



## **Bio Active Compound Analysis of *Croton scabiosus* Bedd: By HPTLC, GC-MS and Evaluation of Anthelmintic Activity and Anticancer Potential on Lung (A549) and Breast (MCF-7) Cancer Cell Lines**

**E. Bhargav<sup>1</sup>, S. Salamma<sup>2</sup>, B. Chandana<sup>3</sup>, S. Harika<sup>3</sup>, M. Vijaya Jyothi<sup>3\*</sup>  
and B. Ravi Prasad Rao<sup>2</sup>**

<sup>1</sup>RERDS-CPR, Raghavendra Institute of Pharmaceutical Education and Research, K. R. Palli Cross, Chiyvedu Post, Anantapuramu-515721, Andhra Pradesh, India.

<sup>2</sup>Department of Botany, Sri Krishnadevaraya University, Ananthapuramu-515003, India.

<sup>3</sup>Department of Pharmaceutical Chemistry, Raghavendra Institute of Pharmaceutical Education and Research, K. R. Palli Cross, Chiyvedu Post, Anantapuramu-515721, Andhra Pradesh, India.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors MVJ, SS and EB performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BRPR managed the analyses of the study. Authors BC and SH managed the literature searches. All authors read and approved the final manuscript.

### **Article Information**

DOI: 10.9734/JPRI/2021/v33i331158

#### Editor(s):

(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.

#### Reviewers:

(1) Safa Abdul Syed Basheerudeen, India.

(2) Amira Mohammed Beltagy, Damanhour University, Egypt.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/65034>

**Original Research Article**

**Received 24 November 2020**

**Accepted 28 January 2021**

**Published 20 February 2021**

### **ABSTRACT**

*Croton scabiosus* Bedd. (Euphorbiaceae) is an endemic plant of Andhra Pradesh, India. Local communities use the verdant parts of this plant as an antidote to treat snake bites and as a remedy to heal leucorrhoea in the form of decoction. In the present study the author found to have a perceptible range of alkaloids, coumarins, glycosides, flavonoids, steroids and gallic tannins as secondary metabolites by phytochemical and spectral studies. Since flavonoids and alkaloids are known for remarkable anticancer and anthelmintic activities respectively, the researcher is interested to document the research findings on these activities. The present research is aimed to evaluate bioactive principles present in acetone, chloroform and ethyl acetate extracts of *Croton*

\*Corresponding author: E-mail: [drmvjyothiriper@gmail.com](mailto:drmvjyothiriper@gmail.com);

*scabiosus* Bedd. by chemical tests and spectral analysis. It is also aimed to study the anthelmintic and anticancer potential of these extracts. Acetone, chloroform and ethyl acetate extracts of *Croton scabiosus* Bedd. were prepared by triple maceration technique. GC-MS and HPTLC analysis were performed to identify chemical constituents. Anthelmintic and anticancer potential was assessed for the three extracts. Owing to the positive results of chemical constituents, the researcher made an attempt to evaluate anthelmintic, cytotoxic potential and anticancer activity on breast adenocarcinoma cells (MCF-7) and lung adenocarcinoma cell line (A549). It was pointed to appraise *Croton scabiosus* Bedd. extracts have considerable Anthelmintic, cytotoxic and anticancer potential.

**Keywords:** Anthelmintic; breast adenocarcinoma cells (MCF-7); *Croton scabiosus* Bedd; cytotoxic potential; lung adenocarcinoma cell line (A549).

## ABBREVIATIONS

ATCC: American Type Culture Collection, DMEM: Dulbecco's Modified Eagle Medium, DMSO: Dimethyl Sulfoxide, EDTA: Ethylenediaminetetraacetic acid, GCMS: Gas Chromatography and Mass Spectroscopy, HPTLC: High Performance Thin Layer Chromatography, IUCN: International Union for Conservation of Nature, MSD: Mass-Selective Detector, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, NIST: National Institute of Standards and Technology, PBS: Phosphate Buffer Saline.

## 1. INTRODUCTION

Herbal medication is a versatile practice generally used by traditional societies. According to the World Health Organization survey of 2000, globally 80% of the world population are practising phytotherapy to cure various diseases. 67% of chemotherapy drugs are from a natural origin [1]. Ayurveda system is also a traditional practice which utilizes plant sources as oral medicine in the form of food, decoctions, powders or external application.

