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Research Article

IN VITRO ANTI-INFLAMMATORY AND CYTOTOXICITY STUDIES OF TWO MANGROVE ASSOCIATED LICHENS, *Dirinaria consimilis* AND *Ramalina leiodea* EXTRACTS

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ABSTRACT

Plan: *To evaluate the anti-inflammatory and cytotoxicity activity of different extracts from two different manglicolous lichens (Dirinaria consimilis and Ramalina leiodea).*
Preface*: Inflammation is the origin of several deadly diseases like cancer, atherosclerosis, Alzheimer's and rheumatoid arthritis. From decades, lichen extracts and its metabolites are well known in treating inflammation and cancer.*
Outcome*: The outcome of protein denaturation method confirmed that the ethyl acetate and acetone extract of R. leiodea depicted better inhibitory profile against protein denaturation with* IC_{50} *values of 268 and 330 µg/mL Furthermore, the results of SRB assay showed that ethyl acetate and acetone extracts of both the lichens acts potently against MCF-7, DLD-1, HeLa and A549. Simultaneously, all the tested extracts depicted low degree of specificity towards NHME, they are less toxic. Hence, further screening of these extracts may lead to the exploration of safe and potent anti-inflammatory and anticancer agents.*

1. INTRODUCTION

Inflammation is a response to any sort of physically contusion like injured cells, pathogens or irritants[1]. Inflammatory process which under usual conditions is self-limiting becomes continuous and chronic inflammatory syndromes flourish eventually[2]. The chronic inflammatory syndrome leading to dangerous diseases like appendicitis, bronchitis, dermatitis, meningitis, sinusitis, sore throat, tonsillitis, aging, Alzheimer's, asthma, atherosclerosis, cancer, Crohn's disease, hepatitis, peptic ulcer, periodontitis, psoriasis, rheumatoid arthritis, sclerosis, sepsis and tuberculosis diseases[3].

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Earlier, steroidal and non-steroidal anti-inflammatory drugs are used to manage inflammation in the human body. Besides the pharmacological activity of these drugs several side effects include bleeding, gastric ulceration and hypertension were noticed. In addition, long term usage of these steroidal and non-steroidal anti-inflammatory drugs also leading to drug-induced toxic/adverse effects[4]. Hence, researchers keep focusing on natural resources for identification and evaluation of safe drugs with limited adverse effects.

The Lichens (a symbiotic organism) belong to the group of epiphytes which have an aptitude to persist on any substratum or geographical region[5]. The lichens that survived particularly or associated on the mangroves are named as manglicolous lichens[6]. Throughout ages, lichens are used for the treatment of several diseases due to its unique survival and their bioactive secondary metabolites. Lichen and their secondary metabolites exert a diverse range of pharmacological actions mainly anti-inflammatory and cytotoxic effects[7]. Especially, manglicolous lichens show difference in their biological components & actions than normal lichens due to stressed physiological adaptation towards the intertidal zone i.e., having both the marine and freshwater environment[6]. So, the researchers have gained interest in the biological screening of particularly manglicolous lichens. Till date, there are a few pharmacological evaluation reports exist on the manglicolous lichens[7].

Dirinaria genus contains 29 species distributed across the world. The pharmacological screening of this genus found to have radical scavenging, antimicrobial and insecticidal effects[8]. On the other hand, *Ramalina* genus has about 246 species distributed around the world, of which only 118 species were investigated for their chemical and biological studies. A diversity of secondary metabolites were isolated, includes dibenzofuran derivatives like usnic acid, depsides, depsidones, fatty acids, sterols and monocyclic aromatic compounds. Moreover, the biological screening of this genus results in identification of antioxidant, anti-inflammatory, anticancer, antimicrobial activities[9]. The present study was performed to evaluate the *in vitro* anti-inflammatory and cytotoxicity properties of two mangrove associated lichens namely *Dirinaria consimilis* and *Ramalina leiodea* using standard procedures and protocols.

