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Bioactivity-guided isolation of Laccaic Acid-A: A potent anti-cancer agent from *Laccifer lacca* (Kerr)

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Laccifer lacca (Kerr) (LL) has widely been used as pigmenting, dying and coloring agent in chemical industry. Although, it has wide range of industrial applications, but unfortunately, due to lesser availability of data, it has been ignored. Keeping in mind, the broad application of *Laccifer lacca* (Kerr), we tried to report the *in vitro* anti-cancer effects along with its chemical characterization. This work is divided into two sections, first section to assess the anti-cancer activity of dye of *Laccifer lacca* (Kerr) employing SRB assay. In second section, the active fraction was processed to isolate the active chemical entities using preparative gradient HPLC-UV. The detector was set at 275 nm and 360 nm as reference wavelength. Results of ethyl acetate fraction (EA) showed promising anti-cancer spectrum for MDA-MB-231 (<10 µg/mL) and SiHa (<10 µg/mL) cell lines. Furthermore, laccaic acid-A was identified from EA of *Laccifer lacca*. In conclusion, our results suggested being the first report for isolation of laccaic acid-A from *Laccifer lacca.* We characterized the isolated compound be UV, IR, NMR and Mass spectrometry. We also propose that this is the first report for isolation of laccaic acid-A using preparative HPLC with a good yield and purity.

Keywords: Laccaic acid-A, *Laccifer lacca,* MDA-MB-231, Preparative HPLC, SiHa

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Lac is a minute crawling insect mostly found in Southeast Asia and India. It is known by different names in different parts of world, i.e., *Laccifer lacca* and *Kerria Lac.* Traditionally, lac has a wide range of applications in different industry like coloring textiles and carpets. In reference to Vedas (ancient religious texts written in India between about 1500 and 1000 BCE) and Sanskrit, lac was a good source of income for the people of India. Lac dye was been traded from Southeast Asia to eastern Iran and has been used to fabricate historical textiles and carpets till eighth or ninth century[1]. The major chemical constituents of lac are resin, dye and wax. The stick lac harvested from lac crop contains proteins, sand, soluble salts, sugar, woody matter, insect body debris, etc. Laccaic acids are the combination of anthraquinone derivatives present in lac dye. The wax portion has long chain esters, alcohols and hydrocarbons. The composition depends on the strain of insect, host plant and environmental conditions[2]. There are different types of laccaic acid ranging from laccaic acid A-F with limited application. Chromatographic techniques such column (cellulose) and preparative paper chromatography were used to isolate laccaic acid-A. IR and UV-Vis[3] were used to characterize laccaic acid-A. The reported pKa value of laccaic acid was 4.4 and 6.5[4]. Minor fraction of laccaic acid-B was isolated using a polyamide column. Structural determination of laccaic acid-B, its methylated product and xantholaccaic acid-B have been deduced with the help of NMR data. Chelated quinine group present in laccaic acid-B[5] was reported by the researchers. Cellulose column was used to isolate laccaic acid-C as a major fraction whereas minor fraction is of laccaic acid-E. Both laccaic acid-C and laccaic acid-E are having same properties like that of laccaic acid-A and B but the only difference is the presence of aside chain (α- amino acid)[6]. Isolation of laccaic acid-D was performed by silica gel column and the mobile phase used was acetone[7]. Chromatographic technique such as high-speed counter-current was used to isolate laccaic acids-A, B, C and E[8] from lac extract, that are the major chromophoric groups of lac-dye. Laccaic acids main components of lac dye have been identified using ESI LC/MS/MS comprising of a volatile mobile phase acetylacetone[9]. Toxicity studies of lac color were performed by developing an

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analytical method to find the traces of lac dye present in diet feed to rats and feces of rats[10]. For the first time, High performance liquid chromatography-Diode array detector (HPLC-DAD) database was developed for the two species of lac dye, *Kerria* and *Paratachardina* genera, with the help of the Principal Component Analysis (PCA) and the statistical analysis[11]. Recently lac dye has proven its biological activity as it has a similar structure with the anti-cancer drug adriamycin (ADR) as both are anthraquinone derivatives. Antioxidant activity was investigated by DPPH assay, reducing power assay and the thiocyanate assay methods for laccaic acids and its aluminum lake[12]. Food coloring is mainly done by lac dye. Auto activations of the PHBP (Plasma Hyaluronan-Binding Protein) proenzyme (IC_{50}=0.35–0.55 g/mL) and the catalytic activity of the active enzyme (IC_{50}=1.1 g/mL) were due to protease inhibition property of lac dye[13]. Laccaic acids are also having a potent anti-cancer activity where it inhibits direct DNA competitive inhibitor of DNA methyl transferase I. *In vitro* methylation assay and alters the expression of methylated genes in MCF-7 breast cancer cells synergistically with 5-aza-2'deoxycytidine[14]. Recent literature survey reveals a comparison between *Paratachardina* and *Kerria* genera, the resinous exudation of lac dye using HPLC-DAD-MS for the identification of laccaic acid-A, B, C and E[15]. However, there is no report available till date for the complete identification and characterization of laccaic acid-A. Moreover, in order to evaluate its biological activity in broader spectrum, we have undertaken other cancer cell lines such as ZR-75-1, BT474, MDA-MB-468 and many others (total fifteen). In this paper, we report for the first time the anti-cancer potential of laccaic acid-A on two different cancer cell lines along with the isolation and characterization of the same with elaborate spectroscopic characterizations. We are hopeful that this can be utilized as a standard data set for characterization of laccaic acid-A isolated from other natural or synthetic sources in future. In addition, the biological activity investigation may help in the future development of drugs which may result in unlocking the new anti-cancer agents from this dye.

