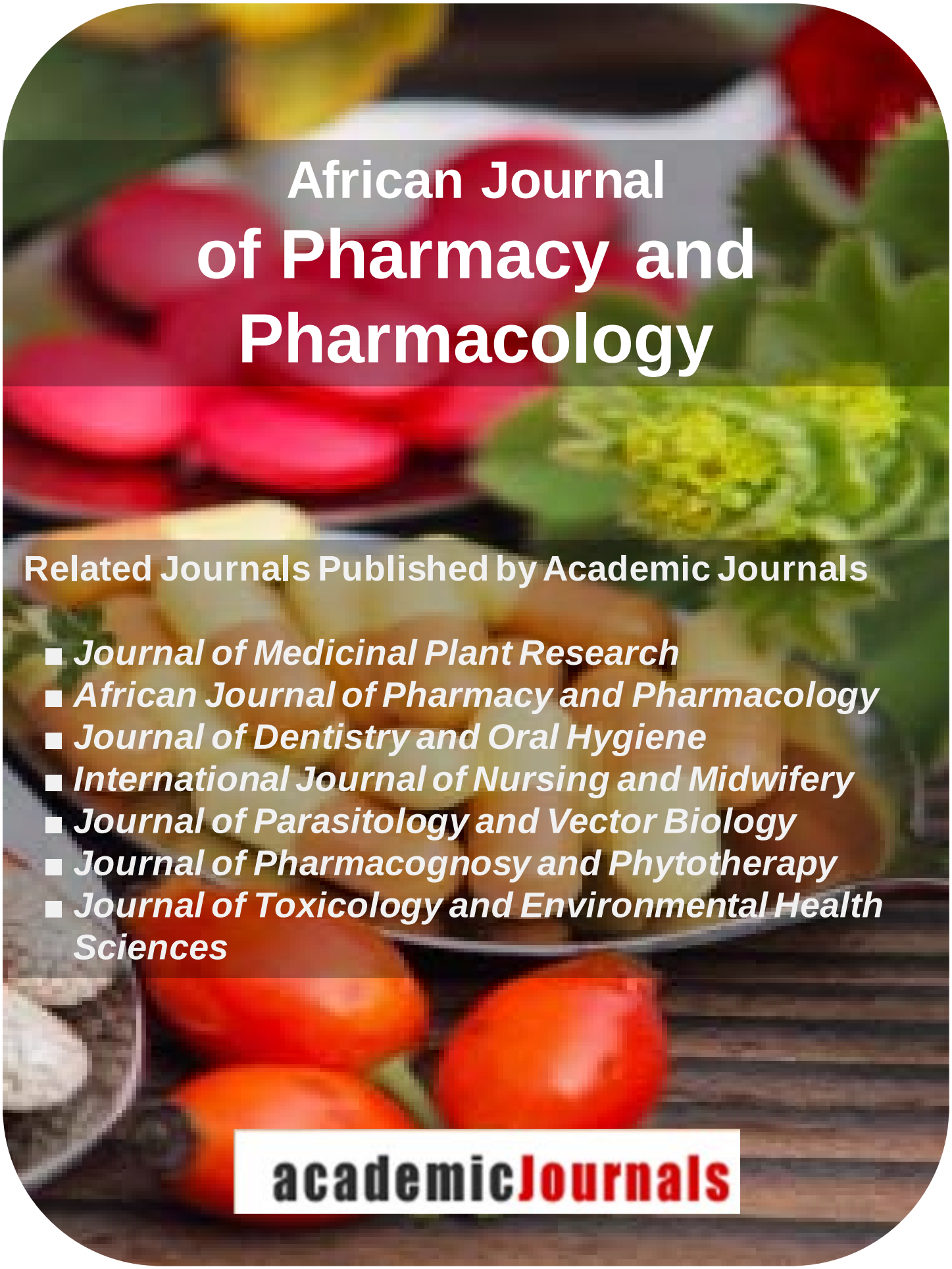
Gouveia L, Batista AP, Sousa I, Raymundo A, Bandarra NM (2008). Microalgae in novel food products. In: Food Chemistry Research Developments. Nova Science Publishers, Inc. ISBN 978-1-60456-262-0

Gouveia L, Veloso V, Reeis A, Fernandes HL, Empis J, Novais JM (1996). Evolution of the colourings in *Chlorella vulgaris* during carotenogenesis. Biores. Technol. 57:157-163

Herrero M, Ibañez E, Cifuentes A, Reglero G, Santoyo S (2006). *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. J. Food Prot. 69:2471-2477.

