Vietnam Journal of Science and Technology ***57*** *(3) (2019) 300-308*

doi:10.15625/2525-2518/57/3/13679

CHEMICAL AND PHARMACOLOGICAL EVALUATION OF MANGLICOLOUS LICHEN *GRAPHIS AJAREKARII* PATW. & C. R. KULK.

Polimati Haritha[1], Sunil Kumar Patnaik[2], Vinay Bharadwaj Tatipamula[1, 3, *]

[1]*Pharmaceutical Sciences Department, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530 003, Andhra Pradesh, India*

[2]*Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Ootacamund- 643 001, Tamil Nadu, India*

[3]*Pharmaceutical Chemistry Department, KL College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram – 522 502, Guntur, Andhra Pradesh, India*

[*]Email: *vinaybharadwajt@gmail.com*

Received: 11 March 2019; Accepted for publication: 23 April 2019

Abstract. The chemical examination of ethanolic extract of Manglicolous lichen *Graphis ajarekarii* **(Ga-Et)** resulted in isolation of three known metabolites i.e. chiodectonic acid **(1)**, graphenone **(2)** and graphisquinone **(3)**. All the isolates **(1-3)** and **Ga-Et** were screened against DPPH, superoxide free radicals and ferric ion reducing power assays, and six different cancer cell lines (MDA-MB-231, SW620, HeLa, FADU, A549, SKOV3) and one normal human cell line (NHME). Compound **1** exhibited equivalent inhibition of superoxide free radical as that of the standard (ascorbic acid) with IC_{50} value of 32.0 µg/mL. Peering at the SRB assay results, it is observed that the better IC_{50} values were determined by **3** of 15.5, 16.5 and 26.50 µg/mL on SW620, FADU and MDA-MB-231, respectively. Addition to, all the tested samples showed low cell lysis on NHME indicate non-toxic. Consequently, the outcomes revealed that the *G. ajarekarii* is the new source to treat oxidative stress and cancer.

Keywords: Manglicolous lichen, antioxidant, cancer, Sulforhodamine B assay.

Classification numbers: 1.1.1; 1.2.1.

1. INTRODUCTION

A symbiotic organism known as the Lichen, belongs to the group of epiphytes which have an aptitude to persist on any substratum or geographical region [1, 2]. Manglicolous lichens are one of the types of lichen, which especially survived on the mangroves [2, 3]. Throughout ages, lichens are used for the treatment of several diseases due to its unique survival and their bioactive secondary metabolites. Lichens with their secondary metabolites employ a diverse range of pharmacological actions, which includes analgesic, antibiotic, anti-inflammatory, antimycotic, antipyretic, antiviral and cytotoxic effects [3, 4]. Chiefly, Manglicolous lichens

display difference in their biological constituents and actions than normal lichens do. It is due to the fact that their physiological adaptation at the intertidal zone (environment in which having both the marine and freshwater) [5]. This lead the researchers to gain interest in the biological screening of particularly Manglicolous lichens. There are a very few pharmacological evaluation reports that exist, till date, on the Manglicolous lichens [2, 4].

As per the general studies, *Graphis ajarekarii* Patw. & C. R. Kulk. belongs to *Graphis* genus which is one of the chief genera of crustose lichens with more than 400 species found across the globe. Pharmacologically, the crude extracts of *Graphis* family possess antioxidant, antimycobacterial, tyrosinase and xanthine oxidase inhibitory activities [6, 7]. Earlier the experiment which we had performed has established the phytochemical analysis along with total flavonoid and phenolic contents, antimicrobial, antioxidant and hypoglycaemic and, anti-hyperglycaemic activities of this mangrove associated lichen *G. ajarekarii* [3]. In addition, we reported the chemical examination of ethyl acetate extract from *G. ajarekarii*, which yielded two known metabolites – atranorin and ribenone [3]. In continuation of our research on chemical and pharmacological evaluation of some Indian mangrove lichens and also based on the aforementioned properties of *G. ajarekarii*, we examined by recollecting the *G. ajarekarii* specimens (in October 2018) for its chemical and pharmacological properties which are reported on this research paper.