2. MATERIALS AND METHODS

2.1 Collection

The specimens of mangrove lichen, *Dirinaria consimilis* (Stirton) D. D. Awasthi was collected on the bark of mangrove plant, *Excoecaria agallocha* from Vainateya Island, Godavari estuary, Andhra Pradesh, India (16°44’48”N and 81°98’19”E with 0 m elevation) in February, 2015, while the specimens of mangrove lichen, *Ramalina leiodea* (Nyl.) Nyl was collected on the twigs of mangrove plant, *Ceriops decandra* from Bhitharkanika Island, Rajnagar, Orissa, India (20°74’N and 86°87’E with 0 m elevation) in March, 2016. Both the mangrove associated lichens, *Dirinaria consimilis* and *Ramalina leiodea* were authenticated by Dr. D. K. Upreti, CSIR-National Botanical Research Institute (NBRI), Lucknow and deposited at Lichen herbarium, CSIR-NBRI, Lucknow, India with accession numbers 15-027173 and 16-027175, respectively.

2.2 Extraction

The lichen extraction is based on the modified procedures of Jug et al., 2017[10] and Usui et al., 2017[11]. The lichen specimens collected from the twigs of respective mangrove plants were shade-dried. The dried lichen materials were powdered using blender and about 50 g of each mangrove associated lichen material was extracted thrice with ethanol-water (1:1) at room temperature. The mixture was filtered through muslin cloth and evaporated under reduced pressure to obtain hydro alcoholic extracts of *Dirinaria consimilis* **(Dc-HA,** 5.45 g**)** and *Ramalina leiodea* **(Rl-HA,** 6.21 g**)**. These hydro alcoholic extracts were re-extracted with solvents (chloroform, ethyl acetate, acetone and methanol) of increased polarity and the obtained respective solvent extract was filtered through muslin cloth and concentrated in vacuum to obtain dry extracts of *D. consimilis* i.e., chloroform **(DC,** 956 mg**)**, ethyl acetate **(DE,** 315 mg**)**, acetone **(DA,** 256 mg**)** and methanol **(DM,** 1.2 g**)**; *R. leiodea* i.e., chloroform **(RC,** 225 mg**)**, ethyl acetate **(RE,** 801 mg**)**, acetone **(RA,** 448 mg**)** and methanol **(RM**, 1.45 g**)** extracts, which were stored in amber coloured vials and preserved at 4°C till further use.

2.3 In vitro anti-inflammatory assay

Protein denaturation method was employed[12] for the determination of *in vitro* anti-inflammatory activity for extracts and its isolates. Bovine serum albumin was used in this study. The protein was solubilized to 1% concentration using sodium phosphate buffer (50 mM, pH 6.4). To 0.2 ml of prepared protein, different concentration (i.e., 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) of test sample/standard drug (Indomethacin, Indo) were added and make up the final volume to 5 mL with buffer, incubated at 37°C for 20 min and then heated to 95°C for 20 min. After cooling, the turbidity was measured at 660 nm by using UV–Visible Spectrophotometer (Model SL 210, Elico India Ltd.). The experiment was performed in triplicate and average values (Mean±SD) were reported. The percentage inhibition of protein denaturation was calculated as follows

$$\textit{Percentage inhibition} = [(C-S)/C] \times 100,$$ *Where C is absorbance of Control, S is absorbance of Sample*

2.4 Cytotoxicity assay

2.4.1 Cancer Cell lines

MCF-7 (Breast), DLD-1 (Colon), HeLa (Cervical), FADU (Head & Neck), A549 (Lung) and normal human mammary epithelial (NHME) cell lines 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˚C for 72 h.

2.4.2 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 1×10^4 cells per.

2.4.3 Sample Preparation

Initially, for primary screening all the crude extracts were dissolved in DMSO to 100 mg/mL. The active extracts (<50% inhibition) were further screened at 3.12, 6.25, 12.5, 25, 50, 75 and 100 µg/mL and their IC_{50} values were determined. The doxorubicin (10 µg/mL) and DMSO were used as a standard and control respectively.