Hetta M, Mahmoud R, El-Senousy W, Ibrahim M, El-Taweel G, Ali G (2014). Antiviral and antimicrobial activities of *Spirulina platensis*. World J. Pharm. Pharm. Sci 3:31-39.
Hoek CD, Mann G, Johns HM (1995). Algae- An Introduction to Phycology. Cambridge University Press.
Ishida K, Matsuda H, Okita Y, Murakami M (2002). Aeruginoguanidines 98-A-98- C: cytotoxic unusual peptides from the cyanobacterium *Microcystis aeruginosa*. Tetrahedron 58:7645-7652
Ishida K, Murakami M (2000). Kasumigamide, an antialgal peptide from the cyanobacterium *Microcystis aeruginosa*. J. Org. Chem. 65:5898-5900.
Jaime L, Rodríguez-Meizoso I, Cifuentes A, Santoyo S, Suarez S, Ibáñez E, Señorans FJ (2010). Pressurized liquids as an alternative process to antioxidant carotenoids' extraction from *Haematococcus pluvialis* microalgae. Food Sci. Technol. Int. 43:105-112
Jaki B, Heilmann J, Sticher O (2000). New antibacterial metabolites from the cyanobacterium *Nostoc commune* EAWAG 122b. J. Nat. Prod. 63:1283-1285
Jaki B, Zerbe O, Hilemann J, Sticher O (2001). Two novel cyclic peptides with antifungal activities from the cyanobacterium *Tolypothrix byssioidea* (EAWAG 95). J. Nat. Prod. 64:154-158
Jaya PGM, Seshikala D, Singara CMA (2007). Antibacterial Activity and Biomolecular Composition of Certain Fresh Water Micro-Algae from River Godavari (India). Sci. World J. 2(3):19-23.
Kannan RRR, Arumugam R, Anantharaman P (2010). In vitro antioxidant activities of ethanol extract from *Enhalus acoroides* (L.F.) Royle. Asian Pac. J. Trop. Med. 3(11):898-901.
Kaushik P, Chauhan A (2008). *In vitro* antibacterial activity of laboratory grown culture of *Spirulina platensis*. Indian J. Microbiol. 48:348-352.
Kellam SJ, Cannell RJP, Owsianka AM, Walker JM (1988). Results of a large scale screening programmed to detect antifungal activity from marine and freshwater micro algae in laboratory culture. Brit. Phycol. J. 23:45-47
Khatun M, Huque KS, Chowdhury SA, Nahar Q (1994). *Chlorella* and *Scenedesmus*: Isolation, identification and mass cultivation for feeding cattle. In: A Report on The Use of Algae as Potential Feed supplements For Cattle. Bangladesh Livestock Research Institute, Savar, Dhaka 1341, Bangladesh. pp. 1-8.
Kim BH, Choi MK, Chung YT, Lee JB, Wui IS (1997). Blue-green alga *Microcystis aeruginosa* Kütz. Bull. Environ. Contam. Toxicol. 59:35-43
Klejdus B, Lojková L, Plaza M, Snóblová M, Stěrbová D (2010). Hyphenated technique for the extraction and determination of isoflavones in algae: ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. J. Chromatogr. A 1217(51):7956-7965.
Kützing FT (1846). Tabulae phycologicae: oder, Abbildungen der Tange. Vol. 1, fasc. 1 pp. 1-8, pls 1-10. Nordhausen: Gedruckt auf kosten des Verfassers (in commission bei W. Köhne).
Lampe MF, Ballweber LM, Isaacs CE, Patton DL, Stamm WE (1998). Killing of *Chlamydia trachomatis* by novel antimicrobial lipids adapted from compounds in human breast milk. Antimicrob. Agents Chemother. 45:1239-1244.
Lawton LA, Morris LA, Jaspars M (1999). A bioactive modified peptide, aeruginosamide, isolated from the cyanobacterium *Microcystis aeruginosa*. J. Org. Chem. 64:5329-5332.
Li AH, Cheng K, Wong C, King-Wai F, Feng C, Yue J (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 102:771-776.
MacMillan JB, Ernst-Russell MA, De Roop JS, Molinski TF (2002). Lobocyclamides, A-C, lipopeptides from a cryptic cyanobacterial mat containing *Lyngbya confervoides*. J. Org. Chem. 67:8210-8215
Mahanom H, Azizah AH, Dzulkifly MH (1990). Effect for different drying methods on concentrations of several phytochemicals in herbal preparation of 8 medicinal plant leaves. Malays. J. Nutr. 5:47-54.