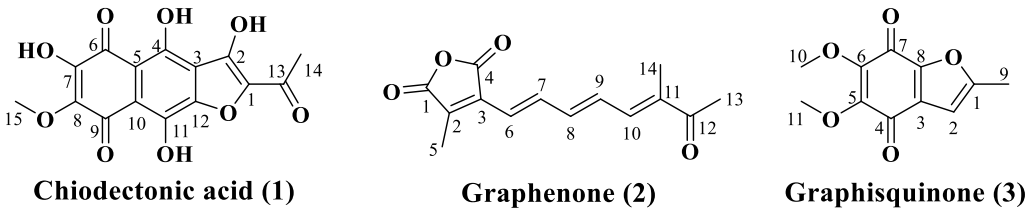

Figure 1. Known secondary metabolites isolated from *Graphis ajarekarii.*

2. MATERIAL AND METHODS

2.1. Collection

The specimens of mangrove associated lichen, *Graphis ajarekarii* Patw. & C. R. Kulk. (micro-lichen) was collected on the twigs of mangrove plant *Avicennia officinalis* from Nagayalanka lighthouse, Krishna Estuary, Andhra Pradesh, India (15°77'N and 80°96'E with 10 m elevation) in 18 October 2018. The *G. ajarekarii* was authenticated by Dr. D. K. Upreti, CSIR-National Botanical Research Institute (NBRI), Lucknow with accession number 15-027174 had been deposited at Lichen herbarium, CSIR-NBRI, Lucknow, India.

2.2. Chemicals and Instruments

All the chemicals used in the present experiment were of analytical grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Sulforhodamine B (SRB) was from Sigma Aldrich (Mumbai, India); doxorubicin from the Avantis Pharma Ltd (Mumbai, India). UV-Visible spectrometer (Electron 420 series spectrophotometer) from S.I. Photonics (Tucson, Arizona).

2.3. Extraction

The lichen specimens were carefully handpicked from the twigs of respective mangrove plants and allowed to shade dry. The dried lichen then (of about 10 g) were powdered, suspended in ethanol for a week, and evaporated under reduced pressure to obtain ethanolic extract from *G. ajarekarii* (**Ga-Et,** 1.10 g, 11 %w/w) as a dark reddish solid. The **Ga-Et** (500 mg) was then subjected to column chromatography by increasing polarity of hexane and ethyl acetate (as a solvent system), which affords three fractions. All those three fractions were subjected to recrystallization by using hexane and acetone. Upon recrystallizing, fraction I (90 mg) obtained in 10 % hexane in ethyl acetate yielded **1** as a red color crystals (60 mg), fraction II (50 mg) obtained in 20 % hexane in ethyl acetate yielded **2** as a yellow needle crystals (25 mg), and fraction III (60 mg) obtained in 30 % hexane in ethyl acetate yielded **3** as a reddish needles (32 mg) (Figure 1).

Additionally, the ethanolic extract of twigs (50 g) of host species (*A. officinalis*, **Ao-Et**, 3.24 g, 6.48 %w/w) of micro-lichen *G. ajarekarii*) was prepared and preserved at 4 ^{o}C. Along with the lichen extract and metabolites, the **Ao-Et** was evaluated to bioactive studies in order to justify the real aptitudes of micro-lichen, *G. ajarekarii*.

1 (Chiodectonic acid): Red color crystals, Mol. Formula: $C_{15}H_{10}O_9$; Yield: 60 mg; R_f: 0.4 (Hexane:ethyl acetate, 1:4); Mp: 296-297 ^{o}C; UV (Ethanol) λ_{max} (log ε) 287 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (1H, s, 2-OH), 2.69 (3H, s, 14-CH_3), 3.79 (3H, s, 15-OCH_3), 5.15 (1H, s, 4-OH), 5.63 (1H, s, 7-OH), 5.84 (1H, s, 11-OH); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 25.13 (C-14), 61.34 (C-15), 111.68 (C-5), 115.55 (C-10), 118.60 (C-3), 129.90 (C-1), 145.69 (C-8), 146.38 (C-11), 148.59 (C-7), 155.94 (C-12), 159.76 (C-2), 158.46 (C-4), 179.37 (C-6), 184.07 (C-9), 188.53 (C-13); CHNS analysis: found C-60.76, H-4.74(%), calcd. C, 60.11, H, 4.41(%); FT-ESI-MS [M+H$^+$] *m/z*: 335.08 (Figure 1) [8].

