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Original Article

IN VITRO CYTOTOXIC ACTIVITY OF CLERODENDRUM INFORTUNATUM L. AGAINST T47D, PC-3, A549 AND HCT-116 HUMAN CANCER CELL LINES AND ITS PHYTOCHEMICAL SCREENING

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ABSTRACT

Objective: Present investigation accounts for scientific evaluation of the plant C*lerodendrum infortunatum* L. for its medicinal efficacy which includes phytochemical screening and anticancer activities.

Methods: Phytochemical screening of *Clerodendrum infortunatum* extracts was performed for the qualitative detection of reducing sugars, terpenoids, flavonoids, saponins, tannins, alkaloids, phlobatannins, steroids, amino acids and glycosides using standard procedures. The sulforhodamine B (SRB), *in vitro* cytotoxic assay, was used to investigate the anticancer activity of hexane, chloroform, ethyl acetate and ethanol extracts of leaves and roots of *Clerodendrum infortunatum* against T47D (Breast), PC-3 (prostate), A549 (lung) and HCT-116 (colon) cancer cell lines.

Results: Secondary metabolites including alkaloids, flavonoids, terpenoids, steroids, tannins, and saponins are present in many extracts. Alkaloids and flavonoids found to be present in almost all the extracts. The best cytotoxic activity has been exerted by hexane of root exhibiting growth inhibition of 72.83 ± 0.44, 85.50 ± 0.29 and 68.17 ± 1.36 % against PC-3, A549 and HCT-116 at a concentration of 100 µg/ml. Further, hexane of root showed a moderate cytotoxic effect of 42.17 ± 0.17 % against T47D at 100 µg/ml. At similar concentration, the chloroform extract is also effective against these three cell lines showing 61.50 ± 0.76, 67.00±0.58, and 68.53 ± 0.80% growth inhibition against PC-3, A549 and HCT-116 cell lines respectively, whereas, T47D cancer cell line showed 46.43 ± 0.30 % growth inhibition. The results have indicated that all the leaf extracts, as well as ethyl acetate and ethanol extracts of root, have exhibited a poor response (≤40 %).

Conclusion: The present findings suggest that the *Clerodendrum infortunatum* extracts are rich in alkaloid, flavonoids and terpenoids. The hexane and chloroform root extracts of *Clerodendrum infortunatum* possess significant anticancer activities which may be due to the presence of these phytochemical groups.

Keywords: Alkaloids, Cancer cell lines, *Clerodendrum infortunatum*, Flavonoids, Growth inhibition.

INTRODUCTION

Cancer is one of the most malignant diseases in which deregulating propagation of abnormal cells plague and disrupt contiguous tissues [1]. Both developed and developing countries express serious public health problems in terms of cancer. It is a mortal disease and presently, about one in five of all deaths is because of cancer [2]. Normal human cells proliferate for a fixed number of generations and then enter a condition of replicative senescence, but cancer cells can multiply endlessly [3]. There are 12.7 million cases of cancer and 7.6 million cancer deaths recorded in 2008; of these 64% of deaths exist in the economically developing countries [4]. The detectable tumors can be evaluated by surgical means, but it may put down nest of cancer cells in the patient, which continue to flourish while radiation therapy provides an inaccurate healing as it can exterminate both cancer and normal cells. The limited success of these therapies in the treatment of cancer indicates that there is an imperative need of alternative strategies in cancer management [5]. The universal fatality rate may increase by nearly 80 % by 2030, with enormous probability in low-and-middle-income countries devoid of instant action cited by the World Health Organization (WHO). Novel therapeutic alternatives to analyze for cancer treatment are of elevated priority for most of the pharmaceutical industries and independent research organizations worldwide. Significant research activity is committed to the innovation of more potent treatments while reducing their toxic side effects. So, there is a precondition of cancer chemotherapy for the development of selective drugs that can kill malignant tumor cells or render them benign without affecting normal cells which may eventually direct to attain the goal [6].

The practical access to this difficulty is the use of terrestrial plants as an application for drug development. Plants have played a leading role in the development of sophisticated traditional medicine systems. About 80% of the populations in some Asian and African countries rely on traditional medicine for main health care as estimated by the WHO. The 70–80% of the populations of developed countries also decides on plant products as a complementary medicine.