Manners DJ, Sturgeon RJ (1982). Reserve carbohydrates of algae, fungi and lichens. In: Plant Carbohydrates I. Springer Berlin Heidelberg. pp. 472-514
Mao SC, Guo YW (2010). Sesquiterpenes from Chinese Red Alga *Laurencia okamurai*. Chin. J. Nat. Med. 8:321-325.
Mendes RL, Nobre BP, Cardoso MT, Pereira AP, Palabra AF (2003). Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorganica Chimica Acta 356:328-334
Mihov SG, Georgiev DI, Minkova KM, Tchernov AA (1996). Phycobiliproteins in *Rhodella reticulata* and photoregulatory effects on their content. J. Biotechnol. 48:251-257
Milner HW (1953). The chemical composition of algae. In: Burlew JH (Eds.), Algae Culture from Laboratory to Pilot Plant. Carnegil Institute, Washington. 600:285-320.
Mowbray S (1957). The antibacterial activity of chlorophyll. Br. Med. J. 1(5013):268–270.
Murugan T (2012). Antibacterial activity of C-phycocyanin against clinical isolates by disc diffusion method. J. Pharm. Res. 5(6):3020-3021.
Muthulakshmi M, Saranya A, Sudha M, Selvakumar G (2012). Extraction, partial purification, and antibacterial activity of phycocyanin from Spirulina isolated from fresh water body against various human pathogens. J. Algal Biomass Util. 3(3):7-11
Naviner M, Berge JP, Durand P, Le Bris H (1999). Antibacterial activity of the marine diatom, *Skeletonema costatum*, against aquacultural pathogens. Aquaculture 174:15–24.
Negri AP, Jones GJ, Hindmarsh M (1995). Sheep mortality associated with Paralytic Shellfish Poisoning toxins from the cyanobacterium *Anabaena circinalis*. Toxicon 33:1321-1329.
Nevin TA, Bibby BG (1954). The effect of sodium copper chlorophyllin on pure cultures of oral-type organisms. J. Dent. Res. 33(4):571-579.
O'Doherty JV, Dillon S, Figat S, Callan JJ, Sweeney T (2010). The effects of lactose inclusion and seaweed extract derived from *Laminaria spp*. on performance, digestibility of diet components and microbial populations in newly weaned pigs. Anim. Feed Sci. Technol. 157:173-180.
Ördög V, Stirk WA, Lenobel R, Bancírová M, Strand M, van Standen J (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. J. Appl. Phycol. 16:309-314.
Ouattara L, Koudou J, Zongo C, Barro N, Savadogo A, Bassole IHN, Ouattara AS, Traore AS (2011). Antioxidant and antibacterial activities of three species of *Lannea* from burkina faso. J. Appl. Sci. 11:157-162.
Ozdemir G, Karabay NU, Dalay MC, Pazarbasi B (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytother. Res. 18:754-757.
Phang SM (1992). Role of algae in livestock-fish integrated farming system. Proceedings of the FAO/IPT Workshop on Integrated Livestock-Fish Production System, 16-20 Dec 1991. University of Malaya, Kuala Lumpur, Malaysia. pp. 49-56.
Pineiro E, Bermejo B, Villar del F (2001). Antioxidant activity of different fractions of *Spirulina platensis* protean extract. Farmacology 59:497-500.
Plaza M, Santoyo S, Jaime L (2010). Screening for bioactive compounds from alga. J. Pharm. Biomed. Anal. 51:450-455.
Pradhan J, Das BK, Sahu S, Marhual NP, Swain AK, Mishra BK, Eknath AE (2012). Traditional antibacterial activity of freshwater microalga *Spirulina platensis* to aquatic pathogens. Aquacult. Res. 43:1287-1295.
Prakash JW, Antonisamy JM, Jeeva S (2011). Antimicrobial activity of certain fresh water microalgae from Thamirabarani River, Tamil Nadu, South India. Asian Pac. J. Trop. Biomed. S170-S173.
Prashantkumar P, Angadi S, Vidyasagar G (2006). Antimicrobial activity of blue green and green algae I. J. Pharm. Sci. 68(5):647-648.