Breast cancers and Lung cancers are the most common cancers in India in terms of prevalence and mortality. According to Globocan 2018 statistics [2] 67,795 new cases and 63,475 deaths occurred due to Lung cancer. In 2018, 1,62,468 new breast cancer cases and 87,090 deaths were reported in India. Chemotherapy with synthetic agents has shown to develop severe side effects. Generally, herbal drugs are used to reduce chemotherapy pertinent side effects and cancer-related effects [3]. The peculiar character of herbal drugs is they can demonstrate better cytotoxic activity only on cancer cells and not on healthy cells by conjugating them with suitable targets. Isoflavones, which were derived from soya bean have accomplished anticancer effects on breast and reduced the probability of developing cancer. Most of the chinese herbal drugs were known to treat Breast cancer, in which most of them have no proven mechanism of action. Medicinal herbs which were popularized as anti-cancer are being

taken as healthy foods or dietary supplements for decades. Pharmacological phytoconstituents isolation, *In vivo* studies and clinical trials should be conducted for specific targeted applications.

*Croton scabiosus* Bedd. is an endemic deciduous tree species belongs to the family Euphorbiaceae, with fortuitous distribution in open dry deciduous forests of Ananthapuramu, Kadapa and Nellore districts of Andhra Pradesh State, India. This plant is locally known as *Puruguchekka*, *Verrichilla*. The species is categorized 'Vulnerable' based on IUCN Red List Categories and criteria [4]. *Croton scabiosus* Bedd. aqueous bark decoction is known as a remedy to heal Leucorrhoea and (helminths) worm infections [5] whereas seed extracts were known to be used as an external patch in snake bite [6] to neutralize the poison. But scientific research evidence is not reported for folklore medical practice.

Since secondary metabolites impart several biological activities, this research is intended to identify phytochemical constituents present in Acetone, ethyl acetate and chloroform bark extracts of *Croton scabiosus* Bedd. by chemical and spectral methods. Hence all three extracts have given the positive result for tannins, alkaloids and saponins as bitter principles; an effort is made to evaluate anthelmintic activity for three extracts. The chloroform and ethyl acetate extracts possess flavonoids and glycosides. These are also tested for cytotoxic potential and a study is made on morphological behaviour

against lung adenocarcinoma cell line (A549) and Breast adenocarcinoma cells (MCF-7) to extend the research on evaluation of the anticancer activity. The research outcomes of these methods are specified in the experimental part, illustrate the considerable anthelmintic, cytotoxic and anticancer activities of *Croton scabiosus* Bedd.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Collection of plant material

The barks of *Croton scabiosus* Bedd. was collected in spring, February 2019 spring from a Reserve forest, 3 km from KK Kottala, Kadapa District, Andhra Pradesh, India. It was authenticated by Professor B. Ravi Prasad Rao, Botanist, Head of the Department of Botany, Sri Krishna Devaraya University Ananthapuramu. The voucher specimens were deposited in S.K. University Herbarium (SKU) with Acc. No. 45704, Ananthapuramu.

(Mention season of collection and scientist who identify the plant).

### 2.2 Methods

#### 2.2.1 Preparation of extracts

Triple maceration technique was used to prepare *Croton scabiosus* Bedd. bark extracts. The bark of *Croton scabiosus* Bedd. was dried then cut into small pieces later crushed to give a moderately coarse powder. Each 250 g of powder was placed in a round bottom flask. To this 750 ml of the solvent acetone (M1) was added as menstruum to the flask. This was allowed to stand for seven days with occasional shaking. The extracts were concentrated to dryness under reduced pressure using a rotary vacuum evaporator. By following the same procedure extraction was carried out by chloroform (M2) and ethyl acetate (M3) separately as the menstruums. These extracts were subjected to auxiliary studies.

#### 2.2.2 Phytochemical tests

Acetone, chloroform and ethyl acetate bark extracts of *Croton scabiosus* Bedd. were subjected to Phytochemical analysis to identify alkaloids, tannins, glycosides, steroids, carbohydrates, flavonoids, vitamins etc [7,8].

