Evaluation of antiangiogenic activity through tubulin interaction of chloroform fraction of the feather star, Lampropetra palmata palmata

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Tubulin binding agents have received considerable interest as potential tumour-selective angiogenesis-targeting drugs. The present study elucidates that chloroform fraction (CC) isolated from methanol extract of the feather star Lampropetra palmata palmata has a tubulin binding property. With the quantification of chick chorioallantoic (CAM) assay, we further demonstrate that CC significantly and dose-dependently inhibits proliferation, migration of endothelial cells and exhibits antiangiogenic effect with ID\textsubscript{50} 10ng/10\textmu l. It showed moderate cytotoxicity with IC\textsubscript{50} 192 \mu g/ml. In addition, CC arrested onion root tip cells at prometaphase phase. We demonstrate that these effects of CC are attributable to its property to inhibit polymerization of tubulin. These findings show that CC is a candidate antiangiogenic agent and needs further purification for the specific compound, which is responsible for all these activities.

[Key words: Bioactive, tubulin, antiangiogenic, sea, cell]

\textbf{Introduction}

The quest of drugs from the sea has yielded an impressive list of compounds. Although marine compounds are under-represented in current pharmacopeias, it is anticipated that the aquatic environment will become an invaluable source of novel compounds in the future\textsuperscript{1}. It is well understood that the marine life consist of primary metabolites such as proteins, lipids, triglycerides, sugars, amino acids, etc. responsible for cellular machinery to work. The added value to marine biota is because of a rich source of low molecular weight bioactive compounds produced as secondary metabolites and are responsible for their bioactive potential\textsuperscript{2}.

Although phylum Echinodermata has great contribution towards the physiologically active saponins from sea stars and sea cucumbers, due to significant cytotoxicity problems, their progress towards bioactive potential is restricted\textsuperscript{3,4}. Feather stars (Phylum – Echinodermata, class-Crinoidea), sessile and sluggish marine invertebrates are extremely vulnerable to mobile predators and the ability of these organisms to chemically defend themselves can play a significant role in regulating predator-prey interactions and thus posses potential for bioactives. Two gymnochrome D and isogymnochrome D isolated from the living fossil crinoid Gymnocrinus richeri are highly potent dengue antiviral agents\textsuperscript{5}.

Angiogenesis or neovascularization is complex developmental processes in which new blood vessels emerge from the pre-existing vasculature\textsuperscript{6}. A highly regulated process, angiogenesis includes coordination of distinct events like endothelial cells proliferation, migration and the establishment of cell-cell contacts to create new functional blood vessels. It is a fundamental biological mechanism that results in serious disease when it goes awry and is known to play in more than seventy pathological conditions\textsuperscript{7}. It is also being well understood that almost all aspect of angiogenesis require participation of cytoskeleton, and accumulating evidence indicate that cytoskeleton plays important role in modulation of the physiological function of blood vessel. The cytoskeleton, especially the microtubule, is highly dynamic and is widely implicated in control of cell proliferation, migration and spreading and elongation of endothelial cells\textsuperscript{8}. Therefore, changes in cytoskeletal dynamics are increasingly recognized as causing endothelial cells to undergo angiogenesis. Thus, microtubules, being dynamic cytoskeletal protein become leading target of new angiogenesis inhibitor.
In present study we have made an attempt to investigate the antiangiogenic potential of a feather star, Lamprometra palmata palmata (L. Palmata palmata) with reference to their interaction with cytoskeletal protein.

**Material and Methods**

**Media and Reagents**
- Hanks Balanced Salt Solution (HBSS), Minimum Essential Medium (MEM), Phosphate Buffer Solution (PBS), Fetal Bovine Serum (FBS), Antimycotic antibiotic solution, Trypsin, Na-pyruvate, Sodium bicarbonate were of tissue culture grade and purchased from Hi-Media, India.
- Sulphorhodamine-B, Tris-HCl buffer, Ethylene glycol-0,0'-bis(2-aminoethyl) N, N, N', N'- tetraacetic acid (EGTA), Magnesium sulfate, Guanosine 5’triphospahate (GTP), Glycerol, Monosodium glutamate , Silica gel G for thin layer chromatography and all other chemicals used were of reagent grade and purchased from Merk, India.
- All sterilized plastic-ware used was of tissue culture grade and was procured from BD Falcon, UK.