Different species of *Clerodendrum* genus have been traditionally used over the centuries, and their antioxidant, and hepatoprotective potential have already been proved [7-9]. *C. infortunatum* is very common throughout the plains of India. The leaves are slightly bitter, cure inflammation, skin diseases and good in smallpox [10]. The plant found to contain triterpenes, steroids, and flavonoids [11, 12]. The antioxidant [13], antimicrobial [14], anti-malarial [15], anthelmintic [16] and analgesic [17] activities of the plant have further created an upsurge in investigations on the plant. Therefore, the objective of the present study was designed to evaluate the anticancer potential of different extracts of *C. infortunatum* against different cancer cell lines.

MATERIALS AND METHODS

Plant source and extract preparation

The plant *C. infortunatum* (Verbenaceae) was collected from the roadside of Bhadra River Channels, Shimoga, Karnataka, India. The plant was authenticated by Dr. V. Krishna, Professor, Department of Biotechnology, Kuvempu University, Karnataka, who is one of the known taxonomists. The leaves and roots (after cutting into small pieces) were shade dried for several days. The plant material was then oven dried for 24 h at a considerably lower temperature (40 °C) for moisture free and better grinding. The air-dried and finely ground leaf (1000g) and root (500g) material of the plant was extracted in a Soxhlet apparatus successively with hexane, chloroform, ethyl acetate and ethanol from low polarity to high polarity. A suitable solvent was added to the flask, and the setup was heated under reflux. The steam of the solvent which, when contacts with the material will dissolve metabolites and brings back metabolites to the flask. The extracts were filtered, pooled and

concentrated to dryness under reduced pressure in a rotary evaporator (Buchi, Flawil, Switzerland) to yield dried hexane, chloroform, ethyl acetate and ethanol extracts. The extracts so obtained from each of solvents were labeled and yield was calculated in terms of grams/weight of the powdered material.

Solubility tests of plant extracts

Solubility tests were carried out for the analysis of solubility of crude extracts in different solvents like hexane, chloroform, ethyl acetate, acetone, DMSO, ethanol, methanol, water, 1N NaOH, and 1N HCl.

Phytochemical analysis of extracts

Phytochemical screening of *C. infortunatum* extracts was performed for the qualitative detection of reducing sugars, terpenoids, flavonoids, saponins, tannins, alkaloids, phlorotannins, steroids, amino acids and glycosides using standard procedures [18, 19].

Chemicals

The chemicals utilized in this analysis comprises RPMI-1640 (Roswell Memorial Park Institute), Minimum Essential Medium, fetal calf serum, trypsin, trypan blue, ethanol, penicillin, streptomycin, gentamycin, dimethyl sulfoxide (DMSO), sulforhodamine B (SIGMA), adriamycin, mitomycin-C, paclitaxel, 5-fluorouracil (5-FU; Sigma Chemical Co., St Louis, MO, USA), phosphate buffer saline (Merck, Darmstadt, Germany); trichloroacetic acid (TCA), distilled water, sodium hydroxide, Tris-EDTA buffer, Tris buffer (Hi-Media, Mumbai, India), acetic acid, sodium bicarbonate, hydrochloric acid (Rankem, New Delhi, India), isopropanol (Sisco, Mumbai, India), and Tris-acetate-EDTA buffer. All other chemicals used in this study were purchased locally and were of analytical grade.

Cell lines and cell culture

The human cancer cell lines used in this study, including T47D (Breast), PC3 (prostate), A549 (lung) and HCT-116 (colon) were obtained from the National Center for Cell Science, Pune, India. The properties of the cell lines under investigation as well as their culture conditions are summarized in table 1.

The cells were sustained in a CO_2 incubator with 5% CO_2 and 95% humidity and supplemented with desired medium and 10% fetal bovine serum (FBS). Glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 mg/ml) were also added to the medium to 1×final concentration from a 100 × stock. After attaining the confluent growth, the cells were trypsinized using Trypsin-EDTA (0.25%) and the number of cells required to perform the assay were seeded into sterile 96-well plates. Then the plates were incubated in a CO_2 incubator with 5% CO_2 and 95% humidity [20, 21].

