Original Article

Bioactivity of Dodecanoic Acid Extracted from *Geitlerinema* sp. TRV57

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ABSTRACT

Aim/Background: Imbalance in oxidant and antioxidant leads to oxidant stress. Increased consumption of exogenous antioxidants helps in neutralizing the oxidant stress and thus protects the cells from oxidant stress. The study focused on the isolation of nutraceutical compound, dodecanoic acid from the marine cyanobacteria *Geitlerinema* sp. TRV57. **Materials and Methods:** Various solvents were used to extract the dodecanoic acid. The ability of the dodecanoic acid to neutralize the oxidant was studied by various assay. **Results:** The GCMS analyses of these extracts revealed the presence of more percentage of saturated fatty acids with dodecanoic acid being the major fraction. These extracts have exhibited appreciable antibacterial, antioxidant and anti-apoptotic activity which can be attributed to the presence of dodecanoic acid. **Conclusion:** This study indicates *Geitlerinema* sp TRV57 as a promising agent for the emollient preparation.

Key words: Fatty acid, Cyanobacteria, *Geitlerinema*, Dodecanoic acid, Bioactivities.

INTRODUCTION

Marine microbes are exploited in recent decades for their biologically active compounds of commercial interest in pharmaceutical, food and agricultural industries.[1-3] Among the marine microbes, cyanobacteria and microalgae are the vital organisms in the production of commercial compounds with industrial applications. Cyanobacteria are photosynthetic, prokaryotic organisms, produce vitamins, pigments, lipids, proteins, sterols and hydrocarbon.

Lipids are esters of fatty acids and alcohols that encompass large group of structurally discrete organic compounds like fats, phospholipids, glycolipids, waxes etc. and lipids of cyanobacteria are esters of fatty acids and glycerol. These fatty acids can be in the form of saturated and unsaturated fatty acids. Depending on the chain length fatty acids are classified as short chain (less than C6), medium chain (C6-C12) and long chain fatty acid (C14 or more than C14).[4] Medium chain fatty acids (MCFA)are caproic acid or hexanoic acid (C6:0), caprylic acid or octanoic acid (C8:0), capric acid or decanoic acid (C10:0) and lauric acid or dodecanoic acid (C12:0). Coconut oil and palm kernel oil are medium chain fatty acid rich food source. Medium chain triacylglycerols are of main nutritional interest because they are readily degraded in the intestine than long chain triacylglyerols.[5] These fatty acids were reported for their antimicrobial activity against bacteria, fungi, viruses and protozoa.[6-9] Medium chain fatty acids and medium chain triacylglycerols suppress the antibiotic resistance genes and provoke low frequency of spontaneous development of resistance in bacteria.[10,11]

Yeast cells are used as model system because of their remarkable similarities at the organelle and molecular level to mammalian cells and were used in the study to understand the mechanism beyond the oxidative stress and apoptosis induced by agents like H_2O_2.[12-14] In this study, *Geitlerinema* sp. TRV57 was used to explore fatty acid composition and their potential antioxidant and antibacterial compounds.

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MATERIALS AND METHODS

Cyanobacterial cultivation and harvesting

Geitlerinema sp. TRV57 (accession number KX710092),[15] a marine cyanobacterial isolate, was grown in F/2 media prepared using a sea water. The cyanobacterial isolate was inoculated in sterile F/2 media (Guillard) and incubated at 27 ± 2°C with 10:14 h light: dark illumination provided by 36 W white fluorescent lamps as per standard laboratory conditions at pH 7.0.[15,16] 15 days old culture was collected and used for the extract preparation.

Preparation of solvent extracts

50ml of solvent was added to 5g of obtained biomass and macerated to get solvent extract. The extraction process was carried out overnight under continuous stirring. The solvents were collected and evaporated at 55°C to get the dried solvent extracts. In this study, solvents like dichloromethane, methanol, hexane, butanol, ethyl acetate were used to prepare solvent extract and labelled respectively.