Methodology

Chemicals and reagents

Analytical grade reagents and chemicals were used for the study. Milli Q water was used for the study obtained from Milli Q water purification system (Millipore, Milford, MA, USA), methanol (Fisher Scientific, Loughborough, Leicestershire, UK) of HPLC grade, HPLC grade acetonitrile (Ranchem), perchloric acid, petroleum ether (60ºC-80ºC), chloroform and ethyl acetate AR Grade (Ranchem). Syringe filter of 0.22 µm and were procured from Merck, Mumbai. Lac-dye was provided from the IINRG (Indian Institute of Natural Resins and Gums), Ranchi, Jharkhand, India. RPMI 1640, fetal bovine serum (FBS), L-glutamine, dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), acetic acid, Sulforhodamine B (SRB) deuterated dimethyl sulfoxide (DMSO-d_6) were purchased from Sigma (St. Louis, MO, USA).

Preparation of Technical grade and sample preparation of lac dye

Technical grade

Wash the stick lac with water, acidify it (hydrochloric acid 0.1% on the volume of water) then the precipitate was allowed to settle down, collect the supernatant liquor by filtration. The mixed filtrate was treated with quicklime or calcium carbonate till colorless liquor appears. Salt of calcium lac dye which has separated out by filtration was effectively collected and repeatedly washed with water. The cold suspension (calcium salt of lac dye) in water was acidified and left for 07 days at room temperature till the dye crystallizes[16,17]. After this it was washed properly then filtered and finally dried (the yield obtained was 0.5% mass of stick lac). The quality and the quantity of the dye depend on the age of stick lac but it is nearly 80-90%[18]. The above technical grade lac dye was prepared according to the method described by Dey and Ghosh, 2018[19].

Sample Preparation

Lac dye of technical grade was obtained in powder form. Further, this lac was again subjected to fractionation. Fractionation was performed according to the polarity of the solvents, non-polar to polar solvents. The sample preparation methodology was followed from the previously reported method of Dey and Ghosh, 2018[19]. At first 5 g of lac was taken in a 50 mL volumetric flask containing 50 mL of petroleum ether (60ºC-80ºC) and allowed to ultrasonicate for 10 min at a temperature of 40°C. Petroleum ether solution containing lac was then transferred to 15 mL centrifuge tube, equally distributed in 5 centrifuge tubes, centrifuged for 10 min at 5000 rpm. The clear supernatant liquid was collected in petri-dishes and allowed to evaporate. The lac dye which has settled down after

centrifugation in the centrifuge tubes were collected and dissolved in 50 mL volumetric flask containing chloroform. Further, the solution was subjected to ultrasonication for 10 min at a temperature of 40°C. Similarly, this procedure was repeated for ethyl acetate and water for the preparation of ethyl acetate and the water fraction. Thus, total 03 sets of fractions were prepared, chloroform (C), ethyl acetate (EA) and water (W) respectively. Fractionation with petroleum ether was mainly done for the defatting purpose to remove the unwanted oils and the unsaturated fatty acids from the samples.

Cell viability assay

Cell culture

Anti-cancer activity of 03 fractions of lac dye were evaluated in 15 different cell lines established from the human breast cancer cell line (MCF-7, ZR-75-1, BT474, MDA-MB-468 and MDA-MB-231), human cervical cancer cell line (ME-180, SiHa and HeLa), human ovarian cancer cell line (A2780, OVCAR-3, PA-1, SK-OV-3 and NCI/ADR-RES) and human prostrate cancer cell line (DO145 and PC-3). The RPMI 1640 medium containing 2 mM L-glutamine and 10% fetal bovine serum was used to grow the cell lines. Plate density depends upon the doubling time of individual cells which were inoculated in a 96 well microtiter plates in100 µL. After the cells were inoculated the microtiter plates were placed in an incubator at 37°C, 5% CO_2, 95% air and 100% humidity for a period of 24 h prior to addition of 03 different fractions[20, 21].