Table 1: Review of cell lines under investigation, their origin, characteristics and culture conditions

Cell Line	Description	Seeding density (Per cm²)	Growth media	ER status	P_{53}Status	Doubling time (h)
T47D	Breast	30,000	DMEM	+	Mu	32
PC-3	Prostate	50,000	DMEM	+	Null	40
A-549	Lung	40,000	RPMI 1640	+	Wt	22
HCT-116	Colon	30,000	RPMI 1640	+	Wt	21

Cytotoxic assay preparation

Trypan blue exclusion method has been employed to determine the viability of the cells. Concisely, 100 µl of cell suspension (10,000 cells) of each cell line was plated in each well of 96-well plates, and incubated for 24 h at 37 °C in a humidified CO_2 (5%) incubator. The growth inhibitory activity of the hexane, chloroform, ethyl acetate and ethanol extracts of both leaf and root were evaluated against four cell line panels consisting of T47D (Breast), PC3 (prostate), A549 (lung) and HCT-116 (colon) using the SRB assay[22]. The lyophilized extracts were dissolved in DMSO and filtrated by 0.2 µm cellulose acetate sterile filter. After 24 h of incubation time of cell lines, an amount of 1, 10 and 100 µg/ml of each extract was added. Several standard anticancer drugs were used as positive control for comparison. The drugs include Adriamycin (10µM) against T47D, mitomycin (1µM) against PC-3, paclitaxel (1 µM) against A549 and 5-Fluorouracil (20µM) against HCT-116 were added as a positive control against desired cancer cell lines per well and incubated for 48 h.

Sulpho rhodamine B assay

After 48 h of incubation with test samples, each well was fixed with 50 µl of 50% trichloroacetic acid solution to stop the reaction and incubated at 4 °C for 1 h followed by washing with distilled water. Excess water was drained off and the plates were air-dried for 24 h. Then cells were stained with 50 µl of 0.4% SRB solution in 1% acetic acid for 30 m at room temperature (27±2 °C). After incubation, the unbound SRB solution was discarded, and the plates were washed with 1% acetic acid. The plates were air-dried, and 100 µl of 10 mM Tris-base solution (pH 10.5) was added to each well to solubilize the dye and shaken for 30 min on a mechanical shaker. The optical density (OD) at 540 nm was determined by the micro plate reader (Bio-Rad, Model 680, USA). All the investigation was performed in triplicates. The percentages of the cell growth inhibition were determined using the following equation:

$$\% \text{ Growth inhibition } = 100 - \frac{\text{OD (test sample)} - \text{OD (blank)}}{\text{OD (Control)} - \text{OD (blank)}} \times 100$$

Statistical analysis

The data from biological assays were subjected to the One-way analysis of variance (ANOVA) procedures which were presented as mean±SEM. $P \leq 0.01$ was considered as significant.

RESULTS

Physical characteristics and percentage yield of extracts

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plants using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. The percent yield of both leaf and root extracts obtained from *C. infortunatum* and their description is outlined in table 2 and 3.

Table 2: Percent yield (w/w) and physical characteristics of *C. infortunatum* Leaf extracts

Samples	Quantity used for extraction		Nature of the extract	% Yield
	Powder (g)	Solvent (ml)		
Hexane	1000	250	Black Sticky mass	3.0
Chloroform	1000	250	Dark Solid	2.0
Ethyl acetate	1000	250	Slightly light green solid mass	3.5
Ethanol	1000	250	Dark sticky brown paste	20.0

Table 3: Percent yield (w/w) and physical characteristics of *C. infortunatum* root extracts

Samples	Quantity used for extraction		Nature of the extract	% Yield
	Powder (g)	Solvent (ml)		
Hexane	500	250	Light yellow mass	1.0
Chloroform	500	250	Brown sticky paste	1.5
Ethyl acetate	500	250	Orange red paste with acidic smell	1.0
Ethanol	500	250	Red paste with acidic smell	4.0

Table 4: Phytoconstituents present in the leaf extracts of *C. infortunatum* L.

Phytoconstituents	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids	+ve	+ve	+ve	-ve
Flavonoids	+ve	+ve	+ve	+ve
Terpenoids	+ve	-ve	+ve	-ve
Steroids	-ve	-ve	-ve	+ve
Tannins	-ve	-ve	-ve	+ve
Phlobtannins	-ve	-ve	-ve	-ve
Saponins	-ve	-ve	-ve	+ve
Carbohydrates	+ve	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve	-ve
Amino acids	-ve	-ve	-ve	+ve

(+ve): Present; (-ve): Absent.

Table 5: Phytoconstituents present in the root extracts of *C. infortunatum* L.

Phytoconstituents	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids	+ve	+ve	+ve	-ve
Flavonoids	-ve	+ve	+ve	+ve
Terpenoids	+ve	+ve	-ve	+ve
Steroids	-ve	-ve	+ve	+ve
Tannins	-ve	-ve	-ve	+ve
Phlobtannins	-ve	-ve	-ve	-ve
Saponins	-ve	-ve	-ve	+ve
Carbohydrates	+ve	+ve	+ve	+ve
Glycosides	-ve	-ve	-ve	-ve
Amino acids	-ve	-ve	-ve	-ve

(+ve): Present; (-ve): Absent.