#### 2.2.3 HPTLC

High-performance Thin-Layer Chromatography was performed using silica gel 60F254 (10 cm×10 cm; 0.25mm layer thickness; Merck) as adsorbent. 25mg/ml of each M1, M2 and M3 extracts were filtered separately using a 0.45-micron filter paper and these concentrates were exposed to HPTLC (CAMAG, Switzerland). Investigation at three extraordinary fixations 4, 8, 12 µg/ml was spotted independently on a silica gel 60F254 (Merck, Darmstadt, Germany) TLC plate. The TLC plate was developed using Hexane: chloroform: methanol (5:4:1 v/v) as mobile phase in a CAMAG-twin-trough glass chamber previously saturated with mobile phase vapour for 20 min. After developing the plate, it was dried at 65°C for 2 min. It was scanned with Scanner 3 (WinCATS 4 software, CAMAG, Switzerland) at 254 nm and 366 nm [9].

#### 2.2.4 GC-MS

According to sangggil choe, *et al* the GC-MS study of the components and development were carried out with slight modifications, equipped with a mass-selective detector (MSD) (HP6890 GC and HP7673) auto sampler, operated at 65eV using acquisition scan mode with HP-5MS (GC capillary column, 0.25 mm×0.25 µm×30 m) at 100°C oven temperature held initially for 1 min and then increased gradually to 280°C by 20°C/min and held for 10 min. Injector temperature was maintained at 250°C and Helium was used as the carrier gas at a constant column flow rate of 1.5 ml/min. 2µl of the sample extract was injected by a splitless mode technique. The average of 10 chromatograms were considered to assess the components and registered by Comp Extractor software [10].

#### 2.2.5 Anthelmintic activity

Acetone, chloroform, ethyl acetate *Croton scabiosus* Bedd. bark extracts were used to evaluate anthelmintic activity on '*Pheretima Posthuma*' worms using Albendazole as a reference standard. Three different concentrations (50, 75 and 100mg/ml) of each 20 ml were stacked and six earthworms (same size) were set in it. Both the test and standard solutions were freshly prepared, then earthworms were kept in both test and standard solution to record paralysis time when no waving of worms observed, unless shaken vigorously.

Death time was noted when worms were not moved after replacement of hot water [11].

## 2.2.6 Cytotoxic activity

### 2.2.6.1 Cell culture

A-549 and MCF-7 cells were sub-cultured in-house in CMCR, VIPER Narsapur. The source of the cell line is ATCC. Selected cells were cultured in corning flasks of 75 cm<sup>2</sup> filled with DMEM. DMEM prepared with 10% fetal bovine serum, 1% non-essential amino acids, 1% Penicillin (1000U/mL), 1% Streptomycin (1000µg/mL) and 1% Amphotericin (250 U/mL). Cells were maintained at 37°C with a humidified atmosphere of 5% CO<sub>2</sub>. 0.25% trypsin - EDTA 1mM was used to passage(30–50) the cells enzymatically in 75 cm<sup>2</sup> corning flasks so as to give 2.2x10<sup>4</sup>cells/cm<sup>2</sup>. Fresh culture medium was supplemented every 2 days. The microbial observance was made periodically to conform 80 % of Cell confluence. After seeding of the cells these were treated with culture medium about 12 hr to prevent cell differentiation.

### 2.2.6.2 Subculturing protocol (volumes used in this protocol are for 75 cm<sup>2</sup> flask)

The culture medium was withdrawn carefully from the Corning flasks. Cell layers were rinsed thoroughly with 0.25% (w/v) Trypsin-EDTA solution to remove trypsin inhibitor impurity which might present in serum. Later, these cells were mixed with 2 to 3 ml of Trypsin-EDTA solution was added to observe the uniform distribution of cells under an inverted microscope usually cells will be settled uniformly within 5 to 15 min. To this 6 to 8 ml of Growth medium was incorporated. Aliquots of cell suspensions were made in new culture flasks to produce the concentrations between 2 x 10<sup>3</sup> and 1 x 10<sup>4</sup> viable cells/cm<sup>2</sup>. Care was taken to do not exceed the cell count than 7 x10<sup>4</sup> cells/cm<sup>2</sup>. Cultured flasks were maintained at 37°C with the concentrations between 6 x 10<sup>3</sup> and 6 x 10<sup>4</sup> cell/cm<sup>2</sup>. 1:3 to 1:8 was followed for sub cultivation process. The culture medium was restored thrice a week with fresh DMEM [12,13].

