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GC-MS analysis, anticancer and anti-inflammatory activities of *Saussurea hypoleuca* spreng. Root

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Abstract: Study has been premeditated to appraise the anticancer and anti-inflammatory activities of a native medicinal plant *Saussurea hypoleuca* Spreng root. Anticancer assays including MTT, Alamar Blue (AB), Neutral Red (NR) & LDH were employed on root methanolic extract (RME) and all fractions to calculate % age of cell viability and cell cytotoxicity. All fractions of plant root were tested for *in vitro* as well as *in vivo* anti-inflammatory assays by reported methods. GC-MS analysis of n-hexane: chloroform fractions in column chromatography has shown isopropyl myristate, hexadecanoic acid, 11-octadecenoic acid, Di-n-octyl phthalate, dioctyl ether, decanedioic acid, 1H-3a,7-Methanoazulene, 3,4-hexanedione and Tetracosapentaene. Percentage of cell viability in anticancer assays was significantly high in all fractions. However, whole results were momentous with ethyl acetate and aqueous fractions owning to excellent profile in evaluating cytotoxicity in each assay. COX-2 inhibition was calculated which was high in RME (68.69%), ethyl acetate (56.52%), aqueous (55.21%) and chloroform fraction (53.47%). Carrageenan and formalin models were developed on rats to investigate *in vivo* anti-inflammatory activity. RME (56.19%, 71.09%, 66.4%, 67.99%) and ethyl acetate (51.36%, 64.97%, 55.63% & 61.01%) produced significant % age inhibition in dose dependent manner at 200 and 400 mg/kg doses respectively. All above findings direct that plant root holds strong anticancer and anti-inflammatory activities.

Keywords: Anticancer, anti-inflammatory, GC-MS, COX-2, MTT, Alamar blue, neutral red, LDH

INTRODUCTION

Cancer is a leading cause of death among various terrible diseases. A lot of research has been done to determine their cause and treatments with effective therapeutical agents but still it remains violent killer. Furthermore, clinically used synthetic chemotherapeutic agents have not been effectively satisfying the population in spite of massive investment on their development. Hence, there is an increasing need to locate effective, innovative and economical tumor inhibiting agents (Solowey *et al*., 2014). Studies have shown that there is corelation between cancer and inflammation.Virchow hypothesized that tumor originate from the site of chronic inflammation (Ngoua-Meye-Misso *et al*., 2018). Inflammation is a physiological defensive mechanism which protects human body from an attack to the body to isolate and repair body tissues (Ben Khedir *et al*., 2016). Inflammatory reactions were treated with clinically available allopathic medicines which are COX inhibitor and generate prostaglandin contributing to edema and pain along with gastric complications. Therefore, for the treatment of inflammation, analgesic and anti-inflammatory agents are required. Now a day's plant derived drugs were used for treating the inflammatory reactions having lesser side effects as compared to synthetic drugs (Saleem *et al*., 2020b).

Traditional medicines are prime importance in all cultures of humanities for the treatment of ailments as they are an ironic source of alternative therapy and offer human to derive innovative medicines from them (Saleem *et al*., 2020a). *Saussurea hypoleuca* belongs to the family Asteraceae locally called as Qust obtained from the mountains of Quetta, Baluchistan, Pakistan. This plant family is dispersed world widely in the tropical and subtropical areas of Central Asia, North America, Southern Africa, Eastern Brazil and Southwestern China excluding Antarctica.

Saussurea hypoleuca was also extant in Himalayan region of Himachal Pradesh, India (Singh *et al*., 2015). Literature review has been extensively explored which confirmed that there is no data available on the isolation of phytoconstituents from this root. A few studies are available and even these studies have been done on the herbal formulation that contained *Saussurea hypoleuca* as the one ingredient. Arvind Kumar Shakya and Sangeeta Shukla evaluated hepatoprotective activity of Majoon-e-Dabeed-ul ward (MD) against acetaminophen induced liver damage (Shakya and Shukla 2011). Proximate analysis and *in vitro* biological assays have shown that roots contain a lot of phytochemicals (Arshad and Ishtiaq, 2019). The objective of the current study was to evaluate the anticancer and anti-inflammatory activities on human hepatic carcinoma cell lines and on rat models.

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MATERIALS AND METHODS

The plant root was collected from Quetta, Baluchistan, Pakistan and authenticated by Prof Dr. Zaheer-ul-din Khan, Department of Botany, GC University Lahore. Specimen was deposited in GC University Sultan Ayyub herbarium, Lahore under the voucher number of GC. Herb. Bot. 3453. Root was pulverized into powder after drying under shade, followed by extraction with cold maceration using methanol for one week. Fractionation of RME was carried out with various solvents according to their polarity order (Arshad and Ishtiaq, 2019). Ethyl acetate was subjected owing to active fraction into column chromatography for isolation of compounds.