Table 6: Cytotoxic effect of leaf extracts of plant on different cancer cell lines

Tissue			Breast	Prostate	lung	Colon
Cell line type			T47D	PC-3	A549	HCT-116
S. No.	Sample	Conc. (µg/ml)	% Growth inhibition			
1.	Hexane	1	15.73±0.64**	NT	NT	1.93±0.52**
		10	20.87±0.35**	1.73±0.43**	1.37±0.20**	3.03±0.15**
		100	28.43±0.49**	13.93±0.61**	43.83±0.55**	22.10±1.15**
2.	Chloroform	1	NT	NT	NT	NT
		10	NT	NT	NT	NT
		100	NT	14.20±0.44**	35.70±0.65**	1.67±0.24**
3.	Ethyl acetate	1	21.10±0.49**	NT	NT	5.83±0.90**
		10	20.87±0.58**	NT	2.37±0.33**	12.83±0.27**
		100	24.43±0.32**	11.33±0.33**	21.23±0.67**	12.27±0.68**
4.	Ethanol	1	NT	NT	NT	NT
		10	NT	NT	NT	NT
		100	18.07±0.58**	11.87±0.24**	10.13±0.09**	11.27±0.37**
5.	Adriamycin	10µM	63.27±0.37	-	-	-
6.	Mitomycin	1µM	-	67.60±0.56	-	-
7.	Paclitaxel	1µM	-	-	76.73±0.82	-
8.	5-Fluorouracil	20µM	-	-	-	50.60±0.31

NT: Non-toxic, Values are expressed as mean±SE of three values. ***P<0.01*; ***p* values were calculated comparing the experimental results to standard results.

From the above tables, it is estimated that the yield of hexane, chloroform, ethyl acetate and ethanol extracts of the leaf was found to be 3.0, 2.0, 3.5 and 20.0% respectively. Likewise, the yield of hexane, chloroform, ethyl acetate and ethanol extracts of root was found to be 1.0, 1.5, 1.0 and 4.0 % respectively. It is evident from the above tables that extraction with ethanol in both leaf and root yields the highest amount of extracts.

Qualitative detection of phytochemical constituents

All the solvent extracts from leaf and root were subjected to preliminary qualitative phytochemical analysis. The various groups of phytochemical constituents found to be present in these extracts are shown below (table 4 and 5). The presence of groups of all these different extracts includes alkaloids, flavonoids, terpenoids, steroids,

tannins and carbohydrates. This analysis revealed that the plant possesses alkaloids, flavonoids, carbohydrates in high concentration.

***In vitro* cytotoxic activity of plant extracts**

The *in vitro* cytotoxic effect of eight extracts of leaf and root (four from each part) derived from *C. infortunatum* were evaluated on four human cancer cell lines from different tissues of origin, namely; T47D (Breast), PC-3 (prostate), A549 (lung) and HCT-116 (colon) cancer cell lines. The cytotoxic activity of the extracts was compared with the activity of standard anticancer drugs. The results suggest that among the four different root extracts, hexane and chloroform showed a significant cytotoxic activity against PC-3, A549 and HCT-116 cell lines used and had shown moderate activity against the T47D cell line, whereas, the ethanol and ethyl acetate extracts did not show promising activity when compared to the respective standards used. The results of cytotoxic effects of both leaf and root extracts are summarized in table 6 and 7. The best antiproliferative activity has been exerted by hexane of root exhibiting growth inhibition of 72.83±0.44, 85.50±0.29 and 68.17±1.36 % against PC-3, A549 and HCT-116 at a concentration of 100µg/ml. Further, hexane showed a moderate cytotoxic effect of 42.17±0.17 % against T47D at 100µg/ml. At a similar concentration the chloroform extract is also effective against these three cell lines showing 61.50±0.76, 67.00±0.58, and 68.53±0.80 % growth inhibition against PC-3, A549 and HCT-116 respectively, whereas, T47D cancer cell line showed 46.43±0.30 % growth inhibition. The results have indicated that all the leaf extracts, as well as ethyl acetate and ethanol root, have exhibited a poor response (≤40%).

Percent growth inhibition resulting from the standard drugs on different cell lines used in this study is found between 50 and 77%. The cytotoxic effect of some extracts is comparatively more than that of standard drugs.