### 2.2.6.3 Screening of test samples against morphology of A-549 Cells and MCF-7 Cells

A-549 and MCF-7 Cells were treated with 100 and 250 µg/ml concentration of each sample namely M2 and M3 for morphological study.

Cells were observed for 24, 48, 72 hrs, after treatment of test samples. 10X Axiovert200M phase contrast microscope with Rel. 4.2 software was used to obtain the images.

### 2.2.6.4 Procedure for Cytotoxic concentration (IC<sub>50</sub>) of M2 and M3 samples against A-549 and MCF-7 cells Using MTT Assay

A-549 and MCF-7 cells were plated and cultured (100 µL per well) in a clear bottom 96- well tissue culture plates. (The number of cells was 10<sup>5</sup> cells per well). 5, 10 and 25 µg/ml concentrated Cisplatin solutions was used as standard to measure IC<sub>50</sub> values. The same protocol was used to measure the IC<sub>50</sub> values of the test samples (M2 against both the cell lines and M3 against A-549 only) at concentrations ranging from 25 to 300 µg/ml (25, 50, 100, 150, 200 and 300µg/ml) in triplicate. After 24 hr seeding, incubated the cells for 24, 48 and 72 h period. A volume of 20 µL culture medium was used for all test samples. Then removed the medium and washed cells with PBS twice. Added 15 µL of MTT reagent per well which contained PBS medium to a final concentration of 0.5 mg/mL. Cells were brood for 3 hr at 37°C until intracellular purple formazan crystals were visible under a microscope. Subsequently, MTT reagent was removed then 100 µL of DMSO was added to each well, mixed gently on an orbital shaker for one hour at room temperature. Absorbance was measured to record the optical density at 570 nm for each well on an absorbance plate reader. The results obtained for all three experimental values were expressed as SD±3 [14,15].

## 3. RESULTS AND DISCUSSION

Phytochemical tests revealed that all three extracts contained various secondary metabolites. The chloroform and ethyl acetate extracts mainly contained higher amounts of terpenoids, Alkaloids, Steroids, flavonoids and carbohydrates, saponins. Inorganic esters like phosphates and sulphates were identified in all three extracts, whereas the acetone extract contained the above constituents at lower amounts as shown in Table 1.

### 3.1 HPTLC

HPTLC analysis was carried on three different *Croton scabiosus* Bedd. extracts to identify specific therapeutically active principles of acetone (M1), chloroform (M2), ethyl acetate (M3) at two different wavelengths (254 and 366

nm) before and after derivatization with a UV detector. Various phytoconstituents identified by HPTLC analysis were represented in Table 2. The major Phytoconstituents identified were Astaxanthin, Colchicine, Reserpine and their  $R_f$  values were 0.35, 0.29 and 0.48 respectively upon comparison with standard data as depicted in Fig. 1.

### 3.2 GC-MS

Gas chromatography-Mass spectrometry analysis of acetone (M1), chloroform (M2) and ethyl acetate (M3) extracts of *Croton scabiosus* Bedd. exhibited different peaks and each peak represented principle phyto component of the specific extract. Steroids, fatty acids, terpenes,

isoquinoline alkaloids, essential oils etc., were identified as secondary metabolites of *Croton scabiosus* Bedd. hence they were matched with mass spectra of NIST library. These phytochemicals may accord to the various medicinal activities like anthelmintic, anti-cancer, anti-inflammatory, anti-oxidant etc. The phytoconstituents identified in all the bark extracts of *Croton scabiosus* Bedd. Along with their retention times were represented in Table 3. From GC-MS analysis the major phytoconstituents which possess anticancer activity were detected as Astaxanthin, Colchicine, Reserpine with their retention times 22.47, 24.48, 24.58 min respectively as exhibited in Fig. 2.