**Collection of animals**
- Feather stars, Lamprometra palmata palmata were collected near the rocky shore of Khardanda, Mumbai. Authentication of the crinoid was done by a naturalist Dr. B. F. Chhapgar, Bombay Natural History Society (BNHS), Mumbai.

**Preliminary chemical investigation of Lamprometra palmata palmata**

**Preparation of extract**
- Pieces of feather stars were immersed in Methanol to get 20 % (w/v) suspension. Methanol extract obtained by cold percolation, was concentrated under reduced pressure. This extract was repeatedly washed with diethyl ether to obtain ether soluble components and was labeled ether extract.

**Chemical analysis of bioactive fraction**
- Bioactive fraction CC was further subjected to preliminary chemical evaluation using qualitative chemical test and TLC analysis for detecting presence of the constituents such as alkaloids, glycosides, phenolic compounds, terpenes, steroids, saponins, carbohydrates, proteins, amino acids etc.

**Antiangiogenic Activity**

**Procurement of eggs**
- White crossbreed hens eggs were procured from the Central Poultry Breeding Farm (CPBF), Aarey Colony, Goregoan, Mumbai. They were maintained at 37° C with 85% humidity in the laboratory incubator throughout the experiment.

**Chicken Chorioallantoic Membrane (CAM) Assay**
- Angiostatic activity (inhibition of new capillary growth) was determined using the chick embryo chorioallantoic membrane, which has been described previously. In brief, a window of 1 cm² size was made in 5 days old embryonated eggs, to observe CAM. Agar (1%) discs of known concentrations (50ng, 100ng, and 200ng, 400ng/disc of 10 µl) of ether extract and its fractions were placed on the CAM away from central blood vessel. Eggs were further incubated for 48 h.

- Antiangiogenic response was assessed by measuring avascular zone. Zone diameter of 4 mm or larger in size was taken as positive for antiangiogenic activity. Embryos treated with 60µg/ 10µl of an angiostatic steroid, tetra-hydrocortisone in presence of heparin were used as positive control. Control group received only DMSO discs. At least 15 eggs were used for each concentration and the experiment was performed in triplicate to ensure reproducibility. Based on the measurement of the avascular zone with respect to various concentrations, ID₅₀ values were calculated.
Quantification of CAM Assay
Quantification of antiangiogenic effect was evaluated by the method described by Melkonian et al.\(^\text{11}\). This assessment was restricted to chloroform fraction (CC) as this was the only fraction found to possess antiangiogenic activity. For quantification instead of agar disc 200\(\mu\)l of HBSS solution containing predetermined concentrations of CC were used. After 48 hr treatment, all CAMs were dissected out from eggs and were screened visually for the pattern of vasculature, growth of secondary/tertiary blood vessels and embryo growth. CAM area was measured. Pieces of CAM that included one or two tertiary blood vessels were cut off and were fixed in 10% neutral formalin. These pieces were further processed for standard histological preparation and stained with Ehrlich’s Hematoxyline and Eosin\(^\text{12}\). The assay was performed in triplicate to ensure reproducibility.

Percent of ectoderm subtended by capillary plexus (cp), density of mesodermal blood vessels, degree of compartmentalization of capillary plexus, CAM thickness and density of mesenchymal cells were considered for quantification of antiangiogenic activity. Quantification was done histologically using ocular micrometer. To compare the degree of compartmentalization of development, the width and the length of individual compartments were measured.

Antimitotic Assay
Antimitotic effect of antiangiogenic compounds were tested by analyzing mitotic arrest in root tip cells of *Allium cepa* as described by Patil S. et al\(^\text{13}\) and modified in our laboratory. Onion bulbs with suitable root length were kept in solutions of various concentrations of CC (50, 100, 200ng/ml) for 2 hrs. Colchicine (0.4%) was kept as positive control. After 2 hours, roots were cut and fixed in fixative glacial acetic acid- methanol (1:3) for 24hrs. After fixation, roots were preserved in 70% alcohol and were hydrolyzed in 0.1N HCl and stained with 0.1% acetoorcein. Slides were screened for total number of cells, also cells with condensed chromosomes and in four stages of mitosis were counted and recorded. The mitotic index for each slide was examined.