Table 7: Cytotoxic effect of root extracts of plant on different cancer cell lines

Tissue			**Breast**	**Prostate**	**Lung**	**Colon**
Cell line type			**T47D**	**PC-3**	**A549**	**HCT-116**
S. No.	**Sample**	**Conc. (µg/ml)**	**% Growth inhibition**			
1.	Hexane	1	NT	NT	NT	NT
		10	NT	25.33±0.34**	2.00±0.58**	14.60±0.31**
		100	42.17±0.17**	72.83±0.44**	85.50±0.29**	68.17±1.36**
2.	Chloroform	1	NT	1.33±0.33	NT	NT
		10	NT	23.00±0.58	NT	6.83±0.22
		100	46.43±0.30**	61.50±0.76**	67.00±0.58**	68.53±0.80**
3.	Ethyl acetate	1	12.27±0.82**	NT	NT	NT
		10	14.33±0.88**	NT	12.00±0.583	NT
		100	26.43±0.260**	29.80±0.42**	14.00±0.58**	15.07±0.41**
4.	Ethanol	1	26.43±0.47**	NT	NT	NT
		10	31.23±0.67**	NT	NT	NT
		100	39.83±0.44**	11.33±0.88**	NT	5.00±0.58**
5.	Adriamycin	10µM	63.27±0.37	-	-	-
6.	Mitomycin	1µM	-	67.60±0.56	-	-
7.	Paclitaxel	1µM	-	-	76.73±0.82	-
8.	5-Fluorouracil	20µM	-	-	-	50.60±0.31

NT: Non-toxic, Values are expressed as mean±SE of three values. ***P<0.01*; ***p* values were calculated comparing the experimental results to standard results.

DISCUSSION

Cancer is the prominent origin of death in efficiently developed countries and second largest death in developing countries [23]. It is estimated that new cases of cancer will rise approximately 25% in each decade, aiming 24 million new cases per year in the year 2050 [24]. The current threat linked with presently existing drugs comprises selectivity, toxicity, resistance, and development of a secondary malignancy. The downsides of these anticancer agents have encouraged the examination of the novel, competent and well resistant drugs against cancer, as natural products, mainly from plants. Keeping this in mind, the present investigations have been carried out to screen the cytotoxic potential of extracts of *C. infortunatum* against different human cancer cell lines.

The plant material was subjected for Soxhlet extraction using solvents of appropriate polarity following the principles of 'like dissolves like' thus non-polar solvents were used to solubilize most lipophilic compounds (e. g., alkanes, fatty acids, pigments, waxes, sterols, few terpenoids, alkaloids). Medium polarity solvents were used to extract compounds of intermediate polarity (e. g., some alkaloids, flavonoids) while more polar solvents were used for more polar compounds (e. g., flavonoid, glucosides, quaternary alkaloids, tannins, etc.). Based on this principle, four different solvents have been used with increasing polarity, i.e., hexane, chloroform, ethyl acetate and ethanol for successive extraction.

Phytochemical screening of *C. infortunatum* extracts was performed to determine the presence of phytoconstituents such as: alkaloids, flavonoids, terpenoids, steroids, carbohydrates, tannins, saponins, glycosides, amino acids. Among these groups alkaloids and flavonoids are found to be very rich in these extracts followed by terpenoids, while as, saponins, tannins, and steroids are present only in ethanol extract. The presence of these groups may direct to comprehend the potent cytotoxic activity of the plant. Earlier reports on medicinal plants suggest that alkaloids [25]and flavonoids [26] extensively play a vital role in the toxicity of cancer cells.

In this study hexane, *C. infortunatum* root is found to be significantly toxic against PC-3 (prostate), A549 (lung) and HCT-116 (colon) cancer cell lines exhibiting growth inhibition of 72.83±0.44, 85.50±0.29 and 68.17±1.36% respectively at a concentration of 100µg/ml in the SRB assay compared to the standard drug. Likewise, hexane, chloroform root extract at the same concentration of 100µg/ml also exhibited considerable growth inhibition of 61.50±0.76, 67.00±0.58 and 68.53±0.80% against same cancer cell lines. It is pertinent to note that, the percentage inhibition is greater than the standard drug used. Both of these extracts exerted moderate cytotoxic activity against the T47D cell line. The growth inhibition may be due to the presence of non-polar and medium polar type of compounds. Hexane and chloroform revealed higher cytotoxic activity than other extracts used which could be due to the high content of alkaloids and flavonoids or may be of terpenoids. Interestingly, both extracts which showed highest anticancer activity are from the root parts of *C. infortunatum* and unveil the presence of alkaloids, flavonoids as well as terpenoids.