2.4.4 Sulforhodamine B (SRB) Colorimetric assay

The SRB assay[13] 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˚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˚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 experiment was performed in triplicate and average values (Mean±SD) were reported. The percentage of growth inhibition was calculated using below formula.

$$\%\ Growth\ inhibition = 100 - [S/C] \times 100$$, *Where S is mean OD value of sample, C is mean OD value of control*

2.5 Statistical Analysis

All the results were expressed as mean±SD and analyzed by using Two-way ANOVA followed by Dunnett's multiple comparison tests. All groups were compared with control.

Table 1: *In vitro* anti-inflammatory activity of all extracts of *D. consimilis* and *R. Leiodea*

Sample	*Percentage inhibition at different concentrations (%)**					
	0.1 mg/mL	0.2 mg/mL	0.4 mg/mL	0.6 mg/mL	0.8 mg/mL	1 mg/mL
DC	15.94±1.03	16.67±0.61*	17.39±1.13*	22.23±0.29*	23.67±1.15*	24.87±1.93
DE	23.67±1.15	24.88±1.92*	33.82±2.64	42.02±0.89*	50.9±1.05*	63.52±1.67
DA	18.12±2.59	22.70±1.53*	32.13±1.56*	42.27±0.53*	51.20±1.73	65.70±0.48*
DM	14.97±1.59	18.35±1.75*	22.46±3.77	23.42±2.95	29.46±1.86	38.88±1.89
RC	20.04±0.83	30.19±3.24	37.19±2.81	46.85±1.99	52.89±1.55*	67.63±2.85
RE	32.12±0.56	41.54±0.59*	54.58±2.97	63.04±3.48	74.63±1.68	82.36±3.15
RA	29.71±2.14	43.71±3.81	61.83±3.75	74.15±2.59	83.57±1.88	89.85±3.07
RM	15.94±2.03	19.80±2.76	23.18±2.41	30.67±1.33*	37.68±1.16*	44.68±2.99
Indo	46.91±2.60	61.52±2.12	73.91±0.45*	81.15±2.29	87.43±1.35	94.45±0.49

**n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05; DC- Chloroform extract of D. consimilis; DE- Ethyl acetate extract of D. consimilis; DA-Acetone extract of D. consimilis; DM- Methanol of D. consimilis; RC- Chloroform extract of R. leiodea; RE- Ethyl acetate extract of R. leiodea; RA-Acetone extract of R. leiodea; RM- Methanol of R. leiodea; Indo- Indomethacin (Standard Drug)*

Table S1: Cytotoxicity studies of ethyl acetate extract of *Dirinaria consimilis* (DE) on different cancer cell lines

Sample	*Percentage inhibition (%) at different concentrations (µg/mL)**						
	3.12	6.25	12.5	25	50	75	100
MCF-7	28.68±0.75	36.34±1.37	50.38±1.79	60.42±0.46*	74.69±0.67*	83.60±1.12	93.35±0.72*
DLD-1	16.85±0.10	26.23±0.75*	38.91±0.52*	56.52±0.37*	71.06±0.32*	84.84±1.28	96.20±0.47*
HeLa	24.79±0.79	35.53±1.17	46.95±1.01	54.15±1.72	60.19±0.56*	77.57±1.11	89.66±0.83*
FADU	6.50±0.23	25.10±0.88*	36.58±0.26*	50.75±1.05	68.30±1.71	86.34±0.39*	97.57±1.90
A549	11.45±0.17	22.09±0.28*	33.67±0.11*	49.86±0.60*	61.05±0.46*	74.94±0.24*	85.50±1.39

**n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05; MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; HeLa: Cervical cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line*

Table S2: Cytotoxicity studies of acetone extract of *Dirinaria consimilis* (DA) on different cancer cell lines