Antibacterial activity of various extract

The antibacterial activity of the solvent extracts was assessed by disc diffusion method against the micro-organisms like *Staphylococcus aureus* (MTCC9542), *Bacillus subtilis* (MTCC1305), *Pseudomonas aeruginosa* (MTCC4673), *Escherichia coli* (MTCC405). Streptomycin was used as standard antibiotic. The zone of inhibition was measured in mm.[16] The bacterial strains used in this study were obtained from the MTCC and the strains were maintained in nutrient agar slants at 4°C.

In vitro Antioxidant activity of the solvent extract

Antioxidant activity of the solvent extracts was studied by various assays like Phosphomolybdenum assay, Reducing power assay, DPPH scavenging assay, Hydrogen peroxide free radical scavenging assay, Antilipid peroxidation assay.[15]

Cytoprotective activity of the extracts by MTT assay

The cytoprotective activity of the extracts against H_2O_2 stress was studied using Baker's yeast (*S. cerevisiae*) cell model as described by Balasubramanian and palghat.[14] 1×10^6 cells were loaded into 96 well plates. Cells were incubated with different concentration of extracts and 100µL of H_2O_2 (200µM) at 37°C for 1h. 50 µL of MTT (5 mg/mL) was added to all the wells and incubated for 3.5 h. 200 µL of DMSO was added and the absorbance was read at 650nm. Untreated cells were used as control and ascorbic acid was used as a standard antioxidant. The percentage cell viability was calculated using the formula:

$$\% \text{ cell viability} = \left(\frac{\text{absorbance of test}}{\text{absorbance of control}}\right)\times 100$$

RESULTS AND DISCUSSION

Geitlerinema sp TRV57,[15] a marine isolate was used in this study to investigate for the presence of potential antibacterial and antioxidant compounds. *Geitlerinema* are thin filamentous cyanobacteria belonging to the order Oscillatoriales.[17,18] *Geitlerinema* are found in different aquatic habitats such as marine and freshwater environments.[19-21] In this study, *Geitlerinema* sp TRV57, a cyanobacterium isolated from Kovalam beach, Chennai, India[15] was used.

GC-MS analysis

GC-MS analysis of crude extracts of various solvent revealed the presence of the saturated (Figure 1) and unsaturated fatty acids, alkanes and alkenes. The results were given in Table 1. The total fatty acid content was found to be 87.06%, 86.75%, 74.51%, 87.72%, 69.19%, 77.58% in the extracts obtained using butanol, dichloromethane, ethanol, ethyl acetate, hexane, methanol as solvents respectively. The total saturated fatty acid content was found to be 86.8%, 82.73%, 74.51%, 87.72%, 69.19%, 78.6% in the extracts obtained using butanol, dichloromethane, ethanol, ethyl acetate, hexane, methanol respectively. Among the various solvent extracts, ethyl acetate and butanol extracts were found to contain more than 84% dodecanoic acid.

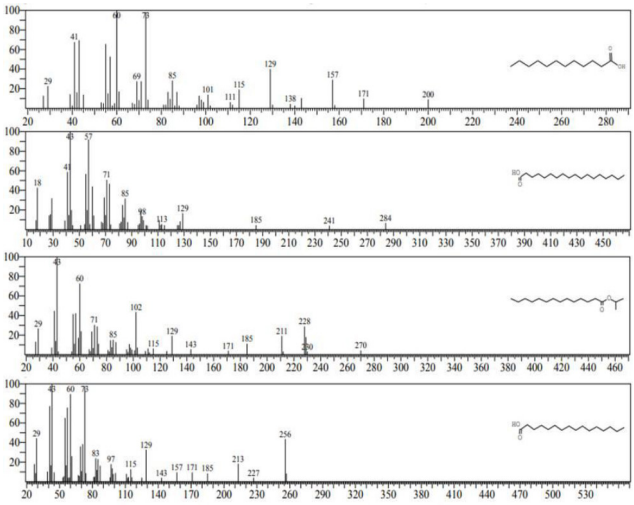

Figure 1: GC MS spectrum of a) dodecanoic acid, b) octadecanoic acid, c) tetradecanoic acid d) hexadecanoic acid obtained from solvent extraction of *Geitlerinema* sp. TRV57.