2 (Graphenone): Yellowish needles, $C_{14}H_{14}O_4$; Yield: 90 mg; R_f: 0.6 (Hexane:Ethyl acetate, 1:1); Mp: 167-168 ^{o}C; UV (Ethanol) λ_{max} (log ε) 290 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.93 (3H, s, 14-CH_3), 2.12 (3H, s, 5-CH_3), 2.34 (3H, s, 13-CH_3), 6.43-6.53 (5H, m, 6,7,8,9-CH), 7.05 (1H, s, 10-CH); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 12.34 (C-5), 12.61 (C-14), 27.54 (C-13), 125.00 (C-6), 128.70 (C-11), 129.02 (C-3), 131.40 (C-2), 131.97 (C-7) 132.65 (C-9), 134.73 (C-8), 143.30 (C-10), 166.28 (C-4), 168.61 (C-1), 200.83 (C-12); CHNS analysis: found C-68.71, H-5.72(%), calcd. C, 68.28, H, 5.73(%); FT-ESI-MS [M+H$^+$] *m/z*: 247.18 (Figure 1) [8].

3 (Graphisquinone): Red needles, Mol. Formula: $C_{11}H_{10}O_5$; Yield: 32 mg; R_f: 0.4 (Hexane:Ethyl acetate, 1:1); Mp: 130-131˚C; UV (Ethanol) λ_{max} (log ε) 228 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38 (3H, s, 9-CH_3), 3.71 (3H, s, 10-OCH_3), 3.75 (3H, s, 11-OCH_3), 7.17 (1H, s, 2-CH); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 14.37 (C-9), 61.33 (C-10/C-11), 104.36 (C-2), 118.91 (C-3), 144.94 (C-5), 150.85 (C-6), 153.57 (C-8), 154.24 (C-1), 176.98 (C-7), 183.80 (C-4); CHNS analysis: found C-59.12, H-4.02(%), calcd. C, 59.46, H, 4.54(%); FT-ESI-MS [M+H$^+$] *m/z*: 223.21 (Figure 1) [8].

2.4. Antioxidant activity

2.4.1. DPPH assay

The antioxidant activity was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay [9] in triplet. Initially, 100 µL of 100 µM of DPPH in ethanol was prepared and reacted with known concentrations i.e. 50, 100, 150 and 200 µg/mL for extracts and 25, 50, 75 and 100 µg/mL for the isolated metabolites and standard (ascorbic acid) samples, incubated for 30 mins. The absorbance was noted at 517 nm on UV-Visible spectrometer (Electron 420 series

spectrophotometer). The percentage inhibition was calculated using the below formula. Simultaneously, the IC_{50} was calculated using linear graph section between percentage inhibition and concentration of tested sample.

2.4.2. Superoxide radical scavenging assay

In the radical method [10], the superoxide radicals generated from non-enzymatic phenazine methosulfate/nicotinamide adenine dinucleotide (PMS/NADH) reduces nitro blue tetrazolium (NBT) to a purple formazan. To 1 mL of reaction mixture contained 20 mM phosphate buffer (pH 7.4), 73 µM NADH, 50 µM NBT, 15 µM PMS added various concentrations i.e. 50, 100, 150 and 200 µg/mL for extracts and 25, 50, 75 and 100 µg/mL for isolates/ascorbic acid and incubated for 10 min at room temperature and the absorbance was noted at 562 nm against blank and the experiment was triplicated and the data was expressed as percentage inhibition.

2.4.3. Ferric ion (Fe^{3+}) reducing power assay

The ferric ion reducing power assay was determined by the modified method of Tohma *et al*. [11] and Khang *et al.* [12]. To 2.5 mL of 0.2 M phosphate buffer (pH 6.6), 2.5 mL of 1 % potassium ferricyanide added various concentrations i.e. 50, 100, 150 and 200 µg/mL for extracts and 25, 50, 75 and 100 µg/mL for isolates/ascorbic acid and incubated for 20 min at 50 ^{o}C. Then 2.5 mL of 10 % trichloroacetic acid and 0.5 mL of 0.1 % ferric chloride were added to the reaction mixture and the absorbance was measured at 700 nm. An increase in the absorbance was considered as greater reducing capacity. The experiment was triplicated and the data was expressed as percentage inhibition.

$$\text{Percentage inhibition (\%)} = (A_c - A_s)/A_c \times 100$$

where A_c is the absorbance of the control; A_s is the absorbance of sample.