Sample	*Percentage inhibition (%) at different concentrations (µg/mL)**						
	3.12	6.25	12.5	25	50	75	100
MCF-7	13.75±0.40	28.30±0.88*	34.38±0.21*	49.89±0.76*	64.15±0.87*	76.06±1.40	87.06±0.40*
DLD-1	29.06±0.69	40.76±1.25	52.79±1.32	62.43±1.23	73.69±0.79*	83.22±0.42*	97.42±0.45*
HeLa	33.25±1.13	42.29±0.21*	51.00±0.83*	63.25±0.57*	72.74±0.76*	81.62±0.67*	94.79±1.54
FADU	3.63±0.55	13.95±0.47*	33.90±1.57	46.14±1.06	67.96±0.74*	77.19±1.13	97.32±0.96*
A549	19.44±0.29	28.59±0.27*	39.43±0.52*	58.00±0.43*	69.31±0.64*	80.69±0.77*	92.61±1.77

**n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05; MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; HeLa: Cervical cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line*

Table S3: Cytotoxicity studies of chloroform extract of *Ramalina leiodea* (RC) on different cancer cell lines

Sample	*Percentage inhibition (%) at different concentrations (µg/mL)**						
	3.12	6.25	12.5	25	50	75	100
MCF-7	17.19±0.75	23.33±1.71	32.69±1.28	39.92±2.50	49.81±1.26	56.31±1.30	71.25±0.10*
DLD-1	12.28±0.48	27.37±1.74	40.12±2.00	49.34±1.56	61.73±0.60*	74.22±0.23*	87.01±0.36*
FADU	3.05±0.06	6.47±0.84*	13.66±0.4*5	26.80±2.06	43.35±0.51*	59.97±0.71*	70.21±0.22*
A549	3.12±0.68	14.15±1.44	21.08±1.21	29.19±1.88	38.00±1.60	45.39±1.02	57.25±1.05

**n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison tests, *p<0.05; MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line*

Table S4: Cytotoxicity studies of ethyl acetate extract of *Ramalina leiodea* **(RE)** on different cancer cell lines

Sample	*Percentage inhibition (%) at different concentrations (µg/mL)**						
	3.12	6.25	12.5	25	50	75	100
MCF-7	13.23±0.61	24.79±1.23	34.60±2.30	46.51±2.42	62.01±1.98	78.26±0.87*	97.30±2.31
DLD-1	16.12±3.00	28.63±3.91	41.39±2.17	53.24±1.49	60.44±1.92	80.33±0.85*	94.79±0.87*
HeLa	48.42±0.87	53.13±0.72*	60.64±1.45	72.56±2.49	81.63±0.12*	88.45±1.58	95.32±0.68*
FADU	25.88±3.71	31.94±1.49	49.26±0.31*	55.14±1.74	66.32±2.25	79.05±0.62*	97.80±1.73
A549	21.41±0.83	35.16±0.11*	40.85±1.35	54.06±0.93*	68.16±1.93	78.99±1.65	90.79±0..77*

**n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05; MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; HeLa: Cervical cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line*

Table S5: Cytotoxicity studies of acetone extract of *Ramalina leiodea* (RA) on different cancer cell lines

Sample	*Percentage inhibition (%) at different concentrations (µg/mL)**						
	3.12	6.25	12.5	25	50	75	100
MCF-7	28.38±0.99	40.21±2.08	51.04±2.48	63.17±2.27	74.02±1.06*	81.37±1.75	94.23±1.05*
DLD-1	18.43±2.11	28.03±1.06*	42.49±1.39*	50.56±1.97	67.61±1.16*	83.86±1.15	98.84±0.53*
HeLa	14.50±1.61	18.50±1.48	29.14±1.77	42.31±2.00	60.53±2.28	72.97±0.98*	89.96±1.41
FADU	16.53±0.34	20.96±1.89	41.06±1.75	56.76±3.29	66.32±2.25	79.05±0.62*	97.80±1.73
A549	13.15±1.41	19.58±1.12*	31.49±1.76	47.63±2.12	62.14±1.49	76.96±1.18	88.08±1.59

**n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05; MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; HeLa: Cervical cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line*

Moreover, the concentration of **RA**, **RE, RC, DA** and **DE** needed for 50% inhibition of bovine albumin protein denaturation were found to be 268, 330, 700, 780 and 800 µg/mL, respectively, while standard (Indo) was 120 µg/mL (Fig.1). From the results it can be concluded that the mangrove associated lichen, *R. leiodea* has potent *in vitro* anti-inflammatory activity when compared to the *D. consimilis*.