**Table 1. Qualitative analysis on phytochemical constituents**

Chemical test	<i>Croton scabiosus</i> Bedd. bark extract		
	Acetone (M1)	Chloroform (M2)	Ethyl acetate (M3)
Mayer's test	+++	++	+++
Dragendorff's test	+++	++	+++
Wagner's test	+++	++	+++
Hager's test	+++	++	+++
Tannic acid test	+++	+	+++
Legal's test	+	++	+++
Froth test	-	+++	+++
Hemolysis test	+	++	++
Gelatin test	+	+	+
Ferric chloride test	+++	+++	+++
Alkaline reagent test	+++	+++	+++
Shinoda test	+	++	+++
Zinc-Hydrochloride reduction test	+	++	+++
Alkaline reagent test	+	+++	+++
Millons test	++	-	++
Ninhydrin test	+	-	+
Molisch's test	++	++	++
Benedicts test	++	++	++
Fehlings test	++	++	++
Terpenoids test	+++	++	+++
Phosphates test	++	++	++
Sulphates test	++	++	++

+++ : High presence of phytochemical constituents; ++: Moderate presence of phytochemical constituents; + : Low presence of phytochemical constituents; - : Absence of phytochemical constituents

**Table 2. Compounds identified by HPTLC**

Extracts	Assigned compounds
M1	a-Tocopherol, Astaxanthin, Chlorogenic acid, Gentisic acid.
M2	Flavanoids (Kaempferol), Saponins, Alkaloids, Apigenin, Quercetin, Vit-C, Astaxanthin, Colchicine, Reserpine
M3	Flavanoids (Kaempferol), Terpene, Saponins, Quercetin, Phenolic compounds, colchicines

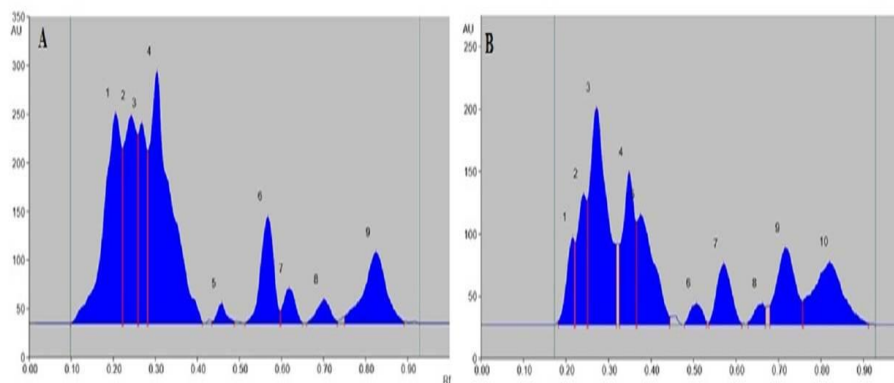


Fig. 1. Typical HPTLC chromatograph of M2 extract at A. 254 nm B. 366 nm

Table 3. Compounds identified by GC-MS

Compound name	Retention time (RT in min)
9,12,15-octadecatrienoic acid	20.04
stannane 1,4 phenylenebis (trimethyl)	22.42
Astaxanthin	22.47
Sa-pregn-16-en-20-one	22.78
3a,12a-dihydroxy acetate	22.78
Cholestan-3-one,cyclic 1,2 –ethanediylaceta,(5a)	22.78
Molybdenum	23.70
Colchicine	24.48
Reserpine	24.88
Alfa-copapene (essential oil)	9.26
Alfa-ylangene (sesqueterpene,essential oil, flavour usage)	9.26
Copaene	9.26
Dodecanoic acid	10.65
Azulene (terpene)	12.92
1-(+) –ascorbic acid 2,6 dihexadecanoate	17.98
Geranyl	21.35
Retinol	24.16
Ingol 12 acetate	19.11

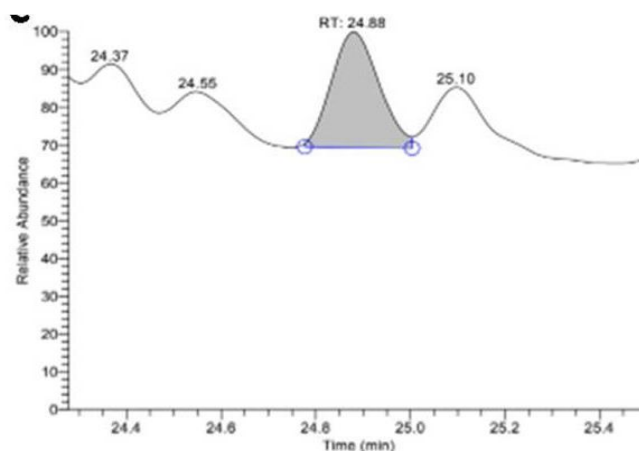


Fig. 2. Typical GC-MS chromatograph of M2 extract representing various phyto active constituent peaks