Sample preparation

The 03 different dried fractions were dissolved in dimethyl sulfoxide at a concentration of 100 mg/mL further dilution was made with water to 1 mg/mL and stored frozen before it was used. Aliquot of the frozen concentration of 1 mg/mL was thawed at the time of addition of different fractions and further diluted to 800 µg/mL, 400 µg/mL, 200 µg/mL and 100 µg/mL with complete medium containing test article. The final drug concentrations were made to 80 µg/mL, 40 µg/mL, 20 µg/mL and 10 µg/mL by the addition of 10 µL from the above drug dilutions to the appropriate microtiter wells containing 90 µL of the medium.

SRB (Sulforhodamine B) assay procedure

The plates were incubated at standard conditions for a period of 48 h after the addition of samples lastly addition of cold TCA marks the termination of the assay. The cells were fixed under *in situ* conditions to which 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) was added and incubated for a period of 60 min at about 4°C. Discard the supernatant and the plates were washed repeatedly 5 times thoroughly by tap water and finally air dried. To each well add 50 µL of sulforhodamine B solution of 0.4% (w/v) in 1% acetic acid. Then the plates were incubated for 20 min at room temperature. Finally, after staining the unbound dye was recuperated followed by elimination of remnants of dye by washing with 1% acetic acid for 05 times. The plates were air dried. Elute the bound stain with 10 mM trizma base. At a wavelength of 540 nm with 690 nm reference wavelength the absorbance was taken on a plate reader. The concentration that inhibits cell growth by 50% (IC_{50}) was found. Adriamycin (ADR) was used as a standard antitumor drug. At each drug concentration levels, the percentage growth was calculated[20,21]. The inhibitory concentration was calculated as:

$$IC_{50}=[Ti/C]x100\%$$

Where, the test growth in presence of the drug at the four concentration levels (Ti), control growth (C) and time zero (Tz)

Chromatographic conditions and instruments

Preparative HPLC

The instrument used was Preparative HPLC (Prep-HPLC) of Agilent 1100 series (Germany) with a binary pump system, having UV, VIS detector along with a fraction collector at the bottom. The column used was Agilent reverse-phase C18 column (250 mm×30 mm×10 µm) the detector was set at 275 nm and reference at 360 nm wavelength. Data were acquired on Agilent Chemstation (Agilent Pvt. Ltd, Germany). The mobile phase consists of methanol: acetonitrile (85:15) as solvent system A and solvent system B consists of 0.15% perchloric acid in water. Finally, the mobile phases were filtered through a 0.22 µm membrane filter. The flow rate was set at 15 mL/min and the runtime was 33 min followed by gradient run. The fraction collector was set in a time base operation, i.e., time set for each vial was 0.41 min and the maximum amount a vial can collect was 5 mL. After the fraction collection, the solvents were evaporated by rota evaporator (Buchi, Germany) then lyophilized and the dried compound was stored at 4ºC for further use.

Characterization studies: UV-VIS, IR, NMR, MS

The study was performed in UV-VIS double beam spectrophotometer (model UV-1800, Shimadzu Limited) with 1 cm matched pair quartz cell. The EA isolated compound from Prep-HPLC was analyzed using Fourier transform infrared spectrometer (FTIR-8400 S, Shimadzu, Japan). Mass spectrometry of EA isolated compound was performed in Thermo Scientific LTQ XL. EA isolated compound was subjected to ^{1}H and ^{13}C NMR, carried out at 400 MHz, using Jeol NMR.

Results

Cytotoxic activity

The cytotoxic activity was determined for water (W), ethyl acetate (EA) and chloroform (C) fraction against MCF-7, ZR-75-1, BT-474, MDA-MB-468, MDA-MB-231, ME-180, SiHa, HeLa, A2780, OVCAR-3, PA-1, SK-OV-3, NCI/ADR-RES, DO145 and PC-3 cancer cell lines, using SRB assay employing Adriamycin (ADR) as a reference drug. All the 03 fractions were tested in 15 cancer cell lines among which EA has promising activity against MDA-MB-231 and SiHa cancer cell lines with IC_{50} value below 10 µg/mL for EA fraction and ADR. At higher concentration, other fractions such as C and W fraction were also found to be active over these two cancer cell lines. Apart from these two cancer cell lines, all the fractions were found to be active on other cell lines too however having IC_{50} values as high as 80 µg/mL or above. These results have been summarized in Table 1-3 & Fig. 1-2.

Chromatographic isolation of ethyl acetate fraction using Preparative HPLC

The optimized chromatogram was observed through an effective gradient run. After the chromatographic run, 03 elution peaks were observed having retention time (R_t) 6.181, 16.749 and 27.625 min respectively (Fig. 3). The peak having R_t 6.181 min was of higher altitude or absorbance when compared to other peaks. The associated compound with R_t 6.181 was effectively collected in the fraction collector of vial no 20, 29, 38 and 47. Samples were collected till sufficient amount was recovered for further analysis.