GC-MS screening

GC-MS screening was done with Agilent GC-MS 2010 network equipped with capillary column DB5 (30 m*250 µm) and mass spectrometer detector having split/splitless injection system. Initial injection temperature was set at 100˚C which was raised to 200˚C and retained for 3 minutes. Helium was used as carrier gas at 60 KPa pressure with flow rate 180 mL/min and linear velocity 34.4 cm/sec. Samples were dissolved in n-hexane and 1 µL of each sample was injected automatically. Spectrum of unknown components were recorded and interpreted by comparing with known standard spectrum stored in MS-NIST profile. Compound name, molecular formula, retention time and SI index were documented (Ezaat *et al*., 2019).

Anticancer assays

Anticancer assays MTT, AB, NR and LDH were employed to investigate the anticancer potential of plant root.

Cell culture method

5% Fetal Bovine Serum (FBS) was mixed with 0.5% Pen-Strept (penicillin-streptomycin) solution and added into Dulbecco's Modified Eagle Medium (DMEM). The medium was used to sustain cell culture in incubator. Incubator was set at 37˚C, 5% CO_2 and 95% relative humidity. T-75 flasks were used for cell culture in anticancer activity and assay was performed when T-75 flask was covered 70-80 % with cells (Myint *et al*., 2019).

Sample preparation

Stocks solutions (1 mg/mL) of RME and fractions were prepared in DMSO and sterilized them through membrane pore filter 0.22 µm using syringe. Further concentrations (3.12, 6.25, 12.5, 25, 50, 100 & 200µg/mL) of stock solutions were made in sterilized DMEM. These were kept at -20˚ C in sterilized sample vials according to the manufacturer's procedures.

MTT assay

Assay was tested to document the anticancer potential of RME and fractions as explained by (Abu-Lafi *et al*., 2019) with slight amendments. Concisely, a 200 µL of cell suspension was seeded in 96 well microplate which was incubated for 24 hours at 37˚C, 95% humidity and 5% CO_2. Next day after 24 hours incubation, cells were examined for expansion under inverted microscope. When almost 70% area of the wells was covered with cells, media was removed without disturbing the cells settled at the bottom of the microplate. The cells were exposed with different concentrations of each sample. 200 µL of each stock solutions of different concentrations were added in labeled microplate wells and plate was incubated again for 24 hours in above mentioned incubator conditions. Next day, 20µL of MTT reagent (5 mg/mL in DMSO) was added by removing 80% contents from each well and incubate it again for 4 hours. After incubation, 150µL of DMSO was added in each well to dissolve the formazan crystals of purple coloured formed with MTT reagent and kept it in incubator for 20 minutes. After incubation absorbance was recorded at 600 nm at ELISA with negative as well as positive controls. Percentage of cell viability was calculated by using the formula:

$$\text{\% Cell viability} = \frac{\text{Absorbance of treated Cells}}{\text{Absorbance of controlled cells}} \times 100$$

AB assay

AB assay was performed to determine the cell viability also called aqua bluer assay. This assay monitors the reducing environment of the living cells. Living cells have active metabolism to reduce resazurine into resorifin a pink coloured fluorescent product as described by (Specian *et al*., 2016). The assay was carried out according to the manufacturer's recommendations. 200 µL cells were seeded in 96 well microplate and kept for 24 hours incubation in similar manner as in MTT Assay. 20µL of resazurin reagent was added in each well and incubated for 2 hours. After incubation, plates were read in florescent plate reader using excitation wave length 544 nm and emission wavelength 590 nm. Percentage of cell viability with respect to negative and positive control was determined using the similar formula as described in MTT assay (Silva *et al*., 2019).

NR assay

NR assay was employed to determine the cell viability as described by (Trintinaglia *et al*., 2015). It was based on the ability of living cells to bind and incorporate the neutral red dye which is weakly cationic and penetrate into cell membrane by passive diffusion where it accrues in lysosomes to interact with anionic and phosphate group of lysosomal matrix. Extent of dye fused into the cells is measured by spectrometry which is directly proportional to the cell number with integral membranes. 200µL cells were seeded in 96 well microplate and kept for 24 hours incubation in a similar manner as in MTT assay. After incubation period, 100 µL of neutral red dye was added in

each well and kept it in incubator for 3 hours at 37˚C. After incubation, absorbance was recorded at 540 nm in spectrophotometer. Percentage of cell viability with respect to DMSO negative control and doxorubicin (DXR) positive control was determined with similar formula as given in MTT. In mammalian cells doxorubicin acts as DNA intercalating agent by blocking DNA and RNA synthesis. It also inhibits DNA topoisomerase II, causing membrane damage and single strand breaks. DXR was used at the concentrations of 0.2 µg/mL.

LDH assay

Cytosolic lactate dehydrogenase catalyzes the conversion of pyruvate into lactate in the presence of NADH. Cells with impaired membrane release LDH into the extracellular medium. The increase in absorbance at 340 nm due to the oxidation of NADH is proportional to the LDH activity in the specimen. 200 µL of cell suspension was seeded in 96 well microplate. The plate was incubated for 24 hours at 37˚C, 95% humidity and 5% CO_2. Next day after 24 hours incubation, cells were examined for expansion under inverted microscope in similar way as in above assays. Next day, after incubations, 50µL of LDH enzyme was added in each well after removing some upper media from the wells. Microplate was incubated for 30 minutes in incubator. After incubation the absorbance was measured at 490 nm with microplate ELISA reader. Assay was determined as reported by (Sridevi *et al*., 2017) with little modifications according to the manufacturers protocols. The percentage of LDH release from cells was evaluated by using previously described formula.