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\text{Mitotic Index (MI)} = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells counted}} \times 100
\]

Antiproliferative Activity
Cell Lines
HeLa cells were purchased from the National Center of Cell Sciences (NCCS), Pune, India. Cell line was grown in MEM supplemented with 10% (v/v) FBS and 1% Antimycotic antibiotic solution at 37°C in 5% CO\(_2\).

Sulphorhodamine B (SRB) Assay
The sensitivity of the cell lines to bioactive fraction was determined using the SRB assay as described previously\(^\text{14}\). In brief, adherent exponentially growing cells were seeded into 96-well plates at 2–5 \(\times\) 10\(^5\) cells/well. Control wells received fresh medium with DMSO. Cells were exposed to the treatment of CC for 24, 48 and 72 hrs. SRB bound to protein was measured by absorbance at a 550-nm wavelength using a Bio- Rad Elisa Plate Reader.

Tubulin polymerization assay
Purified tubulin was prepared with our patented technology as described by Annamma Anil et al using H-CELBEADS column\(^\text{15}\). *In vitro* polymerization assay was conducted in 96 well UV micro-titer plates as described by Thir S. K. et al\(^\text{16}\). The polymerization of tubulin was measured by the change in absorbance at 350nm every 3 min for 25 min on BIO-RAD microplate reader. The chloroform fraction, CC with known concentrations (0.1, 1,10,100,1000 ng/ml) was assessed for their interaction with tubulin using polymerization assay. Each polymerization reaction mixture contained 1mg/ml of purified tubulin in buffer containing 80 mM Tris-HCl, 0.1mMGTP, 1mM EGTA, 3mM MgSO\(_4\) (pH 6.8) and 1 M sodium glutamate. The area under curve was used for calculation\(^\text{16}\).

Statistical Analysis
The data are expressed as means ±standard deviation (SD). Statistical analysis was done by the two-tailed Students’ \(t\)-test for two unpaired groups. Differences with p values of less than 0.05 were considered statistically significant\(^\text{17}\).

Results
Preliminary chemical investigation of CC
Qualitative tests did not show presence of alkaloids, glycosides, polysaccharides, carbohydrates, sterols, saponins, proteins and amino acids as constituents of CC.

When TLC plate applied with CC spayed with anisaldehyde-sulphuric acid solution, on heating to 100-105° C showed development of green spots. This qualitative investigation indicates presence of terpenes, sugars or steroids\(^\text{18}\). However other qualitative tests did not show presence of steroids and
sugars. Thus preliminary quantitative analysis postulate presence of terpenes as a constituent of CC.

**Antiangiogenic activity of ether extract and fraction**

The CAM of chicken embryo provides a model for investigating the process of formation of new blood vessel and vessel responses to antiangiogenic agent. We examined the *in vivo* antiangiogenic activity of ether extract and its fraction of *L. palmata palmata*.

Ether fraction of *L. palmata palmata*, when assessed for their antiangiogenic potential, showed promising results. It showed typical dose response curve for the antiangiogenic activity (Fig. 1A). However, at higher concentration of 400 ng/disc, it showed toxicity towards chick embryo. Out of all fractions, chloroform fraction (CC) showed development of avascular zone around the agar disc. Thus CC was found to have promising antiangiogenic activity with IC$_{50}$ 10 ng/disc (Fig. 1B).

**CC disturbs vasculature pattern**

In control CAMs, blood vessels are distributed in a tree like pattern in which primary blood vessels give off secondary vessels, which in turn produce tertiary blood vessels (Fig. 2A). In case of CC, the avascular zone was not restricted to 4mm but extended to the greater diameters (Fig. 2B). At lower doses, where tertiary vasculature was present upto some extent; vasculature pattern was not healthy but showed torturous course. At 100 ng and 200 ng, some CAMs even showed bending of the primary blood vessel in the region of the disc.

**Quantification of Antiangiogenic Response of CC**

Once antiangiogenic potential of CC was established, this effect was quantified with the help of histological sections of CC treated CAMs.