3. RESULTS AND DISCUSSION

3.1 In vitro Anti-inflammatory activity

The *in vitro* anti-inflammatory capability of all *D. consimilis* and *R. leiodea* extracts were initially subjected to protein denaturation method[12] using concentrations from 0.1 to 1 mg/mL for standard (indomethacin) as well as extracts and data is tabulated in Table 1. The data evident that the **DE, DA, RC, RE** and **RA** revealed good *in vitro* anti-inflammatory profile. (Table 1). Among all tested samples, the **RA** and **RE** (at 1 mg/mL) depicted prominent inhibitory activity against protein denaturation which is almost nearer to the standard drug (Indo, 1 mg/mL) (Table 1). At 1 mg/mL concentration, the percentage inhibition of bovine albumin protein denaturation for **RA** and **RE** were noticed to be 89.85±3.07 and 82.36±3.15%, respectively, while standard with 94.45±0.49% (Table 1). In addition, the **RC, DA** and **DE** at 1 mg/mL concentration exhibited 67.63±2.85, 65.70±0.48 and 63.52±1.67% inhibition of protein denaturation, respectively.

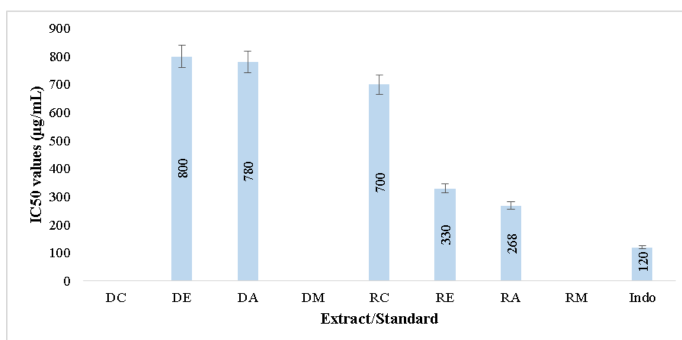

Fig.1: Inhibitory effects of all extracts of *D. consimilis* and *R. leiodea* against protein denaturation.

[DC- Chloroform extract of D. consimilis; DE- Ethyl acetate extract of D. consimilis; DA-Acetone extract of D. consimilis; DM- Methanol of D. consimilis; RC- Chloroform extract of R. leiodea; RE- Ethyl acetate extract of R. leiodea; RA-Acetone extract of R. leiodea; RM- Methanol of R. leiodea; Indo- Indomethacin (Standard Drug)]

3.2 Cytotoxicity studies

Initially, the cytotoxicity studies of all the prepared *D. consimilis* and *R. leiodea* extracts were screened against different human cancer cell lines (MCF-7, DLD-1, HeLa, FADU and A549) at 100 µg/mL concentration by SRB assay[13] using doxorubicin (10 µg/mL) as standard and results of percentage cells growth inhibition was illustrated in Table 2. Simultaneously, the *in vitro* toxicity studies of all extracts were tested on normal human mammary epithelial (NHME) cell line.

Table 2: Cytotoxicity activity of all extracts of *D. consimilis* and *R. leiodea* against different cancer cell lines.

Sample	*Percentage inhibition at 100 µg/mL concentration (%)**					
	MCF-7	DLD-1	HeLa	FADU	A549	NHME
DC	9.31±0.47*	6.82±0.04*	0.36±0.08*	3.14±0.64*	9.08±0.56*	0.15±0.00*
DE	93.34±0.70*	96.20±0.47*	89.66±0.86*	97.57±1.90	85.50±1.39	0.50±0.10*
DA	87.03±0.36	97.42±0.45*	94.79±1.54	97.32±0.96*	92.61±1.77	0.74±0.02*
DM	0.74±0.13*	6.87±0.95*	0.79±0.70*	3.05±0.06*	5.62±0.79*	1.04±0.13*
RC	71.19±0.02*	87.01±0.36*	40.86±0.94*	70.21±0.22*	57.25±1.05	6.53±0.31*
RE	97.30±2.31	94.79±0.87*	95.32±0.68*	97.93±0.35*	90.79±0.77*	8.42±0.09*
RA	94.21±1.06	98.84±0.53	89.96±1.41	97.80±1.73	88.08±1.59	9.20±0.67*
RM	9.67±0.22*	2.32±0.05*	7.47±0.60*	7.47±0.74*	8.34±0.42*	2.30±0.96*
Doxorubicin (10 µg/mL)	81.25±1.56	72.67±0.21	85.55±1.31	98.50±1.21	77.92±0.41*	1.20±0.81*

