CHAPTER-4

Materials, Equipments and Software

4.1 MATERIALS

4.1.1 Chemicals

Table 4.1 List of chemicals

S. NO.	CHEMICALS	SOURCE
1	Curcumin	Sanat Products Pvt. Ltd. New Delhi, India
2	Naringenin	Sisco Research Laboratories Ltd., Mumbai, India
3	Soluthin MD® (A compound of Phosphatidylcholine-Maltodextrin)	LIPOID, Ludwigshafen, Germany
4	Eudragit® E 100	Evonik Degussa India Pvt. Ltd., Mumbai, India
5	Poloxamer 188	Ranbaxy Laboratories Ltd., Dewas, MP, India
6	Ethanol	Merck Ltd., Mumbai, India
7	Mannitol	Sisco Research Laboratories Ltd., Mumbai, India
8	Tween 80	Sisco Research Laboratories Ltd., Mumbai, India
9	Sodium chloride	SD Fine Chemicals Ltd.,

		Mumbai, India
10	Calcium chloride	SD Fine Chemicals Ltd., Mumbai, India
11	Acetonitrile (HPLC-grade)	SD Fine Chemicals Ltd., Mumbai, India
12	Glacial acetic acid (HPLC-grade)	SD Fine Chemicals Ltd., Mumbai, India
13	Sodium Carboxymethylcellulose	Hi Media, Mumbai, India
14	Heparin Sodium Injection	Biological E. Limited, Hyderabad, India
15	4-hydroxybenzophenone	Sigma Aldrich, USA
16	Apigenin	Sigma Aldrich, USA
17	Sodium Bicarbonate	Sigma Aldrich, USA
18	Dulbecco's modified Eagle's medium (DMEM)	PAA Laboratories, Austria
19	Fetal bovine serum (FBS)	PAA Laboratories, Austria
20	Penicilline	Sigma Aldrich, USA
21	Streptomycin	Sigma Aldrich, USA
22	Sulphorhodamine B	Sigma Aldrich, USA
23	Dimethyl sulphoxide (DMSO)	Sigma Aldrich, USA
24	Trypsin	Invitrogen BioServices India Pvt. Ltd, Bengaluru, India
25	EDTA	Sigma Aldrich, USA

26	Phenol Red	Sigma Aldrich, USA
27	Trichloroacetic acid (TCA)	Sigma Aldrich, USA
28	Acetic acid	Sigma Aldrich, USA
29	Tris hydroxyl methyl aminomethane	Sigma Aldrich, USA
30	Sodium hydroxide	Qualigens Chemicals, Mumbai, India
31	Potassium dihydrogen orthophosphate	Qualigens Chemicals, Mumbai, India
32	L-glutamine	Hi Media, Mumbai, India
33	Dialysis membrane (MWCO ≥12 kDa)	Sigma Diagnostics, USA
34	Syringe filters (0.22 µm and 0.45 µm)	Hi Media, Mumbai, India
35	Microcetrifuge tubes	VOLEX Plasticware, India
36	Potassium bromide (KBr)	Hi Media, Mumbai, India
37	Indium	Sigma Aldrich, USA
38	Methanol (HPLC-grade)	SD Fine Chemicals Ltd., Mumbai, India
39	Di ethyl ether	SD Fine Chemicals Ltd., Mumbai, India
40	Rat feed	Pranav Agro Industries, Ltd, Sangli, India

4.1.2 Equipments and Software

The following major equipments as well as software were used in the various stages of the experimental study.

Table 4.2 List of equipments and software

SR. NO.	EQUIPMENTS AND SOFTWARE	SOURCE
1	Pestle and Mortar	Hi Media, Mumbai, India
2	Vacuum desiccator	Hi Media, Mumbai, India
3	Digital magnetic stirrer	IKA®, C-MAG, HS 7, Germany
4	Digital electronic balance	Shimadzu, Japan
5	Digital pH meter	IKON Instruments, New Delhi, India
6	High speed homogenizer	IKA® T25 digital ULTRA-TURRAX®, Germany
7	Lyophilizer	Freezeone, LABCONCO, USA
8	Cooling centrifuge	Refrigerated Centrifuge RC 4100 F, Eltek, Mumbai, India
9	Aluminium foil (freshwrapp®)	Hindalco Industries Ltd., Mumbai, India
10	Vacuum oven	Cintex, Mumbai, India
11	Disposable syringes	Hindustan Syringes & Medical Devices Ltd., Faridabad, India
12	Zeta sizer; DELSA™ NANO C	Backman Coulter, Inc., USA
13	Probe ultrasonicator	UP50H, Hielscher, USA