Table 1: GC MS profile of various solvent extract of *Geitlerinema* sp. TRV57.

Compound	Butanol		Dichloro methane		Ethanol		Ethyl Acetate		Hexane		Methanol	
		Area%		Area%		Area%		Area%		Area%		Area%
Butanoic acid	+	0.08	-	-			-	-	-	-	-	-
Propanoic acid	+	0.180	-	-			-	-	-	-	-	-
Dodecanoic acid or lauric acid (C12:0)	+	84.64	+	79.65	+	56.77	+	86.06	+	64	+	73.45
Octadecanoic acid or Stearic acid (C18:0)	+	0.4	+	0.28	+	2.32	+	0.01	+	1.47	+	1.07
Non-adecanoic acid or nonadecylic acid (C19:0)	+	0.40	-	-			-	-	-	-	-	-
Decanoic acid or capric acid (C10:0)	-	-	+	0.34			-	-	-	-	-	-
Tetradecanoic acid or myristic aicd (C14:0)	+	0.75	-	-	+	4.35	+	0.02	+	0.35	+	0.41
Hexadecanoic acid or Palmitic acid (C16:0)	+	0.61	+	1.46	+	11.07	+	1.63	+	3.37	+	2.65
Eicosanoic acid or Arachidic acid (C20:0)	-	-	+	5.02			-	-	-	-	-	-
Octadecenoic acid or Vaccenic acid (C18:1)	-	-	-	-			-	-	-	-	+	1.02

Table 2: Antibacterial activity of various solvent extracts of *Geitlerinema* sp. TRV57.

Micro-organism	Zone of inhibition in mm			
Extracts (100 µg/mL)	*B. subtilis* MTCC1305	*S. aureus* MTCC9542	*P. aeroginosa* MTCC4673	*E.coli* MTCC405
Ethanol	12±1	11±1.2	11±0.6	11±1.2
Methanol	10±0.8	12±0.9	10±1	11±1.5
Dichloromethane	13±1.5	12±1.0	12±1.2	11±1
Ethyl acetate	14±1.5	12±0.5	10±0.7	10±0.5
Hexane	11±0.3	10±0.7	11±1	10±0.8
Butanol	13±0.5	13±0.4	12±0.8	11±1.1
Standard antibiotic	14±0.3	16±0.4	15±1	14±0.5

Extracts of Dichloromethane, methanol, hexane and ethanol were found to have 79.65%, 73.45%, 64%, 56.77%, respectively. In addition to dodecanoic acid, octadecanoic acid, hexadecanoic acids were also found in all the extracts. Apart from saturated fatty acid, Eicosanoic acid and octadecenoic acid, unsaturated fatty acids, were found in the dichloromethane and methanol respectively.

Antibacterial activity of solvent extracts

Antibacterial activity of the solvent extracts was tested against the MTTC strains; *Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli, Staphylococcus aureus.* The extracts have shown antibacterial activity against the tested gram positive and gram negative organisms. The zones of inhibition of extracts against the bacteria were given in the Table 2. Among the tested extracts, ethyl acetate, butanol and dichloromethane extract had shown maximum zone of inhibition and hexane extract had shown the least zone of inhibition against the tested microorganisms. Maximum zone of inhibition may be due to the dodecanoic acid or saturated fatty acid content. Among the extracts ethyl acetate, butanol and dichloromethane had shown the presence of more than 80% of dodecanoic acid and hexane extract had shown the presence of least saturated fatty acid content of 69.19%.