2.5. Cytotoxicity assay

2.5.1. Cancer Cell lines

MDA-MB-231 (Breast), SW620 (Colon), HeLa (Cervical), FADU (Head & Neck), A549 (Lung), SKOV3 (Ovary) and Normal Human Mammary Epithelial (NHME) (were kindly provided by National Centre for Cell Science, Pune. The cancer cells were maintained in MEM media (containing 10 % fetal calf serum, 5 % mixture of penicillin (100 units) and streptomycin (100 µg/mL) in presence of 5 % carbon dioxide incubator having 90 % humidity at 37 ^{o}C for 72 h.

2.5.2. Cell growth medium

All the cancer cell lines were maintained in minimal essential medium (MEM) (adjusted to 10 % (v/v) FBS, 1.5 g/mL $NaHCO_3$, 0.1 mM MEM non-essential amino acids and 1 mM sodium pyruvate). Three days prior to performing assay, the cells were washed with sterilized PBS and grown using MEM media (supplemented with 0.25 % trypsin in versene-EDTA and 10 % FBS) and mixed to obtain homogeneous suspension of cells. The suspension was taken in a sterilized polypropylene tube and the cell concentration in each well was determined by hematocytochameter chamber under a microscope using 0.4 % trypan blue solution. The minimal seed density must be 1.9×10^4 cells per well.

2.5.3. Sample Preparation

All the crude extracts and standard were dissolved in DMSO to 100 mg/mL and 10 µg/mL, respectively. The doxorubicin and DMSO were used as a standard and control, respectively.

2.5.4. SRB Colorimetric assay

The SRB assay [13, 14] is based on the estimation of cellular protein content. The prepared samples were taken in 96-well tissue-culture plate and added 190 µL screened ideal cell suspension and mixed occasionally and incubate at 37 ^{o}C with 5 % CO_2 and 90 % relative humidity for 3 h. Then add 100 µL cold TCA to each well and incubate at 4 ^{o}C for 1 h. After that the plates were gently washed using water, dried using blow dryer and air-dried at room temperature. To each completely dried well, add 100 µL of 0.057 % SRB solution, kept aside for 30 min and quickly rinse with 1 % acetic acid. To the dried plate add 200 µL of 10 mM Tris base (pH 10.5) solution, shake for 5 min and measure the OD at 510 nm. The blank contains only medium while the control has only cancer cells with no test samples. The percentage of growth inhibition was calculated using below formula.

$$\% \text{ Growth inhibition} = 100 - [(S - B)/(C - B)] \times 100$$

where S is mean OD value of sample; B is mean OD value of blank; C is mean OD value of control.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The shade dried thallus of *G. ajarekarii* was extracted with ethanol at room temperature. The obtained ethanolic extract of *G. ajarekarii* **(Ga-Et)** was subjected to chromatographic purification led to isolation of three metabolites **(1-3)** (Figure 1).

Compound **1** was obtained as a red colour crystals with R_f value 0.4 (hexane: ethyl acetate, 1:4) and the elemental analysis and positive mode of FT-ESI-MS ion peak at *m/z* 335.08 confirmed the molecular formula as $C_{15}H_{10}O_9$. The ^{1}H and ^{13}C NMR data showed the presence of four hydroxyl groups at δ_H 1.41, 5.15, 5.63 and 5.84 with corresponding carbon signals at δ_C 159.76 (C-2), 158.46 (C-4), 148.59 (C-7), and 146.38 (C-11), respectively, one methyl group at δ_H 2.69 with carbon signal at δ_C 25.13 (C-14), and one methoxy group at δ_H 3.79 with corresponding carbon signal at δ_C 61.34 (C-15). In addition, the ^{13}C NMR also revealed the presence of dione groups at δ_C 179.37 (C-6) and 184.07 (C-9), one formyl group δ_C 188.53 (C-13), two quaternary aromatic carbon signals at δ_C 129.90 (C-1) and 145.69 (C-8), and four aromatic methines at δ_C 118.60 (C-3), 111.68 (C-5), 115.55 (C-10), and 155.94 (C-12). Hence, compound **1** was confirmed after corroboration with the existing literature as chiodectonic acid (Figure 1). Compound **1** was earlier isolated from lichens, *Cryptothecia rubrocincta* (Ehrenb.) Thor. and *Pyxine coccifera* (Fee) Nyl. [8].