### 3.3 Anthelmintic Activity

The results shown in Table 4 reveal that the acetone, chloroform and ethyl acetate extracts of *Croton scabiosus* Bedd. Showed compelling anthelmintic activity against earthworms in a dose-dependent manner tested in three different concentrations and it is compared with standard Albendazole drug. Among all the three extracts, ethyl acetate extract showed potent anthelmintic activity compared with other extracts.

### 3.4 Results for Morphological Study

Morphological study for the two extracts of *Croton scabiosus* Bedd. i.e., chloroform and ethyl acetate was performed on A-549 and MCF-7 cell lines. M2 has shown retardation of cellular growth against both A-549 and MCF-7 cells. M3 has shown retardation of cellular growth against A-549 only but not against MCF-7. M2 and M3

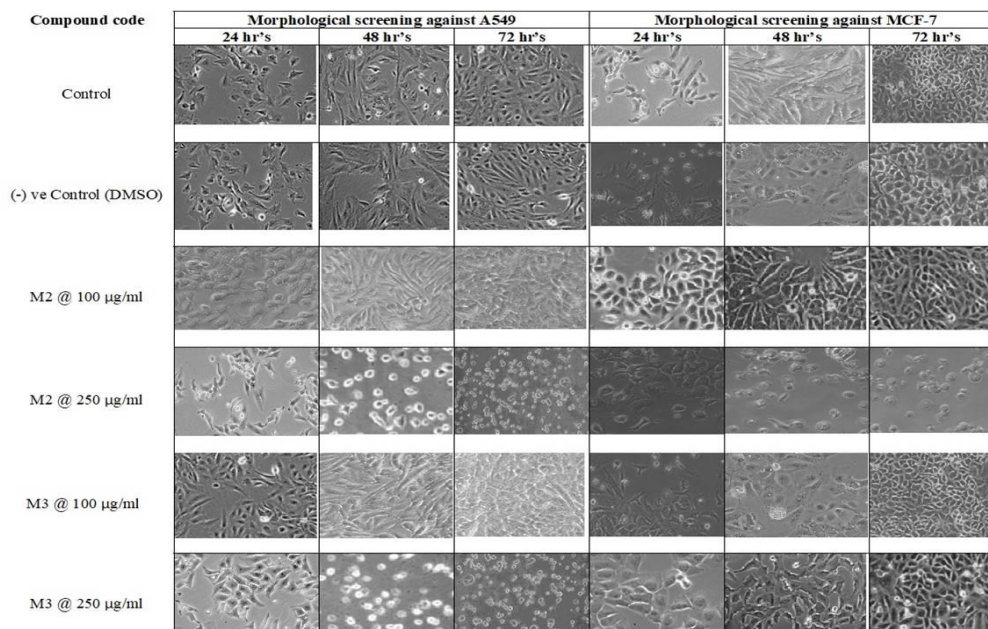
extracts at 100 and 250 µg/ml, has shown significant activity against lung adenocarcinoma cell line (A549) and breast adenocarcinoma cells (MCF-7). M2 has shown potent anti-cancer activity against both A549 and MCF-7 cells but M3 has shown anti-cancer activity only against A-549 cells as exhibited in Fig. 3.

### 3.5 Results for Cytotoxic Assay

Cisplatin had shown cytotoxic activity against both A-549 and MCF-7 cancer cell lines at 10 µg/ml concentration as IC<sub>50</sub>. The chloroform and ethyl acetate extracts of *Croton scabiosus* Bedd. were subjected to cytotoxic assay by MTT assay method against A-549 and MCF-7 cancer cell lines and the research observations were represented in Table 5 and Fig. 4. Upon increase in concentrations from 25µg/ml to 300 µg/ml significant reduction in cytotoxic cells were observed.

