Amer J Med Nat Sci, Volume 1; Issue 1: 25-29. ISSN: 2582-6182

Research Article

American Journal of Medical and Natural Sciences

www.ajmns.com

In-vitro Cytotoxicity study of Manglicolous Lichens, *Graphis ajarekarii* Patw. & C.R. Kulk., and *Parmotrema tinctorum* (Despr. ex Nyl.) Hale.

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Received: 24/02/2020; Revised: 04/03/2020; Accepted: 06/03/2020.

ABSTRACT

Different natural products including lichens, have been using in traditional medicine around the world since the old days. The lichens genera of *Graphis* and *Parmotrema* have a great usage in folklore medicine. The Initially, the hydroalcoholic extracts of mangrove associated lichens, *Graphis ajarekarii* and *Parmotrema tinctorum* were prepared and were used to get different fractions using n-hexane, chloroform, ethyl acetate, acetone and methanol. The prepared extracts was evaluated for cytotoxicity using Sulforhodamine B (SRB) assay on different cell lines such as MDA-MB-231, SW620, HeLa, FADU, A549, SKOV3 and *in vitro* toxicity studies using normal human cell line i.e., Normal Human Mammary Epithelial (NHME). Among all the tested extracts, the ethyl acetate extract from *G. ajarekarii* **(Ga-EA)** and *P. tinctorum* **(Pt-EA)** were active against all tested cancer cells. The SRB assay revealed that **Ga-EA** and **Pt-EA** (100 µg/mL) has good inhibitory profile towards SW620 with 70.51 and 75.18%, than that of the doxorubicin (10 µg/mL, 66.71%). Also, the all the extracts from *G. ajarekarii* and *P. tinctorum* were found to be inactive against NHME indicates non-toxic. Hence, this study revealed that *G. ajarekarii* and *P. tinctorum* has an aptitude to act against cancer.

Keywords: *Graphis ajarekarii; Parmotrema tinctorum; Sulforhodamine B colorimetric assay; Cancer cell lines.*

1. INTRODUCTION

Lichens are symbiotic organisms belongs to epiphytic group, which have grown on any substratum at different geographical regions around the world (Nayaka *e al.,* 2013). As the other natural extracts using in traditional medicines, lichens have been using in the treatment of different diseases throughout ages. The lichens produce different secondary metabolites and they posses' diverse range of pharmacological activities, because of their distinctive endurance. One of such endurance lichens are manglicolous lichens, which are principally grows on mangroves (Kumar *et al.,* 2014; Tatipamula *et al.,* 2018). These manglicolous lichens produces different secondary metabolites compared to normal lichens because of their continued existence at mangrove region i.e. have both marine and fresh water surroundings by adapting their physiological functions (Logesh *et al.,* 2013). But, there was very less research done on manglicolous lichens, because of their distinctive endurance manglicolous lichen getting attention by researchers to isolate different bioactive compounds from them and evaluation of various phamacological activities (Kumar *et al.,* 2014).

Graphis ajarekarii belongs to *Graphis* family, till date the bioactive profile of this species is not established yet. The *Graphis* genus has more than

400 species around the world and there were some earlier reports about some pharmacological activities of different species (Behera *et al.,* 2006; Pittayakhajonwut *et al.,* 2009). Additionally, *Parmotrema tinctorum* belongs to Parmeliaceae, which is the biggest family of foliose lichen. The biological studies of *P. tinctorum* extracts showed antioxidant, antiglycation, α-amylase, α-glucosidase, aldose reductase, tyrosinase and carbohydrate digestive enzymes inhibitory activities (Raj *et al.,* 2014). Keeping these in point of view, this research work is planned to evaluate the *in vitro* cytotoxic, and toxicity studies of *G. ajarekarii* and *P. tinctorum.*

2. MATERIALS AND METHODS

Collection

Lichen *Graphis ajarekarii* Patw. & C.R. Kulk was observed on the twigs of mangrove plant *Avicennia officinalis* at Nagayalnaka light house, Krishna Estuary, Andhra Pradesh, India (15˚77'N and 80˚96'E with 10m elevation) in June 2015 and *Parmotrema tinctorum* (Despr. Ex Nyl.) Hale was observed on twigs of *Excoecaria agallocha* at Bhitharkanika Island, Rajnagar, Orissa, India (20˚74'N and 86˚87'E wirh 0m elevation) in April 2016. The collected species were authenticated by Dr. D.K. Upreti, CSIR-NBRI, Luknow, India and the specimens (15-027174 and 16-027176) were submitted to Lichen herbarium, CSIR-NBRI, Lucknow, India (Figure. 1; Figure 2).