There are many earlier reports suggesting the biological activities of several alkaloids viz., the anticancer effects of vinblastine [27], camptothecin (CPT), a famous topoisomerase-I (TopI) inhibitor [28]. Similarly, berberine inhibits the proliferation of multiple cancer cell lines by inducing cell cycle arrest at the G1 or G_2/M phases and by

apoptosis [29, 30, 31]evodiamine exhibits anticancer activities both *in vitro* and *in vivo* by inducing the cell cycle arrest or apoptosis, inhibiting the angiogenesis, invasion, and metastasis in a variety of cancer cell lines [32, 33]. Piperine and matrine are also considered to be potent anticancer alkaloids [34, 35].

The role of flavonoids in cancer prevention indicates that flavonoids have important effects on cancer chemoprevention and chemotherapy [36]. The various flavonoids which showed anticancer activity on different human cancers include flavanones, daidzein, genistein, quercetin, luteolin are effective against breast cancer[37, 38]. Flavone, quercetin have also been proved to be potent against human lung cancer [39, 40]. Catechin, epicatechin, quercetin, kaempferol, luteolin, genistein, apigenin, myricetin, silymarin possess significant effect against human prostate cancer [41-43]. The flavonoids which are considered to be very effective against human colon cancer exclude flavone, quercetin, genistein, anthocyanin [44, 45].

In addition, of alkaloids and flavonoids, the diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer [46].

Some Species of *Clerodendrum* genus that have been investigated in the anticancer study and have resulted in the innovation of promising drugs include *C. serratum*Spreng. [47, 48], *C. bungei* Study [49, 50], *C. calamitosum*L. [51]. Other species of *Clerodendrum* including *C. phlomidis, C. inerme*, *C. colebrookianum* and *C. trichotomum* also possess significant cytotoxic activity [52, 53]. Some investigations also identified the important cytotoxic activity of leaf extracts of *C. viscosum* [54].

Based on all this information the alkaloids and flavonoids or terpenoids present in *C. infortunatum* may be the actual source of cytotoxic effect against the human cancers investigated in this study.

CONCLUSION

Thus, the findings of this investigation suggest that the *C. infortunatum* extracts are rich in alkaloids, flavonoids, and terpenoids. The hexane and chloroform root extracts of the plant possess significant anticancer activities *in vitro* against PC-3 (prostate), A549 (lung) and HCT-116 (colon) cell lines which may be due to the presence of these alkaloids, flavonoids, and terpenoids. Hence, *C. infortunatum* could be considered as promising plant possessing anticancer properties. Further, extensive studies are required to identify, isolate and characterize specific phyto molecules responsible for bioactivities observed in this study and their inherent mechanism(s) of action.

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CONFLICT OF INTERESTS

We declare that there are no conflicts of interest, financial or otherwise, pertaining to this study.