n=3, Mean±SD, two-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05;* ***DC**- Chloroform extract of D. consimilis;* ***DE****- Ethyl acetate extract of D. consimilis;* ***DA****-Acetone extract of D. consimilis;* ***DM****- Methanol of D. consimilis;* ***RC****- Chloroform extract of R. leiodea;* ***RE****- Ethyl acetate extract of R. leiodea;* ***RA****-Acetone extract of R. leiodea;* ***RM****- Methanol of R. leiodea; MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; HeLa: Cervical cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line; NHME: Normal Human Mammary Epithelial cell lines*

The outcomes of the SRB assay illustrates that the **DE, DA, RC, RE** and **RA** extracts showed good degree of specificity towards the tested panel of cancer cell lines, while rest extracts (**DC, DM** and **RM**) showed very low degree of specificity (Table S1-S5, Table 2). At 100 µg/mL concentration, the ethyl acetate (**DE** and **RE**) and acetone (**DA** and **RA**) extracts of both the lichens exhibited potent percentage of cells growth inhibition on all the tested human cancer cell lines (except on FADU) than that of the standard (doxorubicin) at 10 µg/mL (Table 2).

The **RE, RA, DE, DA** and **RC** showed prominent specificity for the MCF-7 with percentage of cells growth inhibition of 97.30±2.31, 94.21±1.06, 93.34±0.70, 87.03±0.36 and 71.19±0.02% respectively at 100 µg/mL concentration, while standard with 81.25±1.56% at 10 µg/mL (Table 2). Similarly, at 100 µg/mL concentration the **RA, DA, DE, RE** and **RC** exhibited better percentage of growth inhibition on DLD-1 cancer cell lines with 98.84±0.53, 97.42±0.45, 96.20±0.47, 94.79±0.87 and 87.01±0.36% respectively, whereas standard with 72.67±0.21% at 10 µg/mL (Table S1-S5, Table 2).

The percentage cells growth of HeLa cancer cell line was potently inhibited by **RE, DA, RA** and **DE** (100 µg/mL) with 95.32±0.68, 94.79±1.54, 89.96±1.41 and 89.66±0.86%, respectively, while standard (10 µg/mL) with 85.55±1.31% (Table S1-S2; Table S4-S5, Table 2).

Similarly, the FADU cancer cell line was significantly inhibited by **RE, RA, DE, DA** and **RC** with 97.93±0.35, 97.80±1.73, 97.57±1.90, 97.32±0.96 and 70.21±0.22%, besides standard with 98.50±1.21% (Table S1-S5, Table 2). Furthermore, the cells growth of A549 cancer cell line was prominently inhibited by **DA, RE, RA, DE** and **RC** (100 µg/mL) with 92.61±1.77, 90.79±0.77, 88.08±1.59, 85.50±1.39 and 57.25±1.05%, respectively, whereas standard (10 µg/mL) with 77.92±0.41% (Table S1-S5, Table 2).

On the other hand, all the tested extracts depicted less degree of specificity towards NHME cell lines at 100 µg/mL concentrations. The percentage cells growth inhibition of *D. consimilis* and *R. leiodea* extracts on NHME cell lines at 100 µg/mL were in the order: **DC** (0.15±0.00%) < **DE** (0.50±0.10%) < **DA** (0.74±0.02%) < **DM** (1.04±0.13%) < **RM** (2.30±0.96%) < **RC** (6.53±0.31%) < **RE** (8.42±0.09%) < **RA** (9.20±0.67%), besides, standard with 1.20±0.81% at 10 µg/mL concentration (Table 2). This low degree of specificity towards NHME cells indicates that all the extracts were less toxic towards normal human cells. From the data it is also found that the *D. consimilis* is less toxic than the *R. leiodea*.