Identification and elucidation of EA fraction by UV-VIS, IR and MS

The λ_{max} of EA fraction was found to be 494.50 nm for the visible spectrum were as 289 nm in the ultraviolet spectrum (Fig. 4). Laccaic acid-A in the visible spectrum is reported to have maximum absorbance at 497 nm and 287 nm for the ultraviolet region[3]. In IR spectrum the EA fraction showed carbonyl stretching at 1710.92 cm^{-1}. The peak was assigned to a carboxylic acid group, while 1620.26 cm^{-1} peak was assigned for 1, 4-quinone where both carbonyl groups have been chelated by a total of 02 or 03 perihydroxyl groups. The 1670.41cm^{-1} peak was assigned for –C=O amide stretch, 3356.25 cm^{-1} peak was attributed to –OH group, whereas 2924.18 and 2854.74 cm^{-1} were assigned for alkane stretching (-CH_2-CH_2-) (Fig. 5). The MS result showed molecular ion peak of m/z 536.09 $[M-H]^-$ (Fig. 6) which is comparable to laccaic acid-A having a molecular

Table 1 — IC_{50}[a] (µg/mL) of Lac dye fractions obtained from *Laccifer lacca* tested on cell lines

Cell lines	Chloroform Fraction	Ethyl acetate Fraction	Water Fraction	Adriamycin
MCF-7	>80	>80	>80	<10
ZR-75-1	NE*	NE*	NE*	<10
BT474	>80	>80	>80	<10
MDA-MB-468	>80	>80	>80	<10
MDA-MB-231	37.9	<10	60.1	<10
ME-180	NE*	NE*	NE*	<10
SiHa	79.2	<10	57.0	<10
HeLa	>80	>80	>80	<10
A2780	NE*	NE*	NE*	<10
OVCAR-3	NE*	NE*	NE*	<10
PA-1	NE*	NE*	NE*	<10
SK-OV-3	>80	>80	>80	<10
NCI/ADR-RES	NE*	NE*	NE*	<10
DU145	>80	>80	>80	<10
PC-3	NE*	NE*	NE*	<10

[a]IC_{50}: concentration of fraction required to reduce cell survival by 50%; [a]IC_{50} of ≤ 10 µg/mL is considered to demonstrate activity; NE*: Not Estimated

Table 2 — Activity of Lac dye fractions against human breast cancer cell line MDA-MB-231

Human Breast Cancer Cell Line MDA-MB-231

% Control Growth

Drug Concentrations (µg/mL)

	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
C	63.1	43.9	43.6	41.4	68.3	63.3	44.8	52.7	51.8	46.1	37.9	43.8	61.1	51.1	42.1	46.0
EA	46.8	39.4	44.2	46.5	57.2	40.5	39.3	48.2	43.7	40.3	34.8	44.3	49.2	40.1	39.4	46.4
W	60.0	51.7	52.6	45.3	64.5	61.4	47.4	51.4	67.5	47.6	44.7	51.2	64.0	53.5	48.2	49.3
ADR	29.9	39.5	37.6	22.8	43.7	32.3	41.3	40.2	33.8	21.8	28.8	41.5	35.8	31.2	35.9	34.8

Water (W), Ethyl acetate (EA) and Chloroform (C) fraction; Adriamycin (ADR)

Table 3 — Activity of Lac dye fractions against human cervical cancer cell line SiHa

Human Cervical Cancer Cell Line SiHa

% Control Growth

Drug Concentrations (µg/mL)

	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
C	44.8	57.1	45.8	33.5	65.5	56.7	50.1	62.0	70.0	59.6	62.4	57.1	60.1	57.8	52.8	50.9
EA	35.8	29.6	31.3	32.7	59.6	58.0	49.9	49.0	50.3	42.9	38.5	48.7	48.6	43.5	39.9	43.5
W	36.9	39.4	39.5	31.1	91.5	65.0	55.1	53.1	78.2	48.0	55.3	58.6	68.9	50.8	50.0	47.6
ADR	49.5	48.0	39.0	40.2	46.6	46.6	55.4	54.9	26.0	24.2	32.0	43.3	40.7	39.6	42.1	46.1

Water (W), Ethyl acetate (EA) and Chloroform (C) fraction; Adriamycin (ADR)

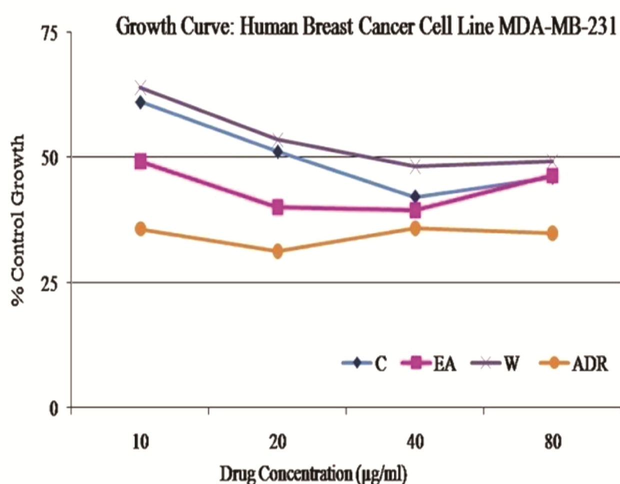

Fig. 1 — Cytotoxicity assessment by SRB assay in MDA-MB-231 (Human Breast Cancer Cell Line) compared to the reference drug. Water (W), Ethyl acetate (EA) and Chloroform (C) fraction; Adriamycin (ADR)