Chemicals

The chemical used in current study were analytical grade. Sulforhodamine B (SRB) and Doxorubicin were from Sigma Aldrich (Mumbai, India) and Avantis Pharma Ltd.

Extraction

The collected lichen species were gently cleaned with water and shade dried. Then the powdered materials (50g) were used to prepare hydroalcoholic extracts with ethanol:water (1:1) using maceration. The obtained extracts of *G. ajarekarii* (Ga-HA, 5.91g) and *P. tinctorum* (Pt-HA, 6.51g) were re-extracted with different solvents by increasing polarity to get different extracts and obtained n-hexane (Ga-Hex, 650mg), chloroform (Ga-Ch, 540mg), ethyl acetate (Ga-EA, 602mg), acetone (Ga-Ac, 444mg) from *G. ajarekarii* and obtained n-hexane (Pt-Hex, 187mg), chloroform (Pt-Ch, 754mg), ethyl acetate (Pt-EA, 514mg), acetone (Pt-Ac, 124mg) from *P. tinctorum.* Similarly, the host species of *G. ajarekarii* i.e. *A. officinalis* hydroalcoholic extract (Ao-HA, 8.24g) was prepared and evaluated for bioactivities along with lichen extracts.

Figure 1: (A) *Graphis ajarekarii* on the twigs of *Avicennia officinalis* (B) *Parmotrema tinctorum* on *Excoecria agallocha*

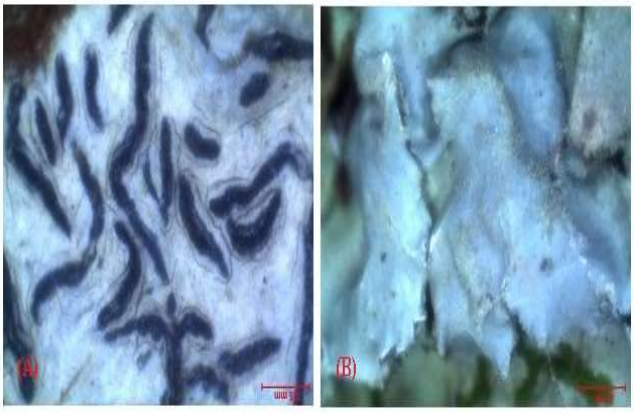

Figure 2: Microscopic images of (A) *Graphis ajarekarii* and (B) *Parmotrema tinctorum*

Samples preparation

The extracts were prepared as 100mg/mL and 10µg/mL using dimethyl sulfoxide (DMSO). The

doxorubicin and DMSO were used as standard and control.

Cytotoxicity assay

The cytotoxicity study was conducted using different cell lines i.e. Breast (MDA-MB-231), Colon (SW620), Cervical (HeLa), Head & Neck (FADU), Ovary and Normal human mammary epithelial (NHME). The cell lines were provided by National Centre for Cell Science, Pune, India and were maintained in minimum essential medium eagle (MEM) media for further study.

Prior to performing the assay using Sulforhodamine B colorimetric assay (SRB assay), the cell lines were prepared as with minimal density 1.9×104cells per vial as below.

Three days before experiment cells were washed with phosphate-buffered saline (PBS) and were grown in MEM media by supplementing with 0.25% trypsin in versene-EDTA and 10% fetal bovine serum (FBS). The cell density was determined by hematocytochameter chamber using 0.4% trypan blue solution.

SRB assay

The SRB assay is primarily based on estimation of cellular protein content (Vichai and Kirtikara, 2006). To the 96 well plate added prepared test samples and 190µL of prepared cell suspension and incubated with 5% CO2 and 90% humidity for 3hrs at 37˚C.

SRB Colorimetric assay: The SRB assay [8] is based on the estimation of cellular protein content. The prepared samples were taken in 96-well tissue-culture plate and added 190µL screened ideal cell suspension and mixed occasionally and incubate at 37˚C with 5% CO2 and 90% relative humidity for 3 h. After the incubation added 100µL of cold trichloroacetic acid and again incubated at 4˚C for 1hr. The plates were washed with sterile water and air dried at room temperature. After drying to each well added 100µL of 0.057% SRB solution and placed a side without any disturbance and then plate was gently rinsed with 1% acetic acid. The dried wells were filled with 200µL 10mM Tris base (pH 10.5) and shake for 5min and the content was used to measure absorbance (OD) at 510nm. During the procedure, the blank have only medium, control have only cancer cell lines without any test samples, standard. The percentage of cellular growth was calculated using below formula.