Pratt R, Daniels TC, Eiler JB, Gunnison JB, Kumler WD, et al. (1944) Chlorellin, an antibacterial substance from *Chlorella*. Science 99:351-352
Pulz O, Gross W (2004). Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65:635-648.
Quoc KP, Pascaud M (1996). Effects of dietary gammalinolenic acid on the tissue phospholipid fatty acid composition and the synthesis of eicosanoids in rats. Ann. Nutr. Metab. 40:99-108.
Ramadan MF, Asker MMS, Ibrahim ZK (2008). Functional bioactive

compounds and biological activities of *Spirulina platensis* lipids. Czech J. Food Sci. 26:211-222
Rattanachaikunsopon P, Phumkhachorn P (2010). Contents and antibacterial activity of flavonoids extracted from leaves of *Psidium guajava*. J. Med. Plants Res. 4:393-396.
Rodr´ıguez-Meizoso I, Jaime L, Santoyo S, Se˜nor´ans F, Cifuentes A, Ib´a˜nez E (2010). Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. J. Pharm. Biomed. Anal. 51(2):456-63.
Rodrı´guez-Meizoso I, Jaime L, Santoyo S, Cifuentes A, Garcı´a-Blairsy RG, Sen˜ ora´ns FJ, Iba´n˜ez E (2008). Pressurized fluid extraction of bioactive compounds from *Phormidium* species. J. Agric. Food Chem. 56:3517-3523.
Sabarinathan KG, Ganesan G (2008). Antibacterial and toxicity evaluation of C-phycocyanin and cell extract of filamentous freshwater cyanobacterium - Westiellopsis sps. Eur. Rev. Med. Pharmacol. Sci. 12:79-82.
Saeki Y, Ito Y, Shibata M, Sato Y, Takazoe I, Okuda K (1993). Antimicrobial action of green tea extract, flavono flavor and cooper clorophyll against oral bacteria. Bull. Tokyo Dent. Coll. 34(1):33-37.
Santoyo S, Rodríguez-Meizoso I, Cifuentes A, Jaime L, García-Blairsy Reina G, Señorans FJ, Ibáñez E. (2009) Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae. LWT–Food Sci. Technol 42:1213-1218.
Shanab SMM (2007). Bioactive Allelo-chemical compounds from Oscillatoria Species (Egyptian isolates). Int. J. Agric. Biol. 9(4):617-621.
Shu Y, Liu Y, Li L, Feng J, Lou B, Zhou X, Wu H (2011). Antibacterial activity of quercetin on oral infectious pathogens. Afr. J. Microbiol. Res. 5:5358-5361.
Silva-Stenico ME, Kaneno R, Zambuzi FA, Vaz MG, Alvarenga DO, Fiore MF (2014). Natural products from cyanobacteria with antimicrobial and antitumor activity. Curr. Pharm. Biotechnol. 14:820-828.
Smith VJ, Desbois AP, Dyrynda EA (2010). Conventional and unconventional antimicrobials from fish, marine invertebrates and micro algae. Mar. Drugs 8:1213-1262.
Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006). Commercial Applications of Microalgae. J. Biosci. Bioeng. 101(2):87-96.
Stotts RR, Namkioshi M, Haschek WM, Rinehart KL, Carmichael WW, Dahlem AM, Beasley VR (1993). Structural modifications imparting reduced toxicity in microcystins from Microcystis spp. Toxicon 31:783-789.
Sudalayandi K, Kumar A, Sessler R, Sayre RT, Falcoa V, Ihemere U, Ndunguru J, Narayanan N (2012) Determination of fatty acids and proteins from the fresh water alga *chlamydomonas reinhardtii* cc 2137 and its antagonism against aquatic bacteria. Pak. J. Bot. 44(6):2139-2144.
Tan LT (2007). Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 68:954-979.
Tandeau-de-Marsac HJ (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol. Rev. 104:119-190.
Vishnu N, Sumathi R (2014). Isolation of fresh water microalgae *Chlorella* sp and its antimicrobial activity on selected pathogens. Int. J. Adv. Res. Biol. Sci. 1:36-43.
Wijesekara I, Pangestuti R, Kim S (2010). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydr. Polym. 84(5):14-21.
Xue CH, Saito Y, Zhang H, Li Z, Cai Z, Ou Y, Lin CH, Imbs AB (2002). Molecular species composition of glycolipids from *Sprirulina platensis*. Food Chem. 77:9-13.
Yamaguchi K (1997). Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. J. Appl. Phycol. 8:487-502.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