Considering the initial cytotoxicity screening of all the extracts, further evaluation of IC50 values were performed for those solvent extract (**DE, DA, RC, RE** and **RA**) which showed good specificity towards cancer cell lines i.e., more than 50% cells growth inhibition. Among all the tested samples, the better IC_{50} values was determined by **RE** of 4.5 µg/mL on HeLa (Fig.2). The **DE** showed IC_{50} value of 12.25, 20.25, 18.02, 24.0 and 25.4 µg/mL on MCF-7, DLD-1, HeLa, FADU and A549, respectively, whereas **DA** depicted IC_{50} value of 25.01, 11.02, 11.79, 30.0 and 19.75 µg/mL, respectively (Fig.2).

In addition, the **RC** showed IC_{50} value of 50.09, 25.04, 70.4 and 85.5 µg/mL on MCF-7, DLD-1, FADU and A549, respectively (Fig.2). The IC_{50} values for **RE** were found to be 31.9, 21.5, 4.5, 13.05 and 21.9 µg/mL on MCF-7, DLD-1, HeLa, FADU and A549, respectively, while **RA** depicted 11.95, 23.75, 35.5, 19.75 and 30.7 µg/mL, respectively (Fig.2).

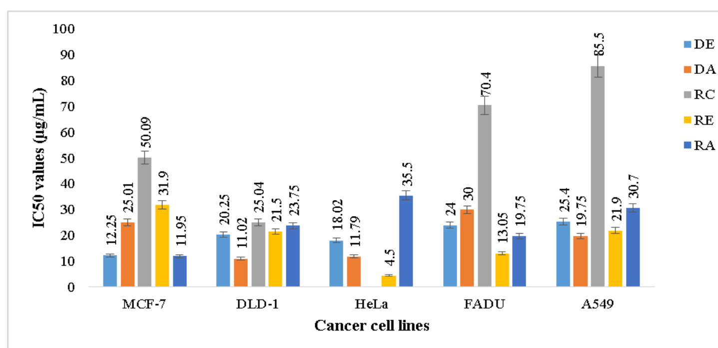

Fig.2 IC_{50} values of **DE, DA, RC, RE** and **RA** extracts against panel of cancer cells.

[MCF-7: Breast cancer cell line, DLD-1: Colon cancer cell line; HeLa: Cervical cancer cell line; FADU: Head & Neck cancer cell line; A549: Lung cancer cell line; ***DE****- Ethyl acetate extract of D. consimilis;* ***DA****- Acetone extract of D. consimilis;* ***RC****- Chloroform extract of R. leiodea;* ***RE****- Ethyl acetate extract of R. leiodea;* ***RA****-Acetone extract of R. leiodea]*

Hence, from the data it can be confirmed that the **DE, DA, RC, RE** and **RA** extracts was very active against Breast (MCF-7), Cervical (HeLa), Colon (DLD-1), Head & Neck (FADU) and Lung (A549) cancer cell lines. In particularly, the **DE** and **RA** were more active against Breast cancer cell line, while **DA** and **RC** against Colon. Furthermore, the **RE** is very potently active against cervical cancer cell lines.

4. CONCLUSION

This is a preliminary report of *in vitro* anti-inflammatory and cytotoxicity profile of mangrove associated lichens, *Dirinaria consimilis* and *Ramalina leiodea*. In this study, the solvent extracts of both lichens were shown to inhibit protein denaturation and also cells growth inhibition against MCF-7, DLD-1, HeLa, FADU and A549 cancer cell lines. Isolation and structural characterization of the pure secondary metabolites that elicited these activities are currently in progress in our laboratory. This study work shows that the both lichens, *D. consimilis* and *R. leiodea* has good amount of anti-inflammatory activity as well as inhibitory profile against cancer cell lines along with low degree of toxicity.

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