% of growth inhibition= 100-[(S-B)/(C-B)] × 100

Where S-Sample' mean OD; B- Blank' OD value; C- Control' OD value.

3. RESULTS AND DISCUSSION

The cytotoxic activity of all the prepared *G. ajarekarii* and *P. tinctorum* extracts (100µg/mL) along with Ao-HA was carried out on different cell lines using SRB assay with doxorubicin (10µg/mL) as standard (Table 1).

The outcomes of the SRB assay illustrates that the Ga-Ch, Ga-Ea, Pt-Ch and Pt-EA extracts showed some degree of specificity towards the tested panel of cancer cell lines, while rest extracts (Ga-Hx, Pt-Hx and Ao-HA) showed low degree of specificity (Table. 1). The Ga-EA and Pt-EA at 100 µg/mL exhibited potent percentage of inhibition particularly on SW620 with 70.51±0.53 and 75.18±0.55%, respectively (Figure 3), while standard with 66.71±0.71% at 10µg/mL (Table 1). In addition, the Ga-EA (100 µg/mL) showed good specificity for the MDA-MB-231 and FADU with percentage of cells growth inhibition of 57.72±0.71 and 63.33±0.96%, respectively (Table 1). Likewise, the Pt-EA (100 µg/mL) exhibited better percentage of inhibition on HeLa, FADU, A549 and SKOV3 cancer cell lines with 56.72±0.77, 60.52±0.89, 63.75±0.11 and 55.35±0.19%, respectively (Table 1). In addition all the tested extracts depicted less degree of specificity towards NHME cell lines. Besides, Ao-HA extract exhibited less percentage of inhibition and *G. ajarekarii* shows solely cytotoxic capacity.

Conclusion

This is the first cytotoxicity reports of lichens, *G. ajarekarii* and *P. tinctorum*. The results depicted

the potential application of *G. ajarekarii* and *P. tinctorum* in acting against different cancer cell lines. However, the chemical constituent in the tested extracts were unclear for cytotoxicity and further investigations are going on to identify bioactive compounds from selected lichens and other marine species.

Table 1: Cytotoxic activity of Lichen extracts.

Extract [a]/ Standard [b]	MDA-MB-231	SW620	HeLa	FADU	A549	SKOV3	NHME [c]
Ga-Hex	2.79 ±0.06	0.16± 0.04	0.63± 0.04	0.17 ±0.05	0.78 ±0.02	1.10 ±0.11	0.52 ±0.01
Ga-Ch	46.59 ±0.36	19.18± 0.97	17.75± 0.66	44.39 ±0.34	40.63 ±0.78	46.76 ±0.16	4.03 ±0.73
Ga-EA	57.72 ±0.71	70.51 ±0.53	30.29 ±0.57	63.33 ±0.96	34.07 ±0.84	48.24 ±0.44	9.40 ±0.33
Ga-Ac	35.67 ±0.18	22.73 ±0.56	18.49 ±0.14	43.85 ±0.93	40.41 ±0.58	35.56 ±0.93	2.91 ±0.31
Pt-Hex	11.69 ±0.11	4.83 ±0.71	1.75 ±0.63	1.31 ±0.25	5.45 ±0.12	3.41 ±0.46	1.73 ±0.42
Pt-Ch	24.53 ±0.56	12.48 ±0.95	26.95 ±0.75	37.86 ±0.99	26.78 ±0.18	41.88 ±0.20	3.12 ±0.52
Pt-EA	45.94 ±0.88	75.18 ±0.55	56.72 ±0.77	60.52 ±0.89	63.75 ±0.11	55.35 ±0.19	8.82 ±0.18
Pt-Ac	8.90 ±0.81	1.79 ±0.15	4.26 ±0.14	6.21 ±0.78	7.12 ±0.95	1.90 ±0.10	2.88 ±0.12
Ao-HA	7.17 ±0.24	11.91 ±0.50	5.12 ±0.18	16.41 ±1.26	14.57 ±0.39	8.14 ±1.05	1.04 ±0.16
Doxorubicin	84.40 ±0.80	66.71 ±0.71	90.71 ±0.13	88.34 ±0.27	65.40 ±0.60	77.05 ±0.22	10.08 ±0.95

All values are mean±SEM (n=3); [a]100µg/mL; [b]10µg/mL; [c] Normal Human Mammary Epithelial