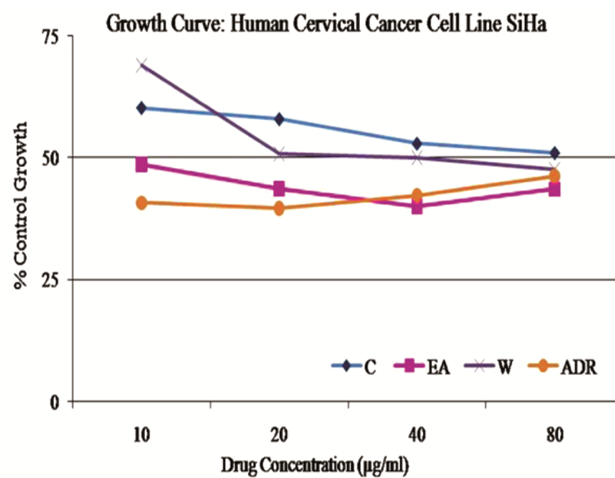

Fig. 2 — Cytotoxicity assessment by SRB assay in SiHa (Human Cervical Cancer Cell Line) compared to the reference drug. Water (W), Ethyl acetate (EA) and Chloroform (C) fraction; Adriamycin (ADR)

weight of 537 amu as reported earlier[8,9]. The base peak was found to be at 492.00 (m/z). We assume that the appearance of this peak has been due to the removal of one molecule of CO_2 (molecular weight 44) from the laccaic acid resulting in the peak at m/z 492.00 $[M\text{-}H\text{-}CO_2]^-$.