The respiratory exchange in the CAM occurs by means of an extensive capillary plexus (cp) that develops initially adjacent to the chorionic ectoderm (Fig. 2C) and later interdigitates between the ectodermal cells of the chorion. The plexus have been reported to be fairly well developed by day six. As the capillary plexus develop, the spaces within the plexus become subdivided. Initially and up to day seven, the major mechanism for this subdivision is by sprouting of pre-existing vessels. The CAM capillary plexus provides an excellent model to study capillary formation quantitatively *in vivo*.$^{11}$

Chloroform fraction (CC) of *L. palmata palmata* showed decrease in capillary plexus count for all treated CAMs but the decrease was not concentration dependent. At 50 ng/10µl dose the reduction in cp count was 72%, which decreased further to 45% for 100 ng/10µl. For higher concentration 200 and 400 ng/10µl there was no further reduction in capillary plexus count (Fig. 2D, 3A). Even a percent area subtended by the cp showed similar trend (Fig 3B). To determine if this inhibition was due to an effect on migration of mesodermal blood vessels to the ectoderm, the number of blood vessels of CAM was determined. Unlike controls, the CC treated CAMs had numerous mesodermal blood vessels (Fig. 3C) that apparently failed to migrate to the ectoderm to form plexus.

To determine if CC affects subdivision of vessels within the capillary plexus, compartmentalization was examined in control and treated CAMs (Fig. 3D). On comparison it was found that none of the treated CAM had compartment with the length of 5 microns. With the increase in the concentration of CC the cp

![Fig 1—Percentage inhibition of angiogenesis. Antiangiogenic response of (A) ether extract of *Lamprometra palmata*; (B) CC. Avascular zone of > 4 mm was considered as an antiangiogenic response. Data representative of three similar experiments with n=15. p<0.001 compared to control.](image)
population was dominated by greater length cp (Fig 3D). At the concentration 400 ng/10µl, all cp were with length more than 15 microns. This pattern of compartmentalization helps in better understanding of mode of action of antiangiogenic activity of CC. Even the width pattern showed similar trend as that of length (Fig 3E). This can be the reason that even though the number of cp reduced the area subtended by cp remained same as that of control.

In normal CAM, formation of the capillary plexus is accompanied by a decrease in CAM thickness and mesenchymal cell density. During antiangiogenesis response there is increase in mesenchymal cell number, as they don’t have to contribute in endothelial cell proliferation, cp division. This increase in number is further associated with the increase in CAM thickness\textsuperscript{11}.

In CC treated CAMs, mesenchymal cells showed irregular behaviour with increase in concentration. For 50 and 100ng doses there was increase in mesenchymal cell number with concentration (Fig 3F). This increase is responsible for dose dependent increase in CAM thickness (Fig. 3G).

Above findings of quantitative antiangiogenic assay demonstrate that the CC must be affecting the migration and proliferation of endothelial cells toward ectoderm.

**Antiproliferative activity of CC with non-endothelial cells**

Agents affecting endothelial cells are known for the differences in their sensitivity to the non-endothelial cells. In this attempt we established effect of CC on cervix cancer cell line HeLa. HeLa cells on treatment with CC showed antiproliferative effect (Fig. 4). CC mediated inhibition of proliferation of HeLa cells was dose and time dependent. The IC\textsubscript{50} value for CC is 192 µg/ml (±0.003).

Mitotic studies using onion root tip model provided insights on antiproliferative activity of CC. Table 1 shows the results of total number of onion root tip cells analyzed, at interphase and the number of cells in mitosis at different phases at different treatment modalities. 2000 cells population was counted for each treatment, which included cells, both in mitosis as well as in interphase. Seventeen percent increase in percentage of mitotic index of CC as compare to control demonstrates the antimitotic behaviour of CC (Table 1). This antimitotic behaviour is concentration dependent. 4% cell population reach telophase at

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**Fig. 2**—Effect of isolated antiangiogenic fractions on CAM vasculature. (A) Circled region represents control CAM; (B) Circled CAM treated with CC 100ng/disc. Histological sections of CAM showing capillary plexus. (C) control; (D) CC (100ng/CAM) 1. Ectoderm; 2. Endoderm; 3. Capillary plexus (cp); 4. Mesenchymal cells.
Fig 3—Effect of CC on different parameters in quantitative angiogenesis analysis (A) capillary plexus count; (B) percent area subtended by capillary plexus; (C) mesodermal blood vessel no.; (D) capillary plexus length; (E) capillary plexus width; (F) Mesenchymal cell density (G) CAM thickness. Each group is mean ± SD of 5 CAMs. * indicates p<0.005 compared to control.
higher doses of CC and majority of them is getting arrested at prophase and metaphase. Also, the increase in prophase arrest was concentration dependant. Fifty percent cells were in prophase when treated with 50µg/ml, the prophase arrest increased to 63% with increase in concentration (Fig. 5).