Anti-inflammatory activity

Cyclooxygenase activity was performed by using calorimetric method of COX-2 screening kit (Cayman no, 760111) as described by (Shaikh *et al*., 2016). Stock solutions of RME and fractions were made in 1% DMSO to develop further concentrations (20, 40, 60, 80 & 100 µm/mL). Celecoxib was served as standard.150µL Tris HCI buffer, 10µL hematin and 10µL DMSO were added in background wells initially. 140µL buffer, 10µL hematin, 10µL DMSO and 10µL COX were added in control wells. Standard (celecoxib) concentrations were also formed similar to the sample concentrations and 140 µL buffer, 10µL hematin, 10µL celecoxib and 10µL of COX were added in wells. Sample wells were prepared by adding 140µL buffer, 10µL hematin, 10µL sample and 10µL of COX. 20µL of TMPD and 20µL of Arachidonic acid were added in each well. Test was performed in triplicate to minimize the errors. Plate was gently shaken and incubated for 5 minutes at RT. Absorbance was recorded by microplate ELISA reader at 540 nm. Following equation was used to calculate the percentage inhibition.

$$\% \text{ age inhibition } \frac{a-b}{a} \times 100$$

a = (control-background well)
b = (Standard or sample- background well)

Animals

Albino Wistar rats of either sex (150-200gm) were procured from Department of Pharmacology, University College of Pharmacy, Punjab University, Lahore, Pakistan. Animals were kept in metal cages at controlled RT; 25±2˚C and relative humidity; 55-65%. Each animal was free to food and water *ad libitum.* They were acclimatized in laboratory conditions for one week before the start of the experiment. Treatment was performed with respect to permission obtained from animal ethical committee of University College of Pharmacy, Punjab University, Lahore, Pakistan under reference no: 88.

In-vivo anti-inflammatory activity

RME and various fractions were screened for anti-inflammatory activity by comparing its results with standards drug.

Carrageenan model

Protocoals for carrageenan model was developed by (Uddin *et al*., 2019). The animals were distributed in fourteen groups having six rats in each group. Paw edema was induced by injecting freshly prepared 0.1 mL of 1% carrageenan in normal saline into the sub plantar region of the right hind paw of rats in all groups. Extracts and standard drugs were administered orally before 1 hour of carrageenan injection. Group I was considered as control and received normal saline (5 mL/kg, orally), Group II and Goup III recieved (200 & 400 mg/kg) RME, Group IV & V (200 & 400 mg/kg) chloroform, Group VI & VII (200 & 400 mg/kg) n-hexane, Group VIII & IX (200 & 400 mg/kg) E.A, Group X & XI (200 & 400 mg/kg) n-butanol, Group XII & XIII (200 & 400 mg/kg) aqueous fraction and Group XIV received diclofenic sodium (50 mg/kg) called reference group. Paw thickness was measured by plethysmometer before and 1, 2, 3 and 4 hours after the adminstration of carrageenan injection. Anti-inflammatory activity was determined as the percentage inhibition of paw edema in extract treated groups with comparison to the standard and carrageenan control by the following formula:

$$\% \text{ age inhibition } = T_0 - \frac{T_t}{T_0} \times 100$$

T_t is the thickness of the rat paw edema of the test extract at the corresponding time and the T_0 is the thickness of the paw edema of the carrageenan control group at the same time.

Formalin model

Formalin model was developed by (Zhao *et al*., 2018). 0.2 mL of 2% V/V freshly prepared formalin in distilled water was given to all rats on first day. Formalin model

was conducted on the basis of the results of carrageenan model. In this model animals were divided into six groups having six rats each. Group 1& II received (200 & 400 mg/kg) E.A while Group III & IV received (200 & 400 mg/kg) RME, Group V received (50mg/kg) diclofenic sodium as standard drug and Group VI was formalin control received only normal saline. Paw thickness was measured at zero time before and after formalin adminstration daily at a fixed time consectively for seven days using plethysmometer or vernier caliper. Percentage inhibition was calculated by using similar formula as in carrageenan model.

STATISTICAL ANALYSIS

All results were expressed as Mean ± SD. To determine the level of significant difference between various calculations one-way variance (ANOVA) between groups and column data were employed using graph pad prism version 5.01 software. Value of $p<0.05$ considered to be significant.

RESULTS

GC-MS Screening

In GC-MS screening, components eluted with mobile phase n-hexane: Chloroform from column chromatography were identified by using GC-MS techniques are depicted in tables 1-2 with chromatographs in figs. 1&2 respectively.