GC MS revealed the presence of dodecanoic acid in all the extracts. Dodecaonic acids also called as lauric aicd, are saturated fatty acid and medium chain fatty acid containing C-12 carbons. Febri *et al.*[22] reported the antibacterial activity of lauric acid against *Staphylococcus*

aureus, Bacillus cereus, Salmonella typhimurium and *Escherichia coli* at a concentration of 5%. Mustapha and Runner,[23] reported the antibacterial activity of the non polar component containing hexane and chloroform extract of *Albizia adianthifolia* and *Pterocarpus angolensis*. Extracts had shown better activity against *E.coli* compared to the other tested bacteria like *P. aeruginosa, B. subtilis, S. aueus*. He also reported that the extracts were found to contain saturated fatty acid. Agoramoorthy *et al.*[24] reported the presence of more percentage of saturated fatty acid in *Excoecaria agallocha* and this saturated fatty acid were found to have antibacterial activity against gram positive bacteria than the gram negative bacteria. Lauric, palmitic, linolenic, linoleic, oleic, stearic and myristic acids were known to have potential anti bacterial and antifungal agents.[25,26] Manivachagam *et al.*[27] reported the presence of relatively more percentage of saturated fatty acid especially lauric acid in *Salicornia brachiata* which exhibited maximum antibacterial and antifungal activity.

Mechanism of action of fatty acid against *Bacillus subtilis*, a gram positive bacterium was reported by Tsuchido *et al.*[28] They have suggested that lysis of *Bacillus subtilis* was due to the induction of autolytic enzymes by fatty acids. Galbraith and Miller[29] have also recorded lysis of protoplast by fatty acid. According to Saito and Tomioka,[30] the antibacterial activity of the fatty acids is by insertion of non polar moieties into phospholipids layer of cell membrane thus causing changes in cell membrane permeability, altering cell membrane protein activity and uncoupling of the oxidative phosphorylation system. McGaw *et al.*[25] reported that though the mechanism of action of fatty acid is unclear, most of the Gram positive bacteria are sensitive to fatty acid and few gram negative species being susceptible.

Antioxidant activity of various solvent extracts

Imbalance in oxidants and antioxidants level leads to oxidative stress which further causes pathological conditions like cancer, neurological disorders, atherosclerosis, hypertension, ischemia, psoriasis, acute respiratory distress, asthma, idiopathic pulmonary fibrosis, chronic obstructive pulmonary diseases.[31] Oxidative stress arises due the overproduction of ROS by metabolic reactions. These ROS are highly reactive compound that readily react with the biomolecules and alter their functions.[31] Currently, many studies are focused on exploring and utilizing the natural anti-oxidants to neutralize this ROS.

Phosphomolybdenum assay

The total antioxidant activity of the various solvent extracts was estimated by phosphomolybdenum assay method, a spectrometric quantitative determination of antioxidant capacity. In this assay, molybdenum (VI) was reduced to molybdenum (V) by the action of antioxidant. The reduction can be observed by formation of green phosphate molybdenum (V) complex at acidic pH.[32] Reducing ability of various solvent extracts has shown an increasing trend with the increase in the concentration of extracts (Figure 2). The maximum phosphate molybdenum reduction was observed for butanol and ethyl acetate extract, which had shown maximum absorbance of 0.38 and 0.36 respectively at 695nm at 200 µg/mL concentration. Methanol extract has shown maximum absorbance of 0.21 at tested highest concentration. Ethanol and Dichloromethane have shown maximum absorbance of 0.15. Hexane extract has shown the least activity and absorbance was found to be 0.05 at tested highest concentration of 200µg/mL.