**CC inhibits tubulin polymerization in vitro**

We then reasoned that the induction of prophase arrest by CC is attributed to disruption of cytoskeleton. To test this hypothesis the polymerization of the de-polymerized tubulin in presence of CC was evaluated by turbidimetric assay. In this analysis the capacity of the tubulin to polymerize in the presence of glutamate (polymerizing agent) and CC was evaluated. Since colchicine is known tubulin-depolymerizing agent, it was used as a positive control during the assessment.

During this *in vitro* study depolymerized tubulin failed to polymerize due to its interaction with CC (Fig. 6). It can be postulated that the CC must have binded to deplomerized tubulin and thus altered its microtubule formation. CC prevented GTP-glutamate induced polymerization in dose dependant manner. The inhibitory concentration (IC$_{50}$) was 3ng/ml (Fig. 7).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Groups (µg/ml)</th>
<th>Cells</th>
<th>Interphase cells</th>
<th>Mitotic cells</th>
<th>MI (%) (±S.D)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>2000</td>
<td>3621</td>
<td>379</td>
<td>9 (±0.01)</td>
<td></td>
</tr>
<tr>
<td>Colchicine</td>
<td>10</td>
<td>2000</td>
<td>1529</td>
<td>471</td>
<td>23 (±0.05)</td>
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<tr>
<td>CC</td>
<td>50</td>
<td>2000</td>
<td>1665</td>
<td>335</td>
<td>10 (±0.1)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2000</td>
<td>1650</td>
<td>350</td>
<td>21 (±0.7)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2000</td>
<td>1565</td>
<td>435</td>
<td>26 (±0.65)</td>
</tr>
</tbody>
</table>

Fig. 5—Distribution pattern (in percentage) of dividing *Allium cepa* root tip cells in prophase, metaphase, anaphase, telophase with different concentrations of CC.

Fig. 6—Inhibition of microtubule formation by CC. (Data shown is the mean of duplicate reactions at 2 min intervals and representative of numerous experiments).

Fig. 7—Inhibition of tubulin assembly in presence of different concentration of CC. (The percentage of tubulin polymerized was calculated using the AUC of each polymerization curve and normalizing the control AUC to 100%).
Discussion
The development of new blood vessel is a complex multi-step process. Release of degradative enzymes allow endothelial cells to migrate proliferate and finally differentiate to give rise to capillary plexus. Any of these steps may be potential target for pharmacological intervention. The present study demonstrates that the chloroform fraction of methanol extract of feather star, Lamprotometra palmata palmata inhibits theses essential steps of angiogenic process.

The qualitative screening helped us establishing the antiangiogenic potential of ether extracts of L. palmata palmata, when tested for their effect on CAM vasculature. Development of avascular zone indicated the antiangiogenic potency of the ether extract as well as chloroform fraction CC. CC showed a dose dependent increase in antiangiogenic activity with ID50 value 10ng/disc. The low ID50 values are impressive and express their potency as antiangiogenic agents. It significantly altered secondary and tertiary blood vessels branching pattern as well as thinning of vasculature. Melkonian et al have demonstrated that Suramin- an antiangiogenic drug alters the branching pattern of secondary and tertiary blood vessels by inhibiting endothelial cell proliferation. Thus, the alteration of vasculature branching can be attributed to antiangiogenic behaviour of CC.

Indap et al, during their bioactivity guided fractionation studies have demonstrated, ether fraction of marine crustacean Diogenus avarius when column chromatographed, chloroform eluted portion gave oily residue containing four saturated fatty acids viz. lauric, myristic, palmitic, stearic. This mixture of fatty acids posses antiangiogenic and antifertility potential. The chloroform (CC) fraction, which was found to be the most active portion, when screened for its bioactive content and it responded positively to the test of terpene. Marine sterols, steroids, fatty acid are being long known for their antiangiogenic activity. The chloroform (CC) fraction, which was found to be the most active portion, when screened for its bioactive content and it responded positively to the test of terpene. Marine sterols, steroids, fatty acid are being long known for their antiangiogenic activity. The chloroform (CC) fraction, which was found to be the most active portion, when screened for its bioactive content and it responded positively to the test of terpene.