REFERENCES

1. Gennari C, Castoldi D, Sharon O. Natural products with taxol-like anti-tumor activity: synthetic approaches to eleuthero bin and dicty statin. Afr J Pure Appl Chem 2007;79:173-80.
2. Chhajed M, Sowle A, Baraskar R, Parihar N. Buserelin as a wonder drug for prostate cancer: a comprehensive study. Int J Pharm Res Sci 2012;1:150-72.
3. Rao MRP, Adagale UR, Shetty A, Namjoshi P, Gaitonde P, Jain P. Cancer Immunotherapy; 2007. Available from: http://www.pharmainfo.net/ reviews/cancer-immunotherapy. [Last accessed on 20 Sep 2015].
4. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
5. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010;15:7313-52.
6. Sporn MB, Liby KT. Cancer chemoprevention: scientific promise, clinical uncertainty. Nat Clin Pract Oncol 2005;2:518–25.
7. Gopal N, Sengottuvelu S. Hepatoprotective activity of *Clerodendrum inerme* against CCl_4 induced hepatic injury in rats. Fitoterapia 2008;79:24-6.
8. Vidya SM, Krishna V, Manjunatha BK, Mankani KL, Ahmed M, Singh SD. Evaluation of hepatoprotective activity of *Clerodendrum serratum* L. Indian J Exp Biol 2007;45:538-42.
9. Chae S, Kim JS, Kang KA, Bu HD, Lee Y, Seo YR, *et al*. Antioxidant activity of iso acteoside from *Clerodendrontrichoto*mum. J Toxicol Environ Health Part A 2005;68:389-400.
10. Chopra RN, Nayer SL, Chopra IC. Glossay of Indian medicinal plant. (Publication and Information Directorate*, CSIR*, New Delhi; 1992. p. 71.
11. Akihisa T, Matsubara Y, Ghosh P, Thakur S, Shimizu N, Tamura T, *et al*. The 24a-and 24ß-epimers of 24-ethylcholesta-5, 22-dien-3ß-ol in two *Clerodendrum* species. Phytochemistry 1988;27:1169-72.
12. Sinha NK, Seth KK, Pandey VB, Dasgupta B, Shah A. Flavonoids from the flower of *Clerodendrum infortunatum*. Planta Med 1981;42:296-8.
13. Sannigrahi S, Mazumder UK, Pal DK, Parida S. *In vitro* antioxidant activity of methanol extract of *Clerodendrum infortunatum* Linn. Oriental Pharm Exp Med 2009;9:128-34.
14. Rajakaruna N, Harris CS, Towers GHN. Antimicrobial activity of plants collected from serpentine outcrops in Srilanka. Pharm Biol 2002;40:235-44.
15. Goswami A, Dixit VK, Srivastava BK. Anti-malarial activity of an aqueous extract of *Clerodendrum infortunatum*. Bionature 1998;48:45-8.
16. Haris M, Riaz M, Haseeb R, Nazneen. Paralysis and death of *Pheretima posthuma* and fungal growth inhibition by leaf extracts of *Clerodendrum infortunatum* L**.** J Pharmacogn Phytochem 2015;3:188-92.
17. Pal DK, Sannigrahi S, Mazumder UK. Analgesic and anticonvulsant effects of saponin isolated from the leaves of *Clerodendrum infortunatum* Linn. in mice. Indian J Exp Biol 2009;47:743-7.
18. Mehta K, Patel BN, Jain BK. Phytochemical analysis of leaf extracts of *Phyllanthusfraternus*. Res J Recent Sci 2013;2:12-5.
19. Ashok Kumar, Jha KK, Dinesh Kumar, Abhirav Agarwal, Akhil Gupta. Preliminary phytochemical analysis of leaf and bark (Mixture) extracts of *Ficusinfectoria* Plant. Pharma Innovation 2012;1.5:71-6.
20. Sharma PR, Mondhe DM, Muthiah S, Pal HC, Shahi AK, Saxena AK. Anticancer activity of essential oil from *Cymbopogonflexuosus*. Chem Biol Interact 2009;179:160-8.
21. Samanta S, Pain A, Dutta S, Saxena AK, Shanmugavel M. Antitumor activity of nitro naphthol-NU, a novel mixed function agent. J Exp Ther Oncol 2005;5:15-22.
22. Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to the reputed anticancer activity. Methods 2007;42:377-87.
23. World Health Organization. The Global Burden of Disease. Cambridge, MA, USA: Harvard; 2008.
24. Schwartsmann G. Breast cancer in South America: challenges to improve early detection and medical management of a public health problem. J Clin Oncol 2011;19:118-24.
25. Zulak K, Liscombe D, Ashihara H, Facchini P. Alkaloids. Plant secondary metabolism in diet and human health. Oxford*:* Blackwell Publishing; 2006. p. 102–36.
26. Sharma DK. Pharmacological properties of flavonoids from plants. Am J Sci Ind Res 2006;65:477-84.
27. Li YW, Shao L, Hu BM6. A new semi-synthetic Vinca alkaloid exhibits its potent *in vivo* antitumor activities via its high

binding affinity for tubulin and improved pharmacokinetic profiles." Cancer Biol Ther 2007;65:787–94.

