



## Extraction, qualitative determination of phytochemicals and anticancer activity of *Plumbago zeylanica*

Shrishail, Yogashree G D, Madhura S, Rakshitha

Department of PG Studies and Research in Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka, India

### Abstract

*Plumbago zeylanica* is a species of the genus *Plumbago* in the family *Plumbaginaceae*. Different parts of this plant are used in traditional practice of medicine. The objective of the study was to analyse the phytochemical constituents of *P. zeylanica* root and evaluation of anticancer activity. Three different extracts petroleum ether, chloroform and methanolic extracts of *P. zeylanica* roots were prepared and phytochemical screenings were carried out. In the extracts, compounds like alkaloids, flavonoids, phenolic compounds, saponins, tannins and terpenoids are detected by phytochemical analysis. Based on the results of phytochemical assays, methanolic extract was selected for evaluation of its anticancer activity using HeLa and A375 cell lines. The present study showed that *P. zeylanica* root contains bioactive compounds that are known to possess anticancer activity and the methanolic extract caused decline in cell viability as the concentration of extract increased.

**Keywords:** qualitative analysis, alkaloids, plumbago, HeLa and a375, methanol extract

### Introduction

The *Plumbago zeylanica* is a plant species, which is distributed throughout tropical and subtropical nations of the flora and fauna as a weed. It belongs to the family *Plumbaginaceae* include 10 genera and 280 species. The *Plumbago* genus consists of 3 species i.e. *P. indica* L., *P. capensis* L., and *P. zeylanica* L. these are distributed in several portions of India (Rana 2011) [1]. In India the plant widely distributed from central India to west Bengal, Maharashtra and several parts of southern India. This plant is native to south Asia and grows in deciduous woodland, savannas and scrublands from sea level up to 2000 m altitude (Manu *et al.*, 2012).

The works exposes its extensive application in traditional remedies against several sicknesses, as anti-inflammatory, anti-malarial, anti-microbial, anti-oxidant, blood coagulation, wound healing, memory enhancer and anti-cancer. The beneficial bioactive compounds extracted in this plant such elliptinone, zeylanone, sistosterol and plumbagin (Paras *et al.*, 2013).

At present, Investigators mainly focus on modern herbal prescription comprising expected composites, which reduce the oxidative cells damage. Prospective combined effect of these natural composites that increases the resistance appliance of body against the abnormal cells development and also decreases harmfulness of the drug with less side effects (Krishnan *et al.*, 2017) [4].

Cancer is one of the most life threatening diseases and possess many health hazard in both developed and developing countries. As various types of cancers like breast cancer, colon cancer, leukemia etc. and management strategies for their curative aspect mainly are chemotherapy, radiation and surgery. so, to overcome the side effects which are caused by these treatments we should go with the adjuvant therapy that can be achieved by using herbal drugs. As so many herbal drugs are mentioned in Ayurveda for the treatment of cancer. With this preview in this study, the anticancer activity on HeLa cell line taken from cervical cancer cells, and A375 cell line taken from melanoma (skin cancer) cells has been investigated using *Plumbago zeylanica* root extracts.

### Materials and Methods

#### Study area

Shivamogga district of Karnataka state is situated in the heart of the Western Ghats region, which is one of the hot-spots of bio diversity in India. The study area, Bhadravathi, is one of the taluks of Shivamogga district, situated in the midst of the Western Ghats region. Bhadravathi taluk is situated between 13<sup>o</sup>42' and 14<sup>o</sup> 06' N latitude and between 75<sup>o</sup> 35' and 75<sup>o</sup> 52' E longitude in about the mid southwestern part of the Karnataka state at an altitude of 594m from the sea level. The map of study area is shown in image 1.



**Fig 1:** Mapping of sample area

### Collection of plant material

The plant sample of *Plumbago zeylanica* L. were collected from Bhadravathi in Shivamogga District, Karnataka, in the month of December. The shade dried plant material was grinded into a coarse powder using mixer grinder. The powdered plant samples were stored in an air tight polythene cover and used for successive solvent extraction.

### Preparation of the extract

The powdered plant materials were subjected to Soxhlet extraction using petroleum ether, chloroform, methanol, distilled water successively in the increasing order of polarity. Each extraction was carried out for 24 hours (approximately 40 cycles). The extracts were concentrated to dryness by normal air dry then extracts were stored in clean storage vials at 4° c till further analysis.

### Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant *Plumbago zeylanica* was carried out using the standard procedures. (Harborne 1998, parasuraman *et al.*, 2010) [5, 6]

### Anticancer activity

Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours. Add appropriate concentrations of the test agent 50, 100, 150, 200, 250 & 300µg/ml. Incubate the plate for 24hrs at 37°C in a 5% CO<sub>2</sub> atmosphere. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume. Wrap the plate with aluminum foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.) Remove the MTT reagent and then add 100 µl of solubilization solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference

wavelength. The IC<sub>50</sub> value was determined by using linear regression equation i.e.

$$Y = Mx + C.$$

Here, Y = 50, M and C values were derived from the viability graph.

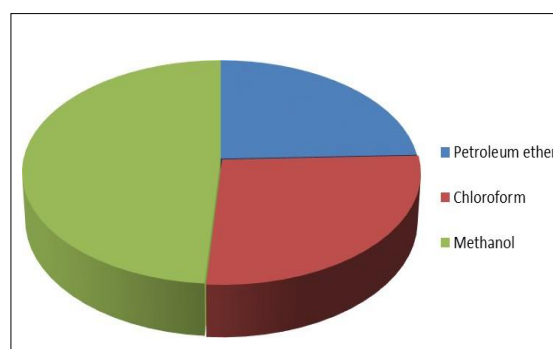
### Result and Discussion

The yield of the plant extract obtained by extracting 30g of plant material by different solvents shown in Table 1.

**Table 1:** Percentage of yield of *P. zeylanica* plant extracts by Soxhlet extraction

Plant sample	Solvent used	% Yield of extracts
<i>Plumbago zeylanica</i>	PETROLEUM ETHER	32%
	CHLOROFORM	35%
	METHANOL	64%

Among the two solvents used methanol was found to be the best solvent generating highest yield (64%) than chloroform (35%) and Petroleum ether (32%). The high efficiency of methanol concentration be attributed to its high polarity leading to the extraction of polar and non-polar compounds. Percentage yield of extracts are plotted and graph is shown in image 2.



**Fig 2:** Graph shows the % yield of extracts of *Plumbago zeylanica*.

### Phytochemical analysis

Preliminary screening of phytochemicals is a valuable steps, in the detection of bioactive principles present in medicinal

plants and subsequently may lead to drug discovery and development. These are naturally present in plants to defend themselves against various pathogenic microbes by showing the microbial activity by inhibition or killing mechanism.

Preliminary phytochemical analysis of *Plumbago zeylanica* methanol extract (PME), *Plumbago zeylanica* chloroform extract (PCE), Petroleum ether extract (PPE) reveals the presence or absence of secondary metabolites.

The preliminary phytochemical analysis of petroleum ether extract shows the presence of phytochemical substances such as steroid, whereas absence of alkaloids, carbohydrates, flavonoids, saponins, tannin, terpenoids. The chloroform extract shows the presence of phytochemical substances such as carbohydrates, steroids. Whereas absence of alkaloids, flavonoids, saponins, tannin, terpenoids. The methanol extract shows the presence of alkaloids, carbohydrates, flavonoids, steroids and tannin. Whereas absence of saponins and terpenoids. The details of qualitative analysis of root extracts of *Plumbago zeylanica* is given in Table 2.

**Table 2:** Preliminary phytochemical analysis of secondary metabolites of *Plumbago zeylanica*

Sl. no	Secondary metabolites	Plant extract		
		Petroleum ether	Chloroform	Methanol
1.	Alkaloids	-	-	+
2.	Carbohydrate	-	+	+
3.	Flavonoids	-	-	+
4.	Steroids	+	+	+
5.	Saponins	-	-	-
6.	Tannin	-	-	+
7.	Triterpenoids	-	+	-