**Table 4. Anthelmintic activity**

Parameter	Standard albendazole solution	Acetone extract (M1)			Chloroform extract (M2)			Ethyl acetate extract (M3)		
Concentration (mg/ml)	10	100	75	50	100	75	50	100	75	50
Death Time (min)	22	100	260	290	80	250	275	35	60	110

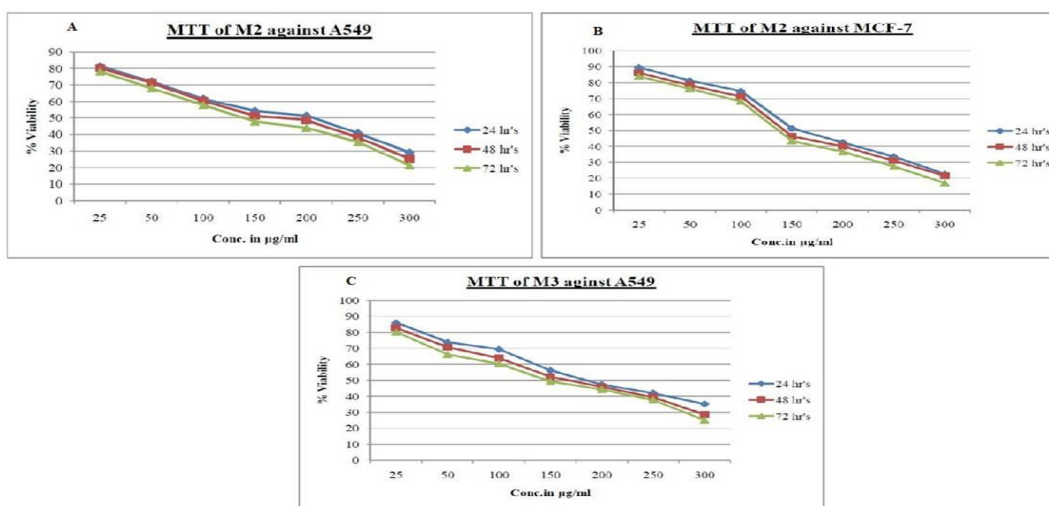


**Fig. 3. Screening of M2 and M3 extract against morphological behaviour of A-549 and MCF-7 cell line lines**

**Table 5. Cytotoxic activity**

Compound name or code	IC <sub>50</sub> in µg/ml at 24 hr*	IC <sub>50</sub> in µg/ml at 48 hr*	IC <sub>50</sub> in µg/ml at 72 hr*
M2 against A549	187.33 ± 1.27	173.65 ± 1.21	155.53 ± 1.7
M2 against MCF-7	179.94 ± 1.35	168.40 ± 1.01	155.33 ± 1.26
M3 against A549	201.89 ± 1.34	179.81 ± 1.5	164.10 ± 1.1

\*All values were expressed as SD±3



**Fig. 4. MTT activity of M2 against A. A-549 cell line B. MCF-7 cell line C. M3 against A-549 cell line**

**4. CONCLUSION**

*Croton scabiosus* Bedd. bark extracts contained Alkaloids, Tannins, Flavonoids, Saponins, Steroids, Vit-A and C as the secondary metabolites and prime biologically active principle constituents. HPTLC and GC-MS analysis led to the identification of Astaxanthin, Colchicine, Reserpine as the responsible principles to demonstrate cytotoxic activity on Lung and Breast cancer cells. It has been found to exhibit considerable anthelmintic activity may due to the presence of bitter principles viz saponins and tannins. Cytotoxic potential and anticancer activity against Lung adenocarcinoma cell line (A549) and Breast adenocarcinoma cells (MCF-7) of M2 extract might be due to the presence of alkaloids like Colchicine, Reserpine and an antioxidant terpenoid like Astaxanthin. Due to absence of Astaxanthin and Reserpine Phytocompunds in M3 extract, it might be active only against Lung cancers (Colchicine), Presence of Astaxanthin and other constituents at lower amounts could not exhibit anticancer activity by M1 extract. It reveals that alkaloids,

flavonoids and terpenes of *Croton scabiosus* Bedd. contribute remarkable biological activities. Hence the work can be continued to explore the anticancer mechanism by DNA fragmentation method. Further Pharmacokinetic studies and targeted nanophytosomal drug delivery systems to treat Lung and Breast cancers can be developed with the identified therapeutic constituents. The above-mentioned research findings demonstrate the necessity of conservation of *Croton scabiosus* Bedd. owing to its medicinal value and threatened status. Further research can be extended to prove the scientific evidence for other traditional practices to treat a snake bite, scorpion sting poisoning and leucorrhoea with *Croton scabiosus* Bedd. decoctions or extracts.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

It is not applicable.



## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Martins Ekor. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2013;4:177.
2. Freddie Bray, Jacques Ferlay ME, Isabelle Soerjomataram MD, Rebecca L Siegel. *Global Cancer Statistics: Globocan Estimates of Incidence and Mortality worldwide for 36 Cancers in 185 Countries.* *CA Cancer J Clin.* 2018; 68:394-424.
3. Shu-Yi Yin, Wen-Chi Wei, Feng-Yin Jian, Ning-Sun Yang. Therapeutic applications of Herbal Medicines for Cancer Patients. *J Evi Based Complement Altern Med.* 2013; 1-15. Available: <http://dx.doi.org/10.1155/2013/302426>.
4. Salamma S, Ravi Prasad Rao B. In vitro propagation of *croton scabiosus* Bedd. (Euphorbiaceae), an endemic and vulnerable tree species. *J Advbiotechnol.* 2014;3(3):13.
5. Venkataratnam K, Venkataraju R. Folk medicine used for common women allignments by Adivasis in the eastern ghats of Andhra Pradesh Indian J tradit know. 2005;4(3):267-270.
6. Padma Y, Sarojinidevi N, VenkaRatnam G, Tirupati Reddy. Herbal/Folk remedies used for Snake bites by tribals/rural people of Rayalaseema region, Andhra Pradesh, India. *J Med Plants Stud.* 2016;4(2):52-56.
7. Peter Masoko. Phytochemical Analysis, Antioxidant and Antibacterial Properties of *Spilanthes mauritiana* used traditionally in Limpopo Province, South Africa. *J Evi Based Complement Altern Med.* 2017; 22(4):936–943.
8. Sarojini Devi N, Mani J, Venkata Raju RR. In vitro antimicrobial activity and phytochemical constituents of *Croton scabiosus* Bedd. *Int J Pharm Res Dev.* 2011;3(6):149-154.
9. Raman Preet, Raghbir Chand Gupta. HPTLC Analysis of *Solanumx anthocarpum Schrad. And Wendl.,* A Siddha Medicinal Herb. *Adv Pharmacol Sci.* 2018;1-6. Available: <https://doi.org/10.1155/2018/8546306>
10. Jitendra Patel, Venkateshwar Reddy, Kumar GS, Satyasai. Gas chromatography and mass spectroscopy analysis of bioactive components on the leaf extract of *Terminaliacoriacea*: A potential folklore medicinal plant. *Int J Green Pharm.* 2017;11(1): S140.
11. Das SS, Monalisha Dey, Ghosh AK. Determination of Anthelmintic Activity of the Leaf and Bark Extract of *Tamarindus indica* Linn. *Indian J Pharm Sci.* 2011; 73(1):104-107.
12. Sangeetha Arullappan, Shamsul Muhamad, Zubaidah Zakaria. Cytotoxic Activity of the Leaf and Stem Extracts of *Hibiscus Rosa sinensis* (Malvaceae) against Leukaemic Cell Line (K-562). *Trop J Pharm Res.* 2013;12(5):743-746.
13. Alioune Dior Fall, Victor Patrick Bagla, Emmanuel Bassene, Jacobus Nicolaas Eloff. Phytochemical screening, antimicrobial and cytotoxicity studies of ethanol leaf extract of *Aphania senegalensis (sapindaceae)*. *Afr J Tradit Complement Altern Med.* 2017; 14(4):135-139.
14. Khushbu N, Thakkar A, Prasad K, JyotiranjanNayak. Antioxidant and *in vitro* cytotoxic activity of extracts of aerial parts of *Cocculus hirsutus* (L) using cell line cultures (breast cell line). *The J Phyto Pharmacol.* 2014;3(6):395-399.
15. Tanzania Ramadhani S, Nondo O, Mainen J. Evaluation of the cytotoxic activity of extracts from medicinal plants used for the treatment of malaria in Kagera and Lindi regions. *J Appl Pharm Sci.* 2015;5(04):07-12.

© 2021 Bhargav et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:  
<http://www.sdiarticle4.com/review-history/65034>