Compound **2** was obtained as yellow needles. Based on the elemental analysis and positive mode of FT-ESI-MS ion peak at *m/z* 247.18 confirmed the molecular formula as $C_{14}H_{14}O_4$. The ^{1}H NMR showed the presence of four singlets for three methyl groups at δ_H 1.93, 2.12, 2.34, and for one alkene proton at δ_H 7.05, and one multiplet for alkene protons at δ_H 6.43-6.53. The ^{13}C NMR revealed the presence of three methyl groups at δ_C 12.34 (C-5), 12.61 (C-14), and 27.54 (C-13), one carbonyl group at δ_C 200.83 (C-12), dione groups at δ_C 166.28 (C-4) and 168.61 (C-

1), two methines carbon signals at δ_C 129.02 (C-3), and 131.40 (C-2), and six alkene carbon signals at δ_C 125.00 (C-6), 128.70 (C-11), 131.97 (C-7) 132.65 (C-9), 134.73 (C-8), and 143.30 (C-10). By corroboration with the existing literature compound **2** was found to be graphenone (Figure 1). Compound **2** was earlier reported from *Graphis scripta* (L.) Ach. var. *serpentina* Meyer [15].

Compound **3** was obtained as red needles. Based on the elemental analysis and positive mode of FT-ESI-MS ion peak at *m/z* 223.21 confirmed the molecular formula as $C_{11}H_{10}O_5$. The ^{1}H NMR showed the presence of four singlets for one methyl group at δ_H 2.38, for two methoxy groups at δ_H 3.71 and 3.75, and one methine proton at δ_H 7.17. The ^{13}C NMR revealed the presence of one methyl group at δ_C 14.37 (C-9), two methoxy groups at δ_C 61.33 (C-10/C-11), dione groups at δ_C 176.98 (C-7), and 183.80 (C-4), one methine carbon signal at δ_C 104.36 (C-2), and five quaternary aromatic carbon signals at δ_C 118.91 (C-3), 144.94 (C-5), 150.85 (C-6), 153.57 (C-8), and 154.24 (C-1). Based on the aforementioned data, compound **3** was compared with the existing literature on lichen metabolites and it was confirmed as graphisquinone. Compound **3** was earlier reported from *Graphis desquamescens* (Fee) Hale et Wirth [15].

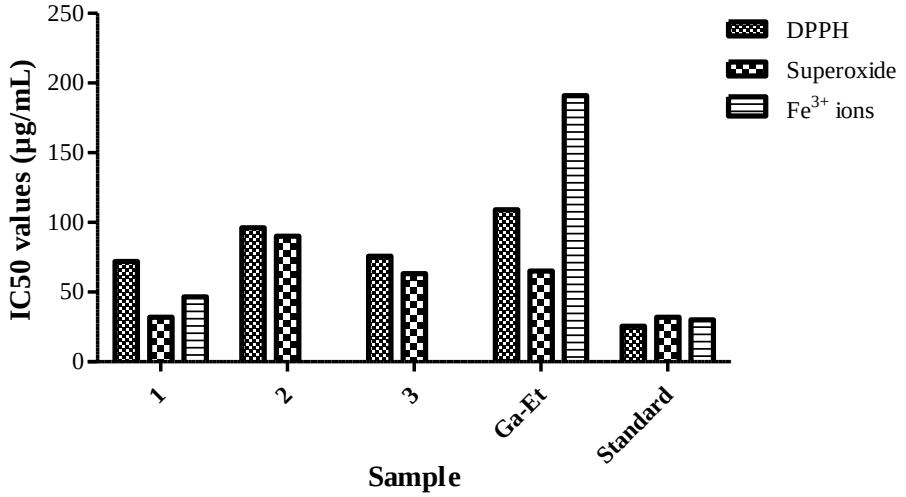

Figure 2. IC_{50} values of **1-3** and **Ga-Et** against different free radicals.