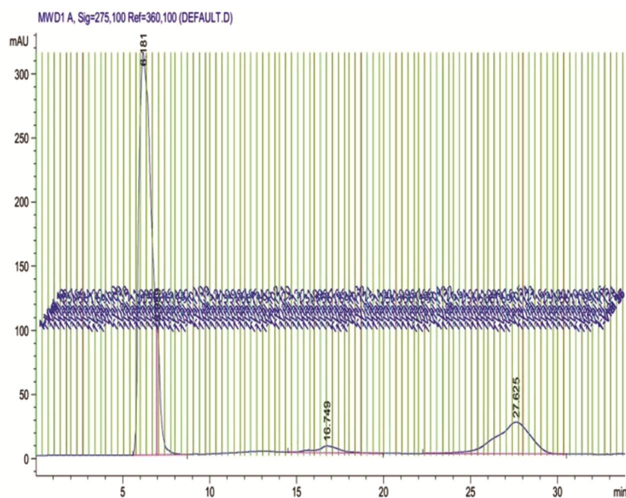

Fig. 3 — Chromatogram of Ethyl acetate fraction of lac dye by Prep-HPLC

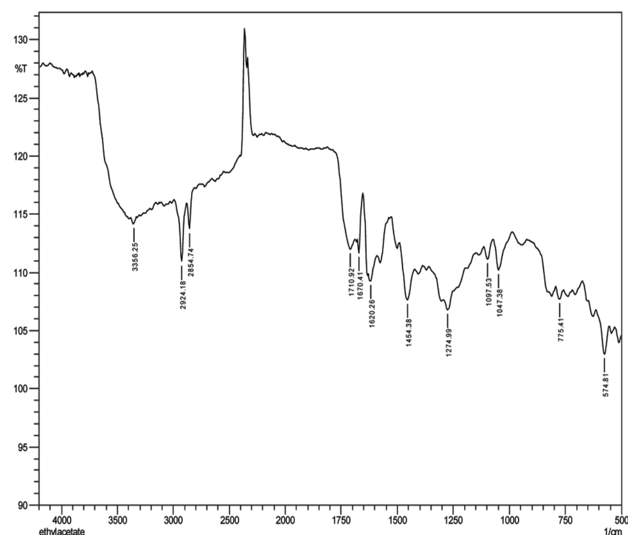

Fig. 5 — IR spectra of ethyl acetate fraction of lac dye

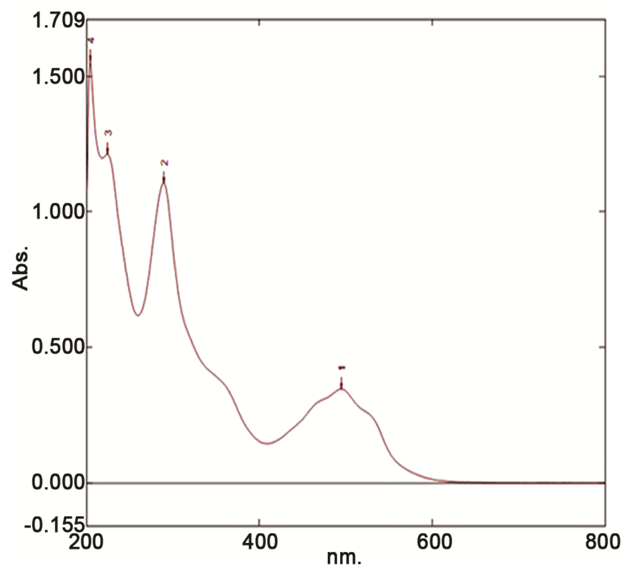

Fig. 4 — UV spectra of ethyl acetate fraction of lac dye

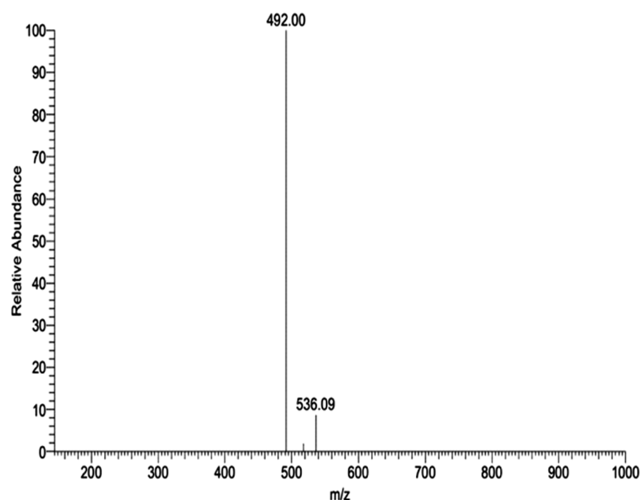

Fig. 6 — HR-ESI-MS of ethyl acetate isolated compound

2D NMR Spectroscopy

The ^{1}H and ^{13}C NMR of isolated compound from ethyl acetate fraction using $(CD_3)_2SO$ (Table 4 and Fig. 7 & Fig. 8). The HMQC (Fig. 9) of EA compound gives the interaction of carbon and hydrogen which are directly attached in the aromatic region that is δH 7.671 (H-8) to δC 114.691 (C-8); δH 6.995 (H-4$^{/}$) to δC 129.864 (C-4$^{/}$); δH 6.846 (H-2$^{/}$) to δC 131.915 (C-2$^{/}$) and δH 6.778 (H-5$^{/}$) to δC 115.922 (C-5$^{/}$) whereas in the aliphatic region the methylene attached to NH can be easily recognized δH 3.518 (H-4$^{//}$) to δC 63.021 (C-4$^{//}$) giving an idea of molecular structure of EA. The DEPT-135 (Fig. 10) of EA compound shows that there are four aromatic carbons, i.e., CH appearing positive δC 131.843 (C-2$^{/}$), 129.697 (C-4$^{/}$), 115.802 (C-5$^{/}$) and 114.604 (C-8). In the aliphatic region, i.e., CH_2 appears negative δC 63.011 (C-4$^{//}$), 34.847 (C-5$^{//}$) and CH_3 appearing positive δC 23.166 (C-1$^{//}$) giving an idea of hybridization of carbon for the molecular structure of EA. The peaks appearing in the region δ 20.981, 66.250, and 14.503 were determined as solvent trace impurities for ethyl acetate[22]. The COSY (Fig. 11) of EA compound shows that there is a correlation between the two protons appearing at δ 3.521 (H-4$^{//}$) and 2.581 (H-5$^{//}$) showing there is a spin-spin coupling between the two methylene groups ($-CH_2-CH_2-$).Thus the compound identified as laccaic acid-A (Fig. 12).

Table 4 — ^{1}H and ^{13}C NMR data of EA compounds in DMSO-d_6.

Position	δ_H (*J* in Hz)	δ_C
1		138.005
2		148.642
3		104.945
4		154.008
4a		111.806
5		125.011
6		119.127
7		159.413
8	7.665 (s, 1H)	114.604
8a		136.165
9		186.532
9a		123.286
10		183.121
10a		120.354
11	13.573 (s, 1H)	168.881
12	13.153 (s, 1H)	167.271
13	9.122 (s, OH, 1H)	
14	10.703(s, OH, 1H)	
15		
16		
17	5.270 (s, OH, 1H)	
1′		123.171
2′	6.847 (s, 1H)	131.853
3′		130.032
4′	7.007 (d, 7.2, 1H)	129.649
5′	6.779 (m, 1H)	115.792
6′		153.625
1″	1.744 (s, 3H)	23.166
2″		169.609
3″	7.877 (s, 1H)	
4″	3.537 (t, 6.6, 2H)	63.011
5″	2.596 (t, 7.6, 2H)	34.837
CH_3 (Ethyl Acetate)	1.944 (s)	20.981
CH_2CH_3 (Ethyl Acetate)	4.007 (q)	
CH_2CH_3 (Ethyl Acetate)	1.18 (t)	
CH_2 (Ethyl Acetate)		66.250
CH_3 (Ethyl Acetate)		14.503