Anticancer activity

Anticancer assays including MTT, AB, NR and LDH were recorded on RME and fractions to investigate the plant root anticancer activity. Results demonstrated that all fractions have anticancer potential at lowest concentrations as compared with positive and negative controls while aqueous and ethyl acetate fractions have highest potential when compared with other fractions. On the basis of results, LDH assay was performed on these two fractions (fig. 5). Results are depicted in percentage of cell viability and cytotoxicity which are indirectly proportional at same concentrations as given in figs. 3 & 4 respectively.

Anti-inflammatory activity

Different concentrations of RME and organic fractions were investigated for COX-2 inhibition activity against standard celecoxib COX-2 inhibitor. % age inhibition of COX-2 was high in RME (68.69%), E.A (56.52%), aqueous (55.21%), chloroform (53.47%) and n-butanol (52.1%). All fractions show dose dependent effects at different concentrations. The results of COX-2 inhibition are summarized in table 3.

Carrageenan induced rat paw edema

Acute anti-inflammatory effect of RME and various fractions was estimated by carrageenan induced rat paw edema using diclofenac sodium as standard drug. % age inhibition was calculated at 200 and 400mg/kg doses. All fractions (400mg/kg) and standard show significant results after 4th hour when compared to carrageenan control with RME (71.09%) and E.A (64.97%) highest % age inhibition while n-butanol (63.71%), aqueous (62.73%), n-hexane (55.91%) and chloroform (55.32%) have comparable anti-inflammatory potential as summarized in table 4.

Formalin model

Formalin model was employed on the bases of outcomes from acute carrageenan model for seven days. Only RME and E.A were selected for formalin model. %age inhibition was calculated. Both fractions show strong anti-inflammatory potential when compared with standard as summarized in table 5.

DISCUSSION

Cancer is becoming a high profile disease in developed and developing countries. A lot of synthetic drugs and treatments are pre-existing but due the limitations of these chemotherapeutical methods causing toxic effects on non-targeted cells putting human further health issues. Increasing demand for medicinal herbs is putting pressure on high-value medicinal plant (Padmaharish and Lakshmi, 2017). To analyze cell viability and cytotoxic properties of potential new medicinal herb, the best approach is to adopt several methods to obtained reliable results and minimized the confounding factors (Abdullha *et al*., 2014). In current study MTT, AB, NR and LDH assays were employed to evaluate the cytotoxicity at different cellular targets. Results of all assays have indicated that there is an inverse relation between the cell viability and test sample concentration. Different assays yield different results as each assay have different functions (Specian *et al*., 2016). However, whole results were momentous with aqueous & E.A fractions owning to excellent profile in evaluating cytotoxicity in each assays. All above findings specified that *Saussurea hypoleuca* root extract have strong anticancer activity which needs to be chemically characterized for future prospective (figs. 3, 4 & 5).

Developing novel, effective and safe anti-inflammatory agents have remained a main reliance area in the leading streams to locate alternative of NSAIDS. However, some of the NSAIDS inhibit both the constitutive (COX-1) and inducible (COX-2) isoforms of the enzymes, leading to side effects such as gastrointestinal bleeding. Selective inhibition of COX-2 could alleviate the side effects associated with inhibiting the COX-1 isoform as anti-inflammatory agent, and that was set as the initial goal for this study (Chirisa and Mukanganyama, 2016). Natural products, specifically medicinal herbs and drug discovery remained a successful combination for the inventorization of new therapeutical agents (Ezzat *et al*., 2019).

Table 1: Components Detected in (1:99) n- Hexane Fraction by GC-MS Screening

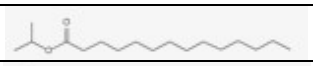
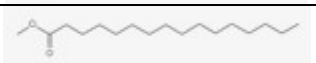
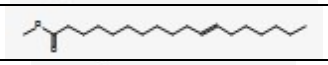
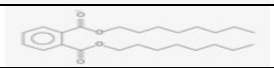
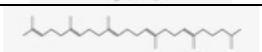
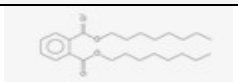
S No.	SI	R. Time	Mol. Wt	Formula	Compound Name	Structure
1	88	23.042	270	$C_{17}H_{34}O_2$	Isopropyl Myristate	
2	92	24.425	270	$C_{17}H_{34}O_2$	Hexadecanoic Acid	
3	91	26.658	296	$C_{19}H_{36}O_2$	11-Octadecenoic acid	
4	92	31.517	390	$C_{24}H_{38}O_4$	Di-n-octyl phthalate	
5	77	36.667	206	$C_{15}H_{26}$	1H-3a,7-Methanoazulene	
6	81	37.517	412	$C_{30}H_{52}$	Tetracosapentaene,	

Table 2: Components Detected in (5:95) n- Hexane Fraction by GC-MS Screening

S No.	SI	R. Time	Mol. Wt.	Formula	Compound Name	Structure
1	87	14.558	296	$C_{19}H_{36}O_2$	11-Octadecenoic acid	
2	89	23.592	390	$C_{24}H_{38}O_4$	Di-n-octyl phthalate	
3	78	29.758	242	$C_{16}H_{34}O$	Dioctyl ether	