**Note:** ‘-’ indicates the absence of constituents, presence of constituents: ‘+’

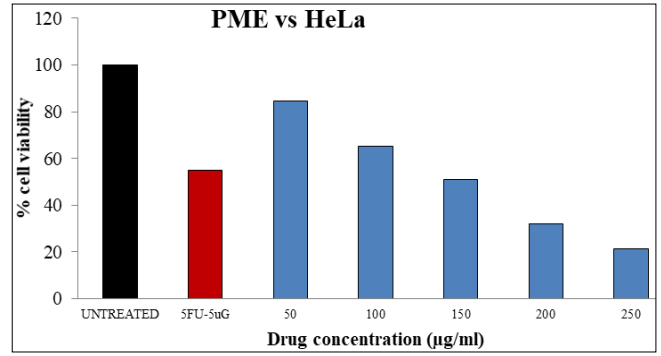
**Anticancer activity**

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm (Alley, M. C *et al.*, 1986, Mosmann *et al.*, 1983) [8]. Test compounds are evaluated to analyses the cytotoxicity effect on HeLa and A375 cell lines. The concentrations of the test compound used to treat the cells are depicted in Table 3:

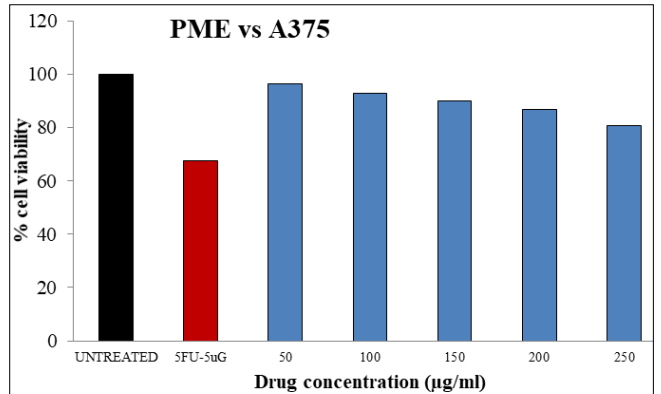
**Table 3:** Details of test compounds concentrations

Sl. no	Test compounds	Cell line	Concentration treated to cells
1	Untreated	HeLa and A375	No treatment
2	Standard (C)	HeLa and A375	5µg/ml
3	Blank	-	Only Media without cells
4	PME	HeLa and A375	50, 100, 150, 200, 250 & 300µg/ml

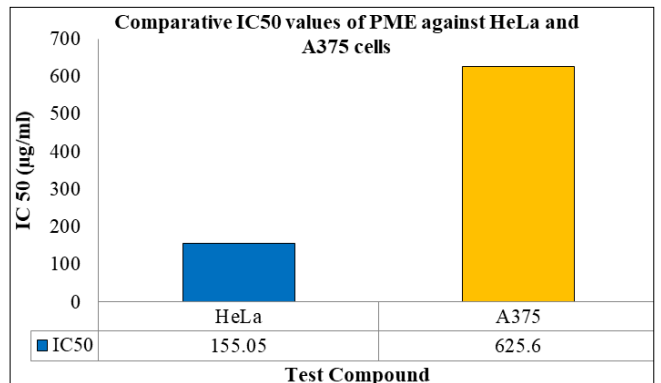
The Graph representing the comparative IC50 values of PME against HeLa and A375 cells are as shown in image 5. The percentage viability of both cell lines against different drug concentrations is as shown in image 1 and image 2.



**Fig 3:** The percentage viability of HeLa cell lines against different drug concentrations



**Fig 4:** The percentage viability of A375 cell lines against different drug concentrations



**Fig 5:** Graph represents the comparative IC50 values of PME against HeLa and A375 cells.

**Table 4:** IC 50 values of root methanol extract of *Plumbago zeylanica*

Sl. no	Cell line	IC 50 (µg/ml)
1	HeLa	155.05
2	A375	625.60

The results of cytotoxicity study (MTT assay) suggested that test compound PME showed significant cytotoxicity on HeLa cells with IC50 value at 155.05µg/ml respectively. On A375cells PME showing moderate toxicity with higher IC50 value than HeLa cells. The absorbance readings with calculations are enclosed in MS excel format.

**Conclusion**

Based on the result of this study compounds present in the root extracts of *Plumbago zeylanica* shows the presence of

good anticancer agents to reduce the cancer and to confirm its safety profile further to be designed in to new drugs having better safety and efficacy.

### Acknowledgement

The authors are thankful to the vice chancellor, Prof BP Veerabhadrappe and Registrar Mrs Anuradha G of Kuvempu university for providing Minor project (start up research grant). The authors are thankful to the Chairman Department of PG studies and research in Botany, Kuvempu university, Jnanasahyadri, Shankaraghatta, Shivamogga for providing facilities to conduct experimental work.

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