Ferric Reducing antioxidant power assay

Ferric ion reducing ability of various extracts was studied by ferric reducing antioxidant power assay. The antioxidant donates electron to reduce ferric to ferrous, an indicator of electron donating ability of an antioxidant. Reducing ability was observed by measuring the pearl's Prussian blue complex colour at 700nm. Increase in the absorbance at 700nm indicates increased reduction of Fe^{3+}. The solvent extracts have shown varying Fe^{3+} reducing ability (Figure 3). At the tested high concentration of 200 µg/mL, butanol, dichloromethane and ethyl acetate extract have shown maximum absorbance of 0.69, 0.63 and 0.61 respectively. At low concentration of 5µg/mL, dichloromethane have shown maximum activity of 0.5 whereas butanol and ethyl acetate have shown absorbance of 0.31 and 0.36. Methanol extract have shown maximum absorbance of 0.51 at 200 µg/mL

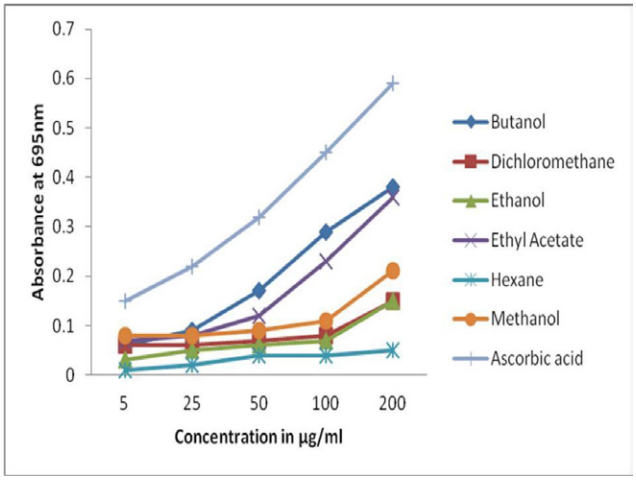

Figure 2: Total antioxidant activity of the various solvent extracts by phosphomolybdenum assay method.

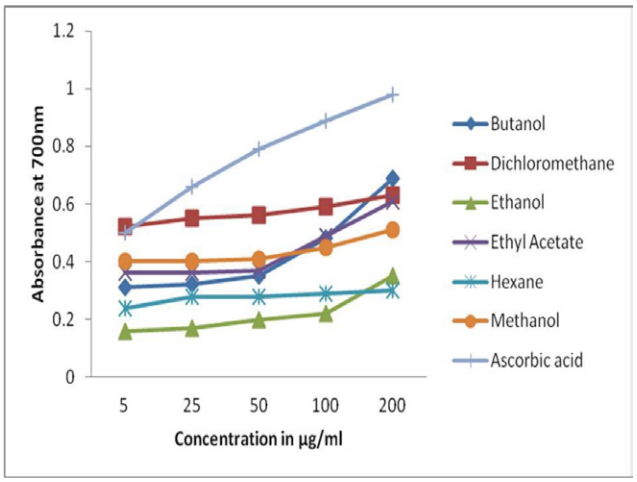

Figure 3: Ferric ion reducing power of various solvent of *Geitlerinema* sp. TRV57.

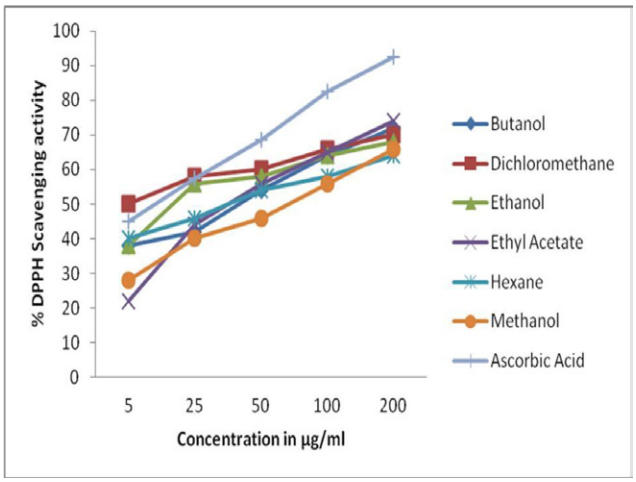

Figure 4: DPPH scavenging activity of various solvent of *Geitlerinema* sp. TRV57.