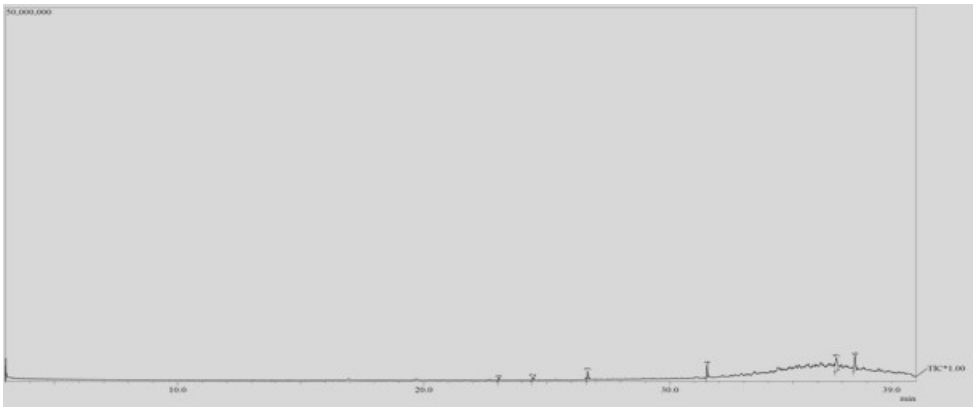


Fig. 1: GC-MS Screening of (1:99) n-Hexane Fraction

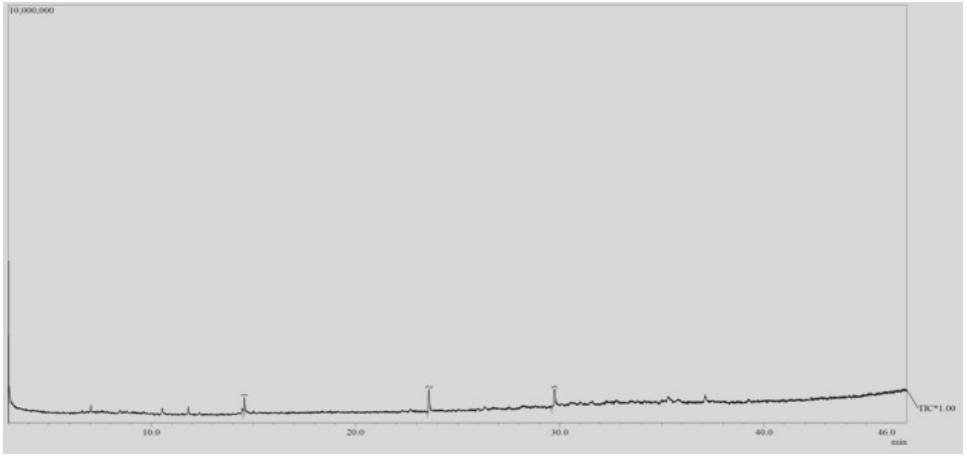


Fig. 2: GC-MS Screening of (5:95) n- Hexane Fraction

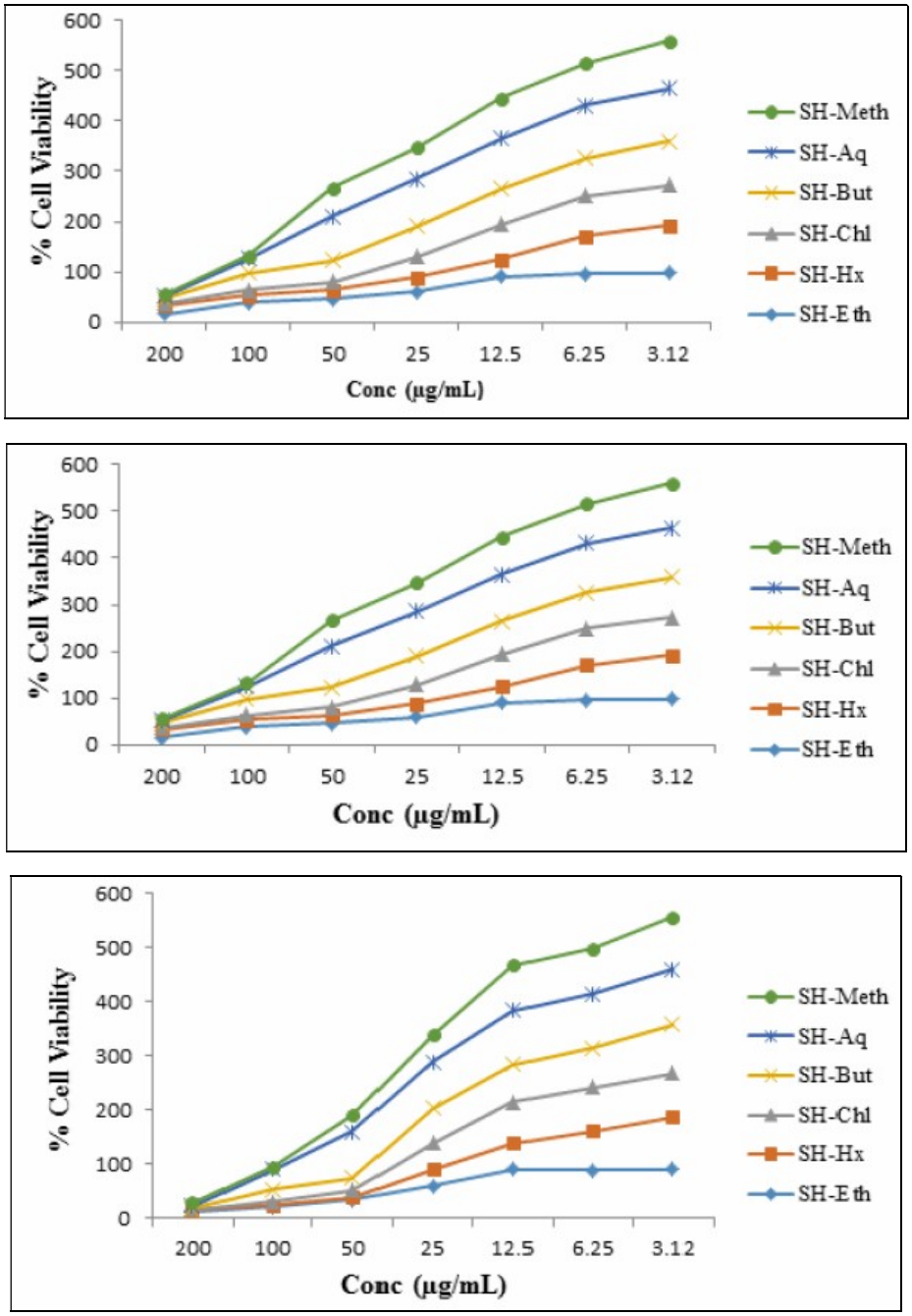


Fig. 3: MTT, AB & NR Assays of RME and Fractions of *Saussurea hypoleuca* Root

The undertaking study evidently demonstrated first time, COX-2 inhibition activity along with *in-vivo* anti-inflammatory activity. RME and fractions of *Saussurea hypoleuca* root were evaluated for COX-2 inhibition *in-vitro* assay as well as *in-vivo* carrageenan and formalin induced paw edema. RME (68.69%), E.A (56.52%), aqueous (55.21%) and chloroform (53.47%) have shown significant COX-2 inhibition compared to standard (table 3). The result of the COX-2 inhibition focuses the significance of selected plant as a new source for sequestration and documentation of COX-2 selective anti-inflammatory agents. Carrageenan induced paw edema is most appropriate method to screened out anti-inflammatory activity of medicinal herbs. It is generally biphasic curve model. In first phase inflammation occur during first hour may be due to the trauma at the injection