28. Huang M, Gao H, Chen Y. Chimmitecan, a novel 9-substituted camptothecin, with improved anticancer pharmacologic pro files *in vitro* and *in vivo*," Clin Cancer Res 2007;13:1298–307.
29. Sun Y, Xun K, Wang Y, Chen X. A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anti-Cancer Drugs 2009;20:757–69.
30. Eom KS, Kim HJ, So HS, Park R, Kim TY. Berberine-induced apoptosis in human glioblastoma T98G Cells Is mediated by endoplasmic reticulum stress accompanying reactive oxygen species and mitochondrial dysfunction. Biol Pharm Bull 2010;33,10:1644–9.
31. Burgeiro A, Gajate C, Dakir EH, Villa-Pulgar JA, Oliveira PJ, Mollinedo F. Involvement of mitochondrial and B-RAF/ERK signaling pathways in berberine-induced apoptosis in human melanoma cells. Anti-Cancer Drugs 2011;22:507–18.
32. Shyu KG, Lin S, Lee CC. Evodiamine inhibits *in vitro* angiogenesis: implication for anti–tumorgenicity. Life Sci 2006;78:2234–43.
33. Zhang Y, Wu LJ, Tashiro SI, Onodera S, Ikejima T. Intracellular regulation of evodiamine-induced A375-S2 cell death. Biol Pharm Bull 2003;26:1543–7.
34. Ji YB. Active Ingredients of Traditional Chinese Medicine: Pharmacology and Application, People's Medical Publishing Hourse Cp., LTD; 2011.
35. Dai ZJ, Gao J, Ji ZZ. Matrine induces apoptosis in gastric carcinoma cells via alteration of Fas/FasL and activation of caspase-3. J Ethnopharmacol 2009;123:91–6.
36. Ren W. Flavonoids: promising anticancer agents. Med Res Rev 2003;23:51934.
37. Pouget C, Lauthier F, Simon A, Fagnere C, Basly JP, Delage C, *et al*. Flavonoids: structural requirements for antiproliferative activity on breast cancer cells. Bioorg Med Chem Lett 2001;11:3095–7.
38. Han D, Tachibana H, Yamada K. Inhibition of the environmental estrogen-induced proliferation of human breast carcinoma MCF-7 cells by flavonoids.*In Vitro* Cell Dev Biol: Anim 2001;37:275–82.
39. Bai F, Matsui T, Ohtani-Fujita N, Matsukawa Y, Ding Y, Sakai T. Promoter activation and following induction of the p21/WAF1 gene byflavone is involved in G1 phase arrest in A549 lung adenocarcinoma cells. FEBS Lett 1998;437:61–4.
40. Caltagirone S, Ranelletti FO, Rinelli A, Maggiano N, Colasante A, Musiani P, *et al*. Interaction with type II estrogen binding sites and antiproliferative activity of tamoxifen and quercetin in human non-small-cell lung cancer. Am J Respir Cell Mol Biol 1997;17:51–9.
41. Knowles LM, Zigrossi DA, Tauber RA, Hightower C, Milner JA. Flavonoids suppress androgen independent human prostate tumor proliferation. Nutr Cancer 2000;38:116–22.
42. Kampa M, Hatzoglou A, Notas G, Damianaki A, Bakogeorgou E, Gemetzi C, *et al*. Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines. Nutr Cancer 2000;37:223–33.
43. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 2000;60:1051–9.
44. Wenzel U, Kunt S, Brendel MD, Daniel H. Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells. Cancer Res 2000;60:3823–31.
45. Kamei, Hashimoto Y, Koide T, Kojima T, Hasegawa M. Anti-tumor effect of methanol extracts from red and white wines. Cancer Biother Radiopharm 1998;13:447–52.
46. Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. Breast Cancer Res Treat 2009;115:223–39.
47. Singh MK, Khare G, Iyer SK, Shavan G, Tripathi Dk. *Clerodendrum serratum*: a clinical approach. J Appl Pharm Sci 2012;2:11-5.
48. Bhangare NK, Pansare TA, Ghongane BB, Nesari TM. Screening for the antifertility and antiallergic activity of Bhargi (*Clerodendrum Serratum*) in animals. Int J Pharmacol Biol Sci 2012;3:245-54.
49. Shi XF, Du DJ, Xie DC, Ran CQ. Studies on the antitumor effect of *Clerodendrum bungei* Steud or *C. foetidum* Bge. Pubmed 1993;18:687-90.
50. Kim SK, Cho SB, Moon HI. Anti-complement activity of isolated compounds from the roots of *Clerodendrum bungei*Steud. Phytother Res 2010;24,1720-3.
51. Cheng HH, Wang HK, Ito J, Bastow KF, Tachibana Y, Nakanishi Y, *et al*. Cytotoxic pheophorbide-related compounds from *Clerodendrum calamitosum* and C. Cyrtophyllum. J Nat Prod 2001;64:915-9.
52. Sannigrahi S, Mazumder UK, Pal D, Mishra SL. Terpenoids of methanol extract of *Clerodendrum infortunatum* exhibit anticancer activity against Ehrlich's ascites carcinoma (EAC) in mice. Pharm Biol 2012;50:304-9.
53. Bhatnagar S, Pattanaik SR. Comparative analysis of cytotoxic and antioxidant activities of leaf and bark extracts of *Clerodendrum viscosum* and *Clerodendrum phlomidis*. Int J Biomed Adv Res 2012;3:385-90.
54. Islam MS, Mogha MMR, Ahamed SK, Ahmed J, Islam MA. A study on cytotoxic and anthelmintic activities of crude extract of leaves of *Clerodendrum viscosum*. Int Res J Pharm 2013;4:99-102.