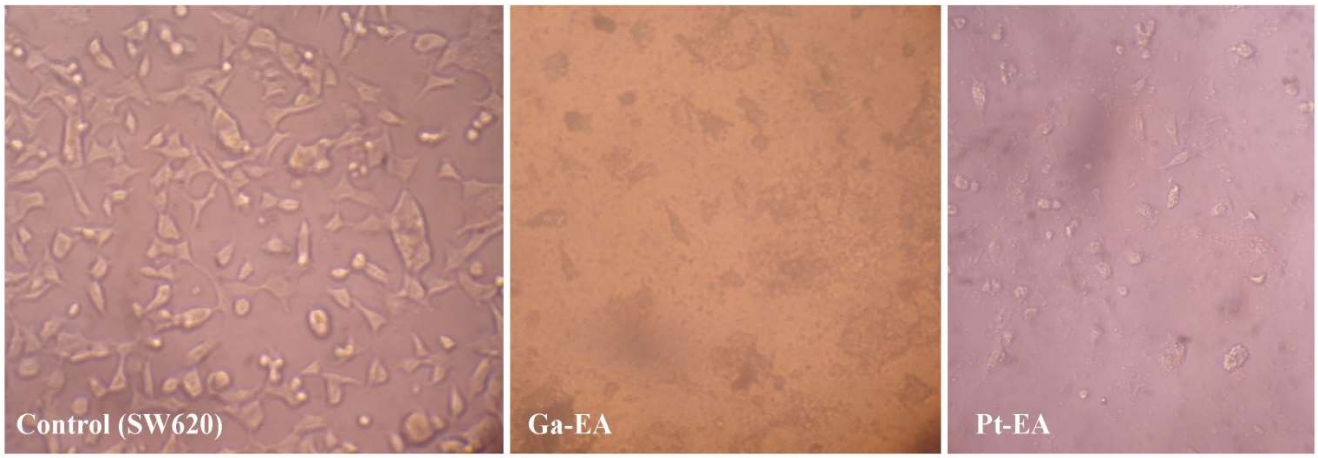

Figure 3: Inhibition of SW620 cell lines by Ga-EA and Pt-EA.

Acknowledgements

The authors are thankful to Ministry of Earth Sciences, India (A.V (1)/MOES-2/DS/6/2017) for financial support and Directors, CSIR-NBRI & CSIR-CDRI, Luknow, India for providing necessary facilities.

Conflicting of Interests

There is no conflict of interest between any of the authors.

References

1. Nayaka S, Ingle KK, Bajpai R *et al.,* Lichens of Gujarat state, India with special reference to coastal habitats. *Current Research in Environmental & Applied Mycology*. 2013; 3(2): 222-229.
2. Tatipamula VB, Vedula GS, Rathod BB *et al.,* Study of Phytochemical Analysis, Total flavonoid and phenolic content, Antimicrobial properties and chemical constituents of two manglicolous lichens extracts. *Inventi Rapid: Planta Activa*. 2018; (2): 1-6.
3. Kumar J, Dhar P, Tayade AB *et al.,* Antioxidant capacities, phenolic profile and cytotoxic effects of Saxicolous lichens from Trans-Himalayan cold Desert of Ladakh. *PLoS One*. 2014; 9(6): e98696.
4. Logesh AR, Kalaiselvam M, Upreti DK *et al.,* Mangroves – an abode for unique lichens. *Cocasal Ecosystems of India–Special Publication*, 2013; 39-44.
5. Behera BC, Adawadkar B, Makhija U. Tissue-culture of selected species of the *Graphis* lichen and their biological activities. *Fitoterapia*. 2006; 77(3): 208-215.
6. Pittayakhajonwut P, Sri-Indrasutdhi V, Dramae A *et al*., Graphisins A and B from the lichen *Graphis tetralocularis*. *Australian Journal of Chemistry*. 2009; 62(4): 389-391.
7. Raj PS, Prathapan A, Sebastian J *et al., Parmotrema tinctorum* exhibits antioxidant, antiglycation and inhibitory activities against aldose reductase and carbohydrate digestive enzymes: An *in vitro* study. *Natural Product Research*. 2014; 28(18): 1480-1484.
8. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocol.* 2006; 1(3): 1112-1116.

Cite this article: Alekhya K, Santhoshi AHV, Hymavathi K, Vinay Bharadwaj T. *In-vitro* Cytotoxicity study of Manglicolous Lichens, *Graphis ajarekarii* Patw. & C.R. Kulk., and *Parmotrema tinctorum* (Despr. ex Nyl.) Hale. *Amer J Med Nat Sci.* 2020; 1(1): 25-29.