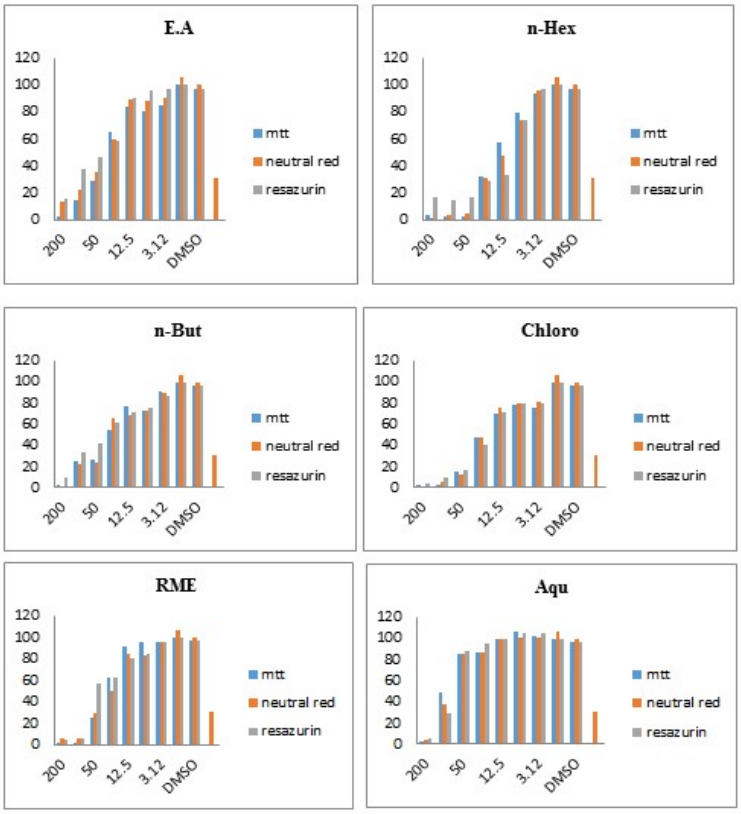


Fig. 4: Comparison of Anti-Cancer Assays (MTT, NR & AB) of *S. hypoleuca* Root

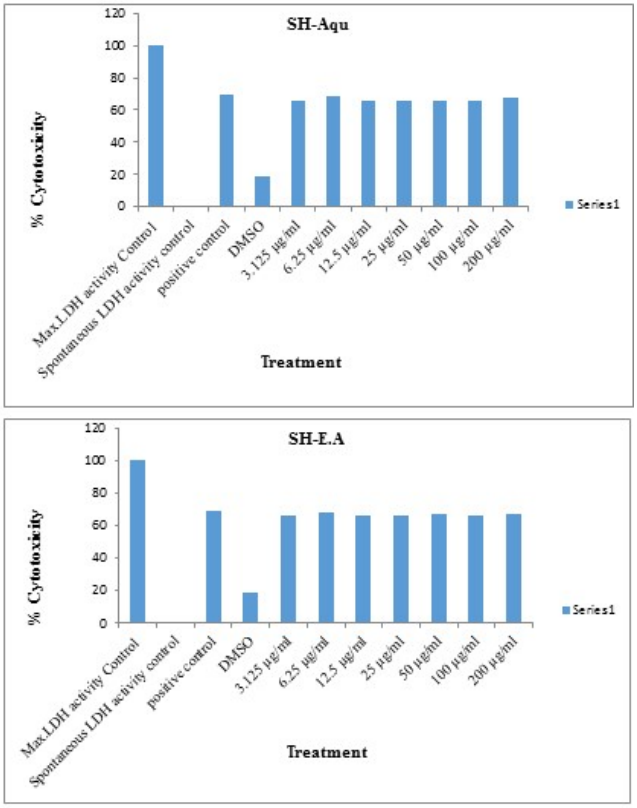


Fig. 5: LDH Assay of aqueous and Ethyl acetate Fractions of *S. hypoleuca* Root

Table 3: COX-2 %age Inhibition of RME and Fractions of *Saussurea hypoleuca*

Groups	20µM Mean ±SD	%age inhibition	40µM Mean ±SD	%age inhibition	60µM Mean ±SD	%age inhibition	80µM Mean ±SD	%age inhibition	100µM Mean ±SD	%age inhibition
Control	-		-		-		-		-	
Celecoxib	0.329±0.016	48.69	0.308±0.001	57.82**	0.298±0.003	62.17**	0.293±0.002	64.34**	0.276±0.007	71.73***
n-Hex	0.408±0.002	14.34	0.392±0.004	21.30	0.372±0.005	30	0.345±0.002	41.73	0.328±0.004	49.13
n-But	0.392±0.005	21.30	0.373±0.004	29.56	0.356±0.001	36.95	0.335±0.003	46.08	0.321±0.002	52.1*
Chloro	0.382±0.002	25.65	0.364±0.003	33.47	0.342±0.002	43.04	0.325±0.004	50.43	0.318±0.002	53.4*
E.A	0.366±0.005	32.60	0.351±0.001	39.13	0.334±0.003	46.52	0.319±0.001	53.04*	0.311±0.001	56.5**
Aqu	0.385±0.003	24.34	0.364±0.003	33.47	0.338±0.006	44.78	0.328±0.008	49.13	0.314±49.842	55.21**
RME	0.36±0.001	35.21	0.342±0.001	43.04	0.316±0.002	54.34*	0.299±0.002	61.73**	0.283±0.003	68.69***

Table 4: Anti-inflammatory Effects of RME and Fractions at 200mg/kg on Carrageenan Induced Rat Paw Edema

Groups	1st hr.Mean±SD	%inhibition	2nd hr.Mean ±SD	%inhibition	3rd hr.Mean ±SD	%inhibition	4th hr.Mean±SD	%inhibition