3.2. Antioxidant activity

In general, natural antioxidants retain substantial tolerance with the mankind. Even in present study, the anti-radical assays of all the samples **(1-3, Ga-Et** and **Ao-Et)** against DPPH, and superoxide radicals, and ferric ion reducing power assay were illustrated in Table S1. The inferior IC_{50} values indicate higher inhibition of free radicals. From the antioxidant results it is confirmed that compound **1** and **Ga-Et** exhibited promising anti-radical scavenging capacities.

In DPPH radical scavenging assay, the antioxidant substances reduce the stable purple coloured DPPH radical to a yellow coloured non-radical DPPH-H form. In general, the DPPH radical quenching activity of antioxidants are subjected to their hydrogen (donating) capacities [16, 17]. As depicted in the Figure 2, the IC_{50} values remained 72.0, 93.2 and 75.8 µg/mL for **1, 2** and **3**, respectively, while standard ascorbic acid continued to exist at 25.5 µg/mL. In addition to that, the IC_{50} values for **Ga-Et** remained 109.0 µg/mL, while the IC_{50} values for **Ao-Et** found to be above 200 µg/mL.

The superoxide radical generally fetch from metabolic process/ROS, which further interact with other substrates in presence of enzyme/metal catalysed processes to generate hydroxyl radicals, H_2O_2 and $^1O_2$. These radicals persuade oxidative damage in DNA, lipids and proteins [16, 17]. In the case of superoxide radical-quenching assay of all the samples **(1-3, Ga-Et** and **Ao-Et)** were tabulated in Table S1. Form Figure 2, indeed, compound **1** evinced equivalent inhibition of superoxide free radical to that of the ascorbic acid with IC_{50} value of about 32.0 µg/mL. The concentration of **2, 3** and **Ga-Et** which needed for 50 % inhibition of superoxide radical were found to be 90.2, 63.2 and 65.0 µg/mL, respectively, whereas **Ao-Et** was above 200 µg/mL.

The Ferric ion (Fe^{3+}) reducing power assay measures the ability of anti-radicals to reduce the ferric (Fe^{3+}) complex to blue coloured ferrous (Fe^{2+}) complex. Among all the tested samples, only compound **1** and **Ga-Et** showed reducing power of ferric ions with IC_{50} value of 46.5 and 191.0 µg/mL, respectively, while ascorbic acid with 30.0 µg/mL. Furthermore, compounds **2** and **3** depicted to have reducing power of ferric ions of above 100 µg/mL, while **Ao-Et** was found to be above 200 µg/mL.

Subsequently, from the antioxidant assays it can be concluded that **Ao-Et** has delicate free radical quenching capabilities. In addition, there were no reports stating antioxidant properties of host mangrove species *A. officinalis.* Based on the aforementioned factors it can be concluded that, *G. ajarekarii* has a tendency to counteract against free radicals.

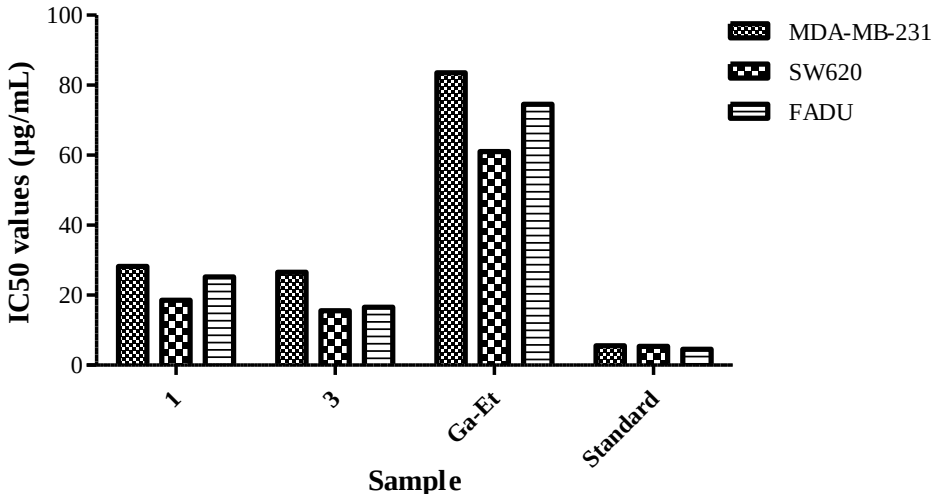

Figure 3. IC_{50} values of **1-3** and **Ga-Et** against series of cancer cell lines.