and 0.4 at 5 µg/mL. Least reducing ability was observed for hexane extract, which have shown absorbance of 0.3 at 200 µg/mL concentrations and 0.24 at 5 µg/mL concentrations. Avery *et al.*[33] reported the reducing ability of medium chain saturated fatty acid rich rice bran oil. In his study, he reported the reducing ability of the lauric acid has increased with an increase in concentration. This assay concludes that the extracts have the ability to reduce ferric ions and it can act as an electron donor to reduce the intermediates of lipid peroxidation process, indicating that it can be used as primary and secondary antioxidants.[34]

DPPH radical scavenging assay

Free radical scavenging activity of the antioxidant can be assessed using DPPH regent.[35] DPPH, stable free radical accepts hydrogen or electron to become non radical form, molecule DPPH-H.[36] It can be visualized by change in the colour of the reaction mixture. The reaction colour changes from purple to yellow and the reduction was observed by measuring absorbance at 517nm. The solvent extracts have shown reduction of free radical DPPH to DPPH-H (Figure 4). Maximum scavenging activity of 74%, 72% and 70% was observed for ethyl acetate, butanol and dichloromethane respectively at 200 µg/mL. Least scavenging activity of 64% was observed for hexane at 200 µg/mL. Ethanol and methanol extract have shown scavenging activity of 68% and 66% at 200 µg/mL. At low concentration of 5 µg/mL, scavenging activity of 50%, 40%, 38%, 28% and 22% was observed for extracts of dichloromethane, hexane, butanol, ethanol, methanol and ethyl acetate respectively. When compared to ascorbic acid, the extracts have shown less activity. Arlee *et al.*[37] also

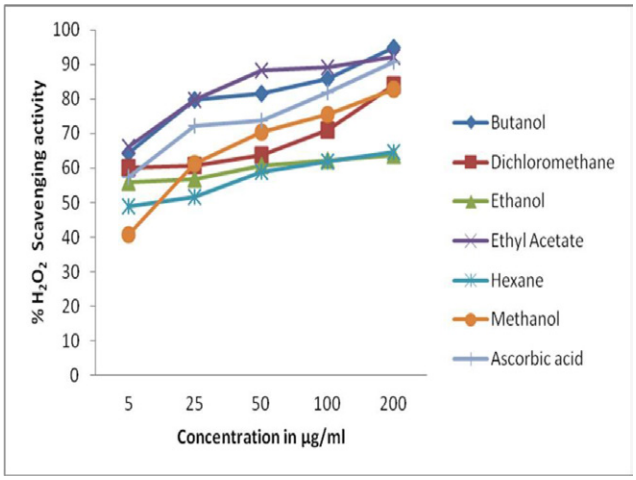

Figure 5: H_2O_2 scavengign activity of various solvent of *Geitlerinema* sp. TRV57.

reported that lauric acid, primary compound in virgin coconut oil, has shown DPPH scavenging activity.

Hydrogen peroxide free radical scavenging assay

Hydrogen peroxide, a strong oxidizing agent, is produced by superoxide dismutase enzyme *in vivo* condition. It crosses the cell membrane and oxidizes the bio molecules. In this study the hydrogen peroxide scavenging activity of the solvent extracts was studied (Figure 5). Among the tested solvents, butane and ethyl acetate have shown maximum activity of 94.77% and 92.03% respectively, which was comparatively greater than ascorbic acid with 90.54% at tested highest concentration. Dichloromethane and methanol have shown activity of 83.92% and 82.69% respectively at tested high concentration. At low concentration more 40% activity was observed for all the extracts. Generally, Hydrogen peroxide is not

reactive, but it can form an oxidant, hydroxyl radical which is toxic to the cell bio molecules. So neutralizing these oxidants like hydrogen peroxide, superoxide and hydroxyl radical, is must in order to protect the bio molecules,[34] which can be achieved by the saturated fatty acid or dodecanoic acid of *Geitlerinema* sp., TRV57.