Control	1.34 ±0.03	-	2.333±0.025	-	3.633±0.019	-	3.255±0.018	-
Diclofenac	0.55±0.028	58.95*	0.811±0.017	65.21**	1.135±0.01	68.75**	0.911±0.014	72.01***
E.A 200	1.117±0.014	16.64	1.576±0.01	32.44	2.168±0.011	40.32	1.583±0.05	51.36*
RME200	1.112±0.017	17.01	1.556±0.021	33.30	2.046±0.022	43.84	1.426±0.013	56.19*
n-Hex 200	1.2±0.02	10.44	1.711±0.014	26.66	2.231±0.017	38.61	1.726±0.021	47.15
Aque 200	1.136±0.012	15.67	1.633±0.012	30.03	2.143±0.013	41.01	1.605±0.013	50.69
Chloro 200	1.146±0.019	14.92	1.658±0.014	33.10	2.178±0.011	40.04	1.668±0.011	48.75
n-But 200	1.163±0.092	13.20	1.638±0.011	29.78	2.16±0.008	40.54	1.593±0.018	51.05
Anti-inflammatory Effects of RME and Fractions at 400 mg/kg on Rat Paw Edema								
Groups	1st hr.Mean±SD	%inhibition	2nd hr.Mean ±SD	%inhibition	3rd hr.Mean ±SD	%inhibition	4th hr.Mean±SD	%inhibition
E.A 400	0.873±0.01	34.85	1.34±0.014	42.56	1.541±0.017	57.58*	1.141±0.026	64.97**
RME400	0.629±0.012	53.73	0.91±0.014	60.99*	1.23±0.014	66.14**	0.941±0.014	71.09***
n-Hex 400	1.145±0.018	14.55	1.628±0.014	30.21	2.026±0.013	44.23	1.435±0.01	55.91
Aque 400	1.035±0.01	22.76	1.441±0.014	38.23	1.731±0.007	52.35	1.213±0.018	62.73*
Chloro 400	1.145±0.019	14.55	1.487±0.007	36.56	1.773±0.016	51.19	1.245±0.016	55.32
n-But 400	1.021±0.014	23.80	1.418±0.014	39.21	1.568±0.019	56.83*	1.181±0.014	63.71*