3.3. Cytotoxicity studies

The cytotoxicity studies of ethanolic extract of *G. ajarekarii* **(Ga-Et)** and its isolates **(1-3)** along with ethanolic extract of host species **(Ao-Et)** were screened against six different human cancer cell lines (MDA-MB-231, SW620, HeLa, FADU, A549, SKOV3) and one normal human cell line such as, NHME by SRB colorimetric assay [10, 11] using doxorubicin as standard. Initially, extracts **Ga-Et** and **Ao-Et** (at 100 µg/mL concentration), isolated metabolites **1-3** (at 30 µg/mL concentration) and doxorubicin (at 10 µg/mL concentration) were screened against selected panel of cancer cell lines (Table S2). The active samples i.e. which remain more than 50 % cell death were further screened at 25, 50, 75 and 100 µg/mL concentrations to extract **(Ga-Et)**; 5, 10, 20 and 30 µg/mL concentrations as for pure compounds **(1** and **3)**; 2.5, 5.0, 7.5

and 10 µg/mL concentrations for standard (doxorubicin). The obtained results of percentage growth inhibition, is plotted against concentrations to obtain IC_{50} values. The lower IC_{50} value indicates better inhibitory profile against cancer cell lines.

From the Table S3, it is clearly evident that the **Ga-Et** showed significant growth inhibitory profile against MDA-MB-231 with IC_{50} value of 83.5 µg/mL (Figure 3). Among the isolates of **Ga-Et,** compounds **3** and **1** showed better IC_{50} values on MDA-MB-231 with 26.5 and 28.2 µg/mL, respectively, while doxorubicin showed with 5.5 µg/mL (Figure 3).

From the outcomes of SRB assay of SW620, it is concluded that **Ga-Et** displayed good cell death rate of SW620 with IC_{50} value of 61.0 µg/mL and, the concentration needed for **1** and **3** for 50 % growth inhibition of SW620 was found to be 18.5 and 15.5 µg/mL, respectively, while doxorubicin to be 5.4 µg/mL (Table S4 & Figure 3).

Based on the cytotoxicity results of FADU, it is observed that **Ga-Et** revealed significant degree of specificity against FADU with IC_{50} value of 74.5 µg/mL (Figure 3). The IC_{50} value of **3** and **1** on FADU were found to be 16.5 and 25.2 µg/mL, respectively, while doxorubicin to be 4.5 µg/mL (Table S5 & Figure 3). The **Ao-Et** at 100 µg/mL exhibited low degree of percentage of cell death on all the tested series of cancer cell lines (Table S2) indicating that the *G. ajarekarii* merely contains cytotoxicity capacity.

Additionally, from the SRB assay results it can be concluded that the anticancer capabilities of **Ga-Et** was mainly due to the presence of compounds **1** and **3.** Also, compounds **1** and **3** showed better cancer cell lysis is due to the presence of higher levels of oxygenated substituents in their chemical structure.

4. CONCLUSION

To wind up, three known metabolites namely chiodectonic acid **(1)**, graphenone **(2)** and graphisquinone **(3)** were isolated from the ethanolic extract of manglicolous lichen *G. ajarekarii* **(Ga-Et)**, which were for the first time reported from *G. ajarekarii*. From the pharmacological screening of the isolates **(1-3)**, it was found that **1** and **3** exhibited better inhibition of superoxide free radicals with IC_{50} value of 32.0 and 63.2 mg/mL, respectively. Correspondingly, these compounds **(1** and **3)** revealed good degree of specificity towards MDA-MB-231, SW620 and FADU and delicate degree of specificity towards normal human cell line, in which, it justifies the compounds containing the antioxidant properties has an aptitude to treat cancer. Hence, this study represents the natural product researchers for further chemical and biological investigations of Manglicolous lichens to discover new bioactive substances.

Acknowledgements. We thank the heads of AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam for providing the necessary facilities. No conflict of interest between any of the authors.