During quantitative screening, increase in mesodermal blood vessels and corresponding decrease in capillary plexus count demonstrate the inhibition of migration of endothelial cells to form capillaries along the ectoderm, when treated with CC. During angiogenesis of CAM, mesenchymal cells participate in endothelial cell proliferation and compartmentalization of capillary plexus to a great extent. According to Melkonian et al, Cytochalasin D, an antiangiogenic drug inhibits both the spreading of mesodermal vessels along the ectodermal basal lamina, which would affect compartment width, and the subdivision of the plexus vessels by sprouting, which would affect the length of compartments. This is correlated with its ability to affect endothelial cell proliferation. Increase in mesenchymal cells, capillary plexus with greater length (due to poor compartmentalization), on the treatment of CC reveal its antiproliferative effect on endothelial cells.

The antiangiogenic effect is further highlighted by its ability to arrest cells at promaphase. Interestingly, the degree of arrest by CC at higher doses was compatible with the results of colchicine a known antimitotic compound. Most of the cells were showing typical chromosomal condensation, characteristics of late prophase. These results further support aforesaid possibility that inhibitory action of CC on HE La cell proliferation involves blockage of cell cycle progression at prophase/metaphase transition. Because G2/M phase is strictly involved in dynamics of cytoskeleton, our findings might shed a new light on mechanistic basis of CC’s angiogenesis-targeting activities.

Rapid microtubules dynamics are especially prominent in mitosis and are essential for proper spindle assembly and function. Any substance that shows a potential inhibition of cell division usually interferes with tubulin/microtubule system. Different mechanistic are involved in these binding studies. Few drugs bind preferentially to polymerized tubulin cause hyper-assembly of microtubules of increased stability in vitro. The other mechanism involves interaction of drug with tubulin dimer (and not microtubule) and inhibit microtubule assembly. These drugs cause disappearance of microtubules in both interphase and mitotically arrested cells.

CC inhibits tubulin polymerization and thus exhibits potential as depolymerizing agent. Depolymerizing agents are known to cause change in the secondary structure of tubulin, by binding with
high affinity site in heterodimer. It can be postulated that CC may causes a change in structure/conformation of the tubulin dimer, which hinders the assembly of tubulin dimers to form microtubule.

Alternatively, recently it is believed that depolymerizing agents bind to dimer and modulates the dynamic property of microtubules and thus inhibit polymerization. During prometaphase, the plus end of mitotic spindle microtubules appears to probe the cytoplasm until linkage with kinetochore is established. Reduced microtubule dynamics in presence of CC can be the reason for the prometaphase arrest of the cells.

The cytoskeleton has long been of interest in cancer chemotherapy because several well-validated antimitotic agents target it. Because the morphological changes in endothelial cells, such as spreading and elongation are crucial for endothelial tube formation. The special role of cytoskeleton in endothelial cell function has received attention as a therapeutic target for antiangiogenic strategies. The vascular targeting agent ZD6126, Combrestatin A-4 phosphate (CA-4-P) a known anticancer drug, affects endothelial cell morphology, destabilizes the tubulin cytoskeleton, and disrupts newly formed vessels. It would be of great interest to study interaction of CC with endothelial cytoskeleton, which even can elaborate on mechanism of antiangiogenic action.

Progress of antiangiogenic compound as antineoplastic agent is hindered since the concentration required to affect endothelial cells exceed those effective on cancer cells and thus responsible for undesired side-effects. The potent tubulin binding drugs vincristine and vinblastine from *Vinca rosea* even though have profound tumour vasculature effects, they are at close to their maximum tolerated doses (MTD). Our finding suggest that CC might be affecting endothelial cells at the much lower concentrations as compare to its cytotoxicity concentration with cancer cells. Hence CC on further purification can contribute as antineoplastic agent.

**Conclusion**

The study revealed that the chloroform fraction of the feather star *Lampropetra palmata palmata* which is terpene in nature, interacts with cytoskeletal protein, tubulin. It also shows antiproliferative property and antiangiogenic activity, which include alteration of endothelial cells migration and proliferation. These findings along with its low toxicity (in vivo and in vitro), indicate that CC is a promising tubulin binding, antiangiogenic entity. Since antimitotic agents that posses antiangiogenesis are of interest because of their potential use in the treatment of human neoplastic and inflammatory diseases, further identification, purification and interaction with endothelial cells of CC will be appreciated.

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