Values expressed in Mean ± SD, n= 6, ***p< 0.001, **p< 0.01 &*p<0.05. Extract treated groups and standard are compared with control.

Table 5: Anti-inflammatory Effects of RME and Fractions on Formalin Induced Rat Paw Edema

Groups	1st d Mean±SD	2nd d Mean±SD	3rd d Mean±SD	4th d Mean±SD	5thdMean±SD	6th dMean±SD	7th dMean ±SD
Control	4.908±0.011	3.918±0.023	3.166±0.012	2.628±0.011	2.018±0.013	1.548±0.023	1.431±0.007
%age Inhibition	-	-	-	-	-	-	-
Diclofenac	3.528±0.014	2.456±0.01	1.73±0.014	1.151±0.011	0.833±0.012	0.521±0.014	0.436±0.012
% age Inhibition	28.01	37.31	45.35	56.24*	59.05*	66.34**	69.53***
E.A 200	3.873±0.02	2.86±0.014	2.205±0.061	1.438±0.014	1.005±0.018	0.726±0.012	0.635±0.019
% age Inhibition	21.09	27	30.35	45.28	50.20	53.10	55.63*
E.A 400	3.821±0.014	2.76±0.008	1.975±0.013	1.346±0.01	0.925±0.01	0.645±0.013	0.558±0.011
% age Inhibition	21.14	29.56	37.61	48.78	54.18	58.33*	61.01**
RME 200	3.718±0.014	2.598±0.011	1.881±0.014	1.253±0.013	0.905±0.018	0.565±0.033	0.486±0.042
% age Inhibition	24.24	33.69	40.58	52.32	55.15	63.50*	66.45**
RME 400	3.636±0.022	2.516±0.016	1.78±0.017	1.19±0.014	0.85±0.02	0.54±0.014	0.458±0.014
% age Inhibition	25.91	35.78	43.77	54.71	57.87*	65.11**	67.99**

Values expressed in Mean ± SD, n= 6, ***p< 0.001, **p< 0.01&*p<0.05. Extract treated groups and standard are compared with control.

site or it may be due to the release of serotonin and histamine (Saleem *et al*., 2017a). Table 4 indicated that there is no significant inhibition of rat paw edema during first hour. Acute carrageenan induced inflammation is sensitive procedure to cyclooxygenase inhibitors and has been used to investigate the effects of NSAIDS which mainly inhibit cyclooxygenase involved in prostaglandin synthesis. It plays vital role in the development of secondary phase of anti-inflammatory effects which were produced in 3rd hour as depicted in table 4. E.A fraction and RME at doses of 400mg/kg produce significant inhibition. Therefore, it was concluded that inhibition of cyclooxygenase activity in carrageenan induced edema was due to the inhibitory effect of E.A fraction and RME which may lead to the inhibition of prostaglandin synthesis.

On the basis of previous results, formalin induced paw edema model was developed on rats. This model is most suitable method to estimate the anti-inflammatory activity chronically as it closely resembles with human arthritis (Sing *et al*., 2010). RME (56.19%, 71.09%, 66.4%, 67.99%) and E.A (51.36%, 64.97%, 55.63% & 61.01%) produced significant %age inhibition in dose dependent manner at 200 and 400mg/kg doses (table 5).

GC-MS is a common confirmation test for the effective analysis of obtained extracts which can be interesting tool for detection of phytoconstituents present in herbs, cosmetic, food and pharmaceuticals. It provides representative spectral data of the compounds that get separated from the sample. The main components present in samples were Isopropyl myristate, Hexadecanoic acid, 11-octadecenoic acid, tetracosapentaene, di-n-octyl phthalate, dioctyl ether, decanedioic acid and 3,4-hexanedione (figs. 1-2).

CONCLUSION

The present study forte the importance of selected plant root. In some cases, compounds separated from herbs may not serve as therapeutical agent but supports to the discovery of novel agents. With prompt identification and characterization of innovative compounds from natural origin having strong anticancer and anti-inflammatory activity may be considered substantial therapeutical agents in the main stream of anticancer and anti-inflammatory drug delivery marathon.

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