Antilipid peroxidation activity

A lipid rich model, egg yolk was used as a substrate in this study. $FeSO_4$ was added to induce lipid oxidation in the reaction mixture which leads to the formation of Malondialdehyde (MDA), a secondary product of lipid oxidation. This MDA reacts with 2 molecules of thiobarbituric acid (TBA) and forms pinkish red chromogen which can be read at 532nm.[15] Antioxidant prevents the formation of MDA molecules which further stops the formation of pinkish red chromogen and affects the absorbance at 532nm. Antilipid peroxidation activity of the solvent extracts of *Geitlerirnema* sp. is presented in Figure 6. Butanol, Ethyl acetate and dichloromethane extract had shown maximum activity of 85.33%, 84.08% and 83.33% respectively at tested high concentration 200µg/mL, whereas the ascorbic acid have shown maximum activity of 89.97%. Methanol, ethanol and hexane extract had shown 77.08%, 77.91% and 75.41% respectively at tested high concentration. At tested low concentration extracts have shown activity of more than 46%. The activity of solvent extracts was found to be almost comparable with that of the standard ascorbic acid.

Cytoprotective activity of extracts by MTT assay

The cytoprotective activity of solvent extracts on H_2O_2 induced stress was studied by MTT assay using yeast as a model system. Figure 7 showed that all the extracts have shown better cytoprotective activity and the activity

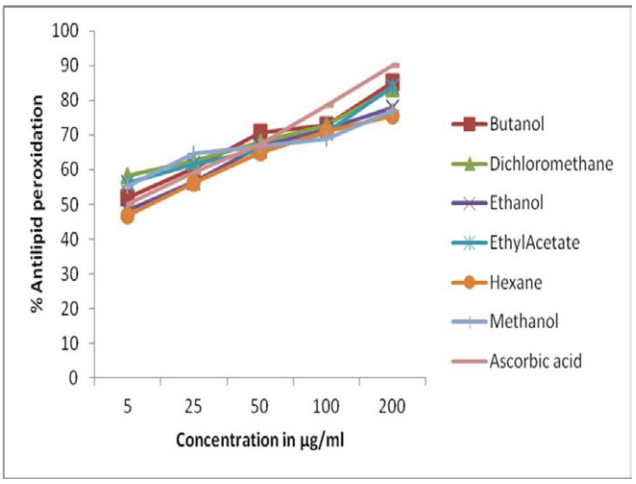

Figure 6: Antilipid peroxidation activity of various solvent of *Geitlerinema* sp. TRV57.

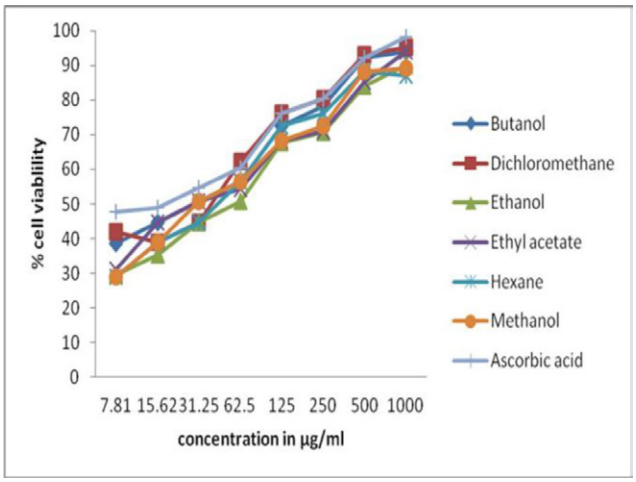

Figure 7: Cytoprotective activity of various extarcts of *Geitlerinema* sp. TRV57.

was more similar to ascorbic acid. With increase in the concentration of extracts the cytoprotective protective effects also have increased. The IC_{50} value was found to be 28.25 µg/mL, 28.5 µg/mL, 34.7 µg/mL, 48.5µg/mL, 50.5 µg/mL and 59.75µg/mL for extracts of butanol, ethyl acetate, dichloromethane, methanol, hexane and ethanol respectively. Among these extracts, butanol, ethyl acetate and dichloromethane extracts have exhibited the least IC_{50} value whereas the standard ascorbic acid has shown IC_{50} value of 24.75µg/mL. This result suggests that the extracts have the ability to protect the cells from the oxidative stress. Similar results were reported by Balasubramanian and Palghat[14] in their study that the extracts of *Z. mays* have ability to protect the yeast cell from induced oxidative stress.