Discussion

The present study describes the anti-cancer potential of ethyl acetate fraction of laccaic acids in 02 different cancer cell lines for the first time. Ethyl acetate fraction of laccaic acids has shown anti-cancer activity in MDA-MB-231 (human breast cancer cell line) and SiHa (human cervical cancer cell line) cancer cell lines at a very minimum concentration below 10 µg/mL which is similar when compared to standard drug ADR. Previously from the literature, it has been reported that laccaic acid-A is having anti-

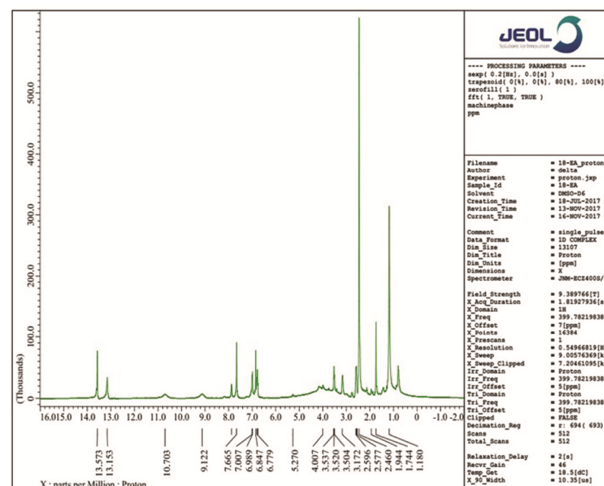

Fig. 7 — ^{1}H-NMR spectrum of ethyl acetate isolated compound (400 MHz – DMSO-d_6)

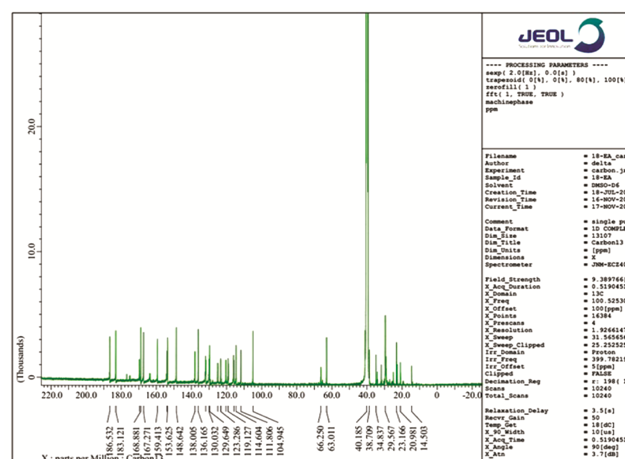

Fig. 8 — ^{13}C-NMR spectrum of ethyl acetate isolated compound (400 MHz – DMSO-d_6)

cancer activity in MCF-7 human breast cancer cell line[14]. The bioactive ethyl acetate fraction was then further processed to isolate the active compound, followed by characterization. The λ_{max} was found to be 494.50 and 289 nm for the visible and ultraviolet region. In the visible region this position is the characteristic feature of anthraquinone with typical 03 peri-hydroxyl groups shows absorption maxima in the region of 480 to 510 nm, for example, Islandicin (2-methyl-1,4,5-trihydroxy), Helminthosporin (2-methyl-4,5,8-trihydroxy) and purpurin (1,2,4-Trihydroxyanthraquinone) has an absorption maxima 492 nm, 480 nm and 482 nm in the visible region[3, 23] showing 03 perihydroxyl groups attached to anthraquinone moiety. This shows that the EA fraction has the presence of anthraquinone ring