REFERENCES

1. Nayaka S., Ingle K. K., Bajpai R., Rawal J. R., Upreti D. K., and Trivedi S. – Lichens of Gujarat state, India with special reference to coastal habitats, Current Research in Environmental & Applied Mycology **3** (2) (2013) 222-229.
2. Tatipamula V. B., Vedula G. S., and Murthy K. S. – A note on the occurrence of lichens on Vainateya Godavari mangroves in East Godavari district of Andhra Pradesh India, Studies in Fungi **3** (1) (2018) 302-308.

3. Tatipamula V. B., Vedula G. S., Rathod B. B., Shetty P. R., and Sastry A. V. S. – Study of phytochemical analysis, total flavonoid and phenolic content, antimicrobial properties and chemical constituents of two manglicolous lichens extracts, Inventi Rapid: Planta Activa **2018** (3) (2018) 129-134.
4. Kumar J., Dhar P., Tayade A. B., Gupta D., Chaurasia O. P., Upreti D. K., Arora R., and Srivastava R. B. – Antioxidant capacities, phenolic profile and cytotoxic effects of saxicolous lichens from Trans-Himalayan cold desert of Ladakh, PLoS One **9** (6) (2014) e98696.
5. Logesh A. R., Kalaiselvam M., Upreti D. K., Nayaka S., and Karthiresan K. – Mangroves-an abode for unique lichens, Cocasal Ecosystems of India-Special Publication (2013) 39-44.
6. Behera B. C., Adawadkar B., and Makhija U. – Tissue-culture of selected species of the *Graphis* lichen and their biological activities, Fitoterapia **77** (3) (2006) 208-215.
7. Pittayakhajonwut P., Sri-Indrasutdhi V., Dramae A., Lapanun S., Suvannakad R., and Tantichareon M. – Graphisins A and B from the lichen *Graphis tetralocularis*, Aust. J. Chem. **62** (4) (2009) 389-391.
8. Huneck S., and Yoshimura I. – Data of lichen substances, Springer, Heidelberg (1996).
9. Talluri M. R., Ketha A., Battu G. R., Tadi R. S., and Tatipamula V. B. – Protective nature of *Aurelia aurita* against free radicals and Streptozotocin-induced diabetes, Bangladesh, J. Pharmacol. **13** (3) (2018) 287-295.
10. Tatipamula V. B., Vedula G. S., Paidi K. R., and Annam S. S. P. – Nutraceutical value of lichens, *Graphis ajarekarii* and *Parmotrema tinctorum* and their implications in diabetes, Inventi Impact: Nutraceuticals **2018** (3) (2018) 189-194.
11. Tohma H., Gülçin İ., Bursal E., Goren A. C., Alwasel S. H., and Koksal E. – Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS, Food Measure **11** (2) (2017) 556.
12. Khang D. T., Dung T. N., Elzaawely A. A., and Xuan T. D. – Phenolic profiles and antioxidant activity of germinated legumes, Foods **5** (2) (2016) 27.
13. Tatipamula V. B., and Vedula G. S. – In vitro anti-inflammatory and cytotoxicity studies of two mangrove associated lichens, *Dirinaria consimilis* and *Ramalina leiodea* extracts, Hygeia. J. D. Med. **10** (1) (2018) 16-26.
14. Vichai V., and Kirtikara K. – Sulforhodamine B colorimetric assay for cytotoxicity screening, Nat. Protoc. **1** (3) (2006) 1112-1116.
15. Miyagawa H., Hamada N., Sato M., and Ueno T. – Pigments from the cultured lichen mycobionts of *Graphis scripta* and *G. Desquamescens*, Phytochemistry **36** (5) (1994) 1319-1322.
16. Sastry A. V. S., Vedula G. S., and Tatipamula V. B. – In-vitro biological profile of mangrove associated lichen, *Roccella montagnei* extracts, Inventi Impact: Ethnopharmacology **2018** (3) (2018) 153-158.
17. Tatipamula V. B., Kolli M. K., Lagu S. B., Paidi K. R., Reddy P. R., and Yejella R. P. – Novel indolizine derivatives lowers blood glucose levels in Streptozotocin-induced diabetic rats: A histopathological approach, Pharmacol. Rep. **71** (2) (2019) 233-242.