14	Fourier transform infrared spectrophotometer	FTIR 8400S system, Shimadzu, Japan
15	Differential scanning calorimeter; TGA/DSC1	STAR® system, Mettler Toledo, Switzerland
16	X'Pert Pro X-Ray diffractometer	PANalytical, Holland
17	High resolution transmission electron microscopy; TECHNAI $20G^2$	FEI Company, Netherlands, Holland
18	Atomic force microscopy; SOLVER next	NT-MDT, Moscow, RUSSIA
19	Confocal laser scanning microscope	CLSM 510 Meta; Carl Zeiss, USA
20	Spectrofluorophotometer	RF-1501, Shimadzu, Japan
21	Bath sonicator	WUC 1.8L, Fisher Scientific, India
22	UV-Visible spectrophotometer	UV-Visible 1800, Shimadzu, JAPAN
23	High performance liquid chromatography (HPLC)	Shimadzu Corporation, koyoto, Japan
24	Vortex mixer	Fisher Scientific, India
25	Foam tape	Camlin Ltd., Mumbai, India
26	96-well clear flat-bottom polystyrene tissue-culture plates	Corning, Sigma-Aldrich, USA
27	CO_2 Incubator	Sanyo CO_2 Incubator, Japan

28	100 mm Tissue culture plates	Corning, Sigma-Aldrich, USA
29	Inverted microscope	TMS, Nikon, Japan
30	Multiwell microplate reader	Wallac Victor V, PerkinElmer,USA
31	Multichannel pipette	Gilson, USA
32	Gyratory plate shaker	Model 4625, Lab-Line, USA
33	GI_{50}-calculation software	SigmaPlot, SPSS, USA
34	Kinetica 5 Trial Version	Fisher scientific, India
35	GrapPad Prism 5	GraphPad Prism Inc., USA
36	Minitab 16	Minitab Inc., USA
37	OriginPro 8	Origin Lab Corporation, USA

4.1.3. Animals and cancer cell line

Table 4.3 List of animals and cancer cell line

SR. NO.	ANIMALS AND CANCER CELL LINE	SOURCE
1	Albino Wister rats (either sex, 200-250 g body weight)	Central Animal House, Institute of Medical Science, Banaras Hindu University, Varanasi, India
2	Colon-26 cancer cells (colorectal cancer cell line)	National Cancer Institute, USA
3	Murine BALB/c mice (Females, 6-8 weeks old and ~18-22 g body weight)	Tata Memorial Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India

4.1.3.1 Animals

Healthy Albino Wister rats (either sex and 200-250 g body weight) and healthy murine BALB/c mice (either sex, 6-8 weeks old and ~18-22 g body weight) were obtained from Central Animal House, Institute of Medical Science, Banaras Hindu University, Varanasi and Tata Memorial Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India, respectively. Wister rats were used for pharmacokinetic studies while murine BALB/c mice were used for *in-vivo* anticancer efficacy of the different formulations.

4.1.3.2 Cancer cell line and their culture conditions

Colon-26 (colorectal cancer cells) cell lines were used for both *in-vitro* and *in-vivo* anticancer studies and were obtained from National Cancer Institute, USA. The Cells were maintained in complete medium containing Dulbecco's modified Eagle's medium, 10% fetal bovine serum and antibiotics (streptomycin and penicillin). The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub-cultured twice a week.

4.1.3.3 Ethics Statement

All the animals were obtained and kept in quarantine for at least one week before starting the experiment. All animals were maintained in accordance with the guidelines suggested by Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India, Delhi, India and were used for the experiments according to protocol approved by the Central Animal Ethics Committee (CAEC), Institute of Medical Sciences, Banaras Hindu University, Varanasi, India ***(CAEC approval number: Dean/13-14/CAEC/200)***. The all animals were provided with food and water *ad libitum* and were housed under normal laboratory conditions at ambient temperature 20-25°C and 55-60% relative humidity with controlled 12 hr light/dark cycles. All efforts were made to decrease animal distress.