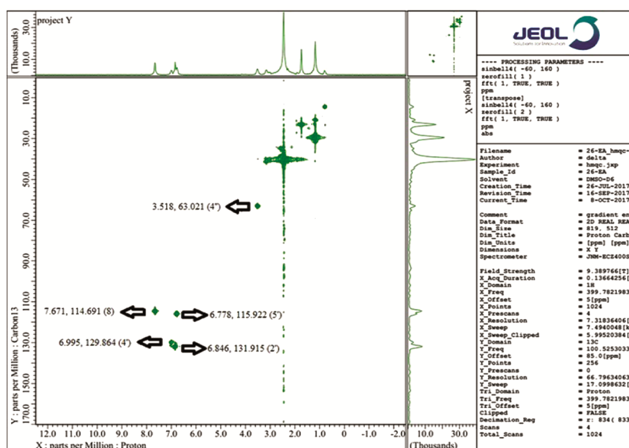

Fig. 9 — HMQC-NMR spectrum of ethyl acetate isolated compound

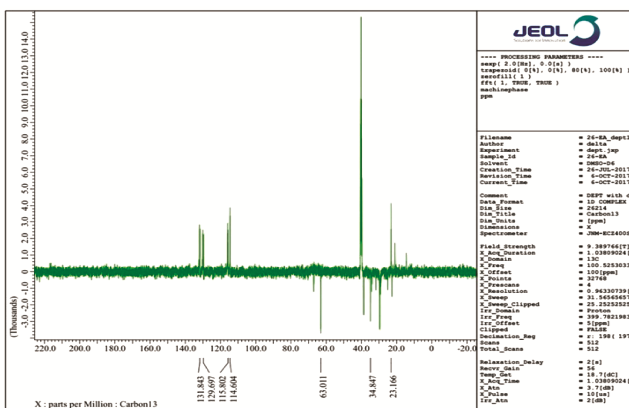

Fig. 10 — DEPT-135-NMR spectrum of ethyl acetate isolated compound

system with 03 perihydroxyl groups. Moreover, this anthraquinone system shows multiple bands between 260 nm and 300 nm[23] which is another characteristic feature of the anthraquinone ring system. The IR of EA fraction shows bands in the region of carbonyl stretching at 1710.92, 1670.41 and 1620.26 cm^{-1}. Literature review reveals our findings for the IR spectroscopy that the bands are present in the region of carbonyl stretching at 1715, 1677, and 1620 cm^{-1} for the laccaic acid-A[3]. The molecular mass of the EA fraction was found to be 537.09 amu. The molecular ion peak was found to be 536.09 $[M-H]^-$ and the base peak was found to be 492.00 $[M-H-CO_2]^-$ due to the removal of one molecule of CO_2, the mass spectrometry data was fully supported by the literature[8,9]. For the first time, the NMR data for laccaic acid A has been reported in this paper. Previously from the literature, it was found that only ^1H NMR data has been reported that too by using tau

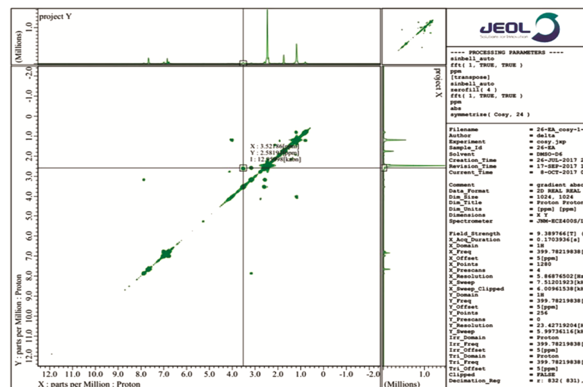

Fig. 11 — COSY-NMR spectrum of ethyl acetate isolated compound

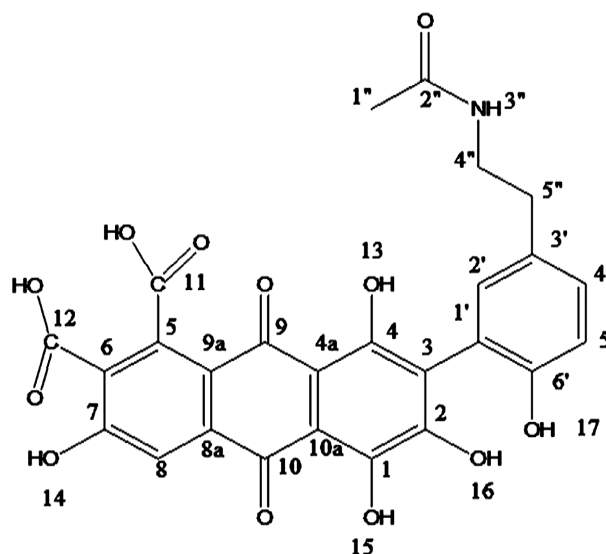

Fig. 12 — Chemical structure of laccaic acid A

(τ) value[4]. In this paper, for the first time, we are reporting the ^1H and ^{13}C of laccaic acid-A in delta (δ) value along with HMQC, DEPT-135 and COSY which provides the structural conformation of the isolated compound as laccaic acid-A having anti-cancer potential.

Conclusion

In conclusion, the compound laccaic acid-A (7-acetyl-6-ethyl-3,5,8-trihydroxy-9,10-dioxoanthracene-1,2-dicarboxylic acid) was isolated for the first time using preparative HPLC from *Laccifer lacca* (Kerr) crude ethyl acetate fraction and characterized by UV, IR, MS and NMR. This isolated compound laccaic acid-A has proven to be an anticancer agent in MDA-MB-231 (human breast cancer cell line) and SiHa (human cervical cancer cell line) cancer cell lines for the first time. These new findings can contribute in

developing a new anticancer drug which can open the gateway for the treatment of cancer from this dye.

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