SUMMARY AND CONCLUSION

GC-MS analysis of the various solvent extract revealed the presence of more percentage of saturated fatty acid and lauric acid or dodecanoic acid in all the extracts. Ethyl acetate extract was found to contain maximum percentage of saturated fatty acid followed by butanol and dichloromethane. Of the saturated fatty acid, dodecanoic acid was found to form the major fraction. Increase in antibiotic resistant strains and undesirable side effects of the available antibiotics leads to the increase in search of new antimicrobial agents. In this study, the extracts have shown better antibacterial activity. It may be due to the presence of more percentage of saturated fatty acid or dodecanoic acid in the extracts. Therapeutic use of fatty acid to cure skin infections which is caused by bacteria and fungi by topical applications have been reported.[25] Further study is required to study the mechanism of action of lauric acid on these tested bacteria.

Antioxidant studies revealed that the extracts have the ability to neutralize the free radical or oxidant in the test solution by acting as an electron/ proton donor. Antibacterial and antioxidant activity may be due to the presence of the saturated fatty acid in particular due to the more percentage of dodecanoic acid in the extracts. Compound which has antioxidant and antibacterial activity can be used to treat skin diseases like psoriasis, which is caused due to oxidative stress or Cellulitis, Erysipelas, Folliculitis, Erythrasma, Hidradenitis suppurativa, Methicillin resistant *Staphylococcus aureus* infection which are caused due to bacterial infection. Further study is required to study the effect of fatty acid especially lauric acid on these diseases.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

GC-MS: Gas chromatography–mass spectrometry; **MCFA:** Medium chain fatty acids; **H_2O_2:** Hydrogen peroxide; **MTCC:** Microbial Type Culture Collection and Gene Bank; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **DMSO:** Dimethyl sulfoxide **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **µM:** micro molar; **µL:** micro liter; **mg/Ml:** milligram per millilitre; **h:** hour; **nm:** nanometer; **µg/mL:** microgram/millilitre; **$FeSO_4$:** Ferrous sulphate; **MDA:** Malondialdehyde; **TBA:** Thiobarbituric acid; **IC_{50}:** Half maximal inhibitory concentration; **°C:** degree Celsius.

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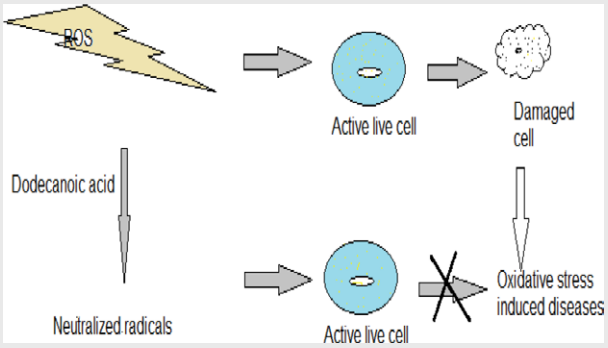
PICTORIAL ABSTRACT



SUMMARY

Dodecanoic acid commonly called as lauric acid, is a medium chain fatty acid. In this study dodecanoic acid was extracted from *Geitlerinema* sp. TRV57. Among the solvent used ethyl acetate extract was found to contain 84% of dodecanoic aicd. The deodecanoic acid rich extracts had shown ability to neutralize the oxidant species *in vitro* assays. Also it had shown ability to protect the yeast cell from the H_2O_2 stress. With further study it can either used as a anutracetuical compound to neutralize the oxidant in order to cure diseases which are caused due to oxidant stress.

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