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Evaluation of anticancer activity using leaf extract of *Simarouba glauca* on leukemic cancer cell lines

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Abstract
The present study was designed to evaluate anticancer activity of crude extract of *Simarouba glauca* in three cancer cell lines viz., K-562, MOLT-3 and KG-1. Different concentrations (2 to 64 µg/ml) of leaves extract were tested for cytotoxicity using MTT assay. Methanolic extract of *Simarouba glauca* leaves showed significant anticancer activity against MOLT-3 and K-562 as compare to KG-1 cell line. Concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. IC_{50} values of *Simarouba glauca* methanolic leaf extract with respect to K-562, MOLT-3 and KG-1 were found to be 74.21, 69.69 and 131.1µg/ml respectively. The methanolic leaf extract of *Simarouba glauca* exhibit effective activity against leukemic cancer cell lines. Leaf of *Simarouba glauca* can be recommended for cancer treatment especially for leukemic cancers.

Keywords: *Simarouba glauca*, MTT assay, cytotoxicity, K-562, MOLT-3, KG-1, cell viability

1. Introduction
In human population, cancer is one of the threatening diseases, which is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body and characterised by cells in the human body continually multiplying with the inability to be controlled or stopped [1, 2]. In modern treatment, serious side effects occur due to chemotherapy, surgical and radiations. Nowadays, pharmacological research is focused on Indian traditional system of medicine, which are natural and safe. Drugs from plants is the alternative form of medicine which has less toxicity and shows less side effects to patients [3]. Phytochemicals from medicinal plants is a common alternative for cancer treatment in many countries around the world [4, 5]. The phytochemicals such as alkaloids, flavonoids and others are used in the treatment of various cancers [6]. Approximately 60% of drugs used for cancer treatment have been isolated from natural products currently [7].

Simarouba glauca belongs to family Simaroubaceae, commonly known as "The Paradise Tree" or "King Oil Seed Tree" or "Laxmitaru Tree", is a versatile multipurpose evergreen tree having a height of 7-15 m with tap root system. It is a poly-gamo-dioecious tree and a potential source of biodiesel [8]. This plant is well known for its different types medicinal and pharmacological properties. The bark and leaf extract of *S. glauca* is well known for its different types of pharmacological properties such as haemostatic, antihelmentic, antiparasitic, antidysenteric, antipyretic and anticancerous [9]. The leaf, fruit, pulp and seed of *S. glauca* are known to possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic and vermifuge [10].

According to Rivero-Cruz *et al.* (2005), four alkaloids derivatives (canthin-6-one) isolated from *Simarouba glauca* shows cytotoxic activity against human colon cancer, human oral epidermoid cancer, human hormone-dependent prostate cancer and human lung cancer cells [11]. Furthermore, Jiang and Zhou (2008) also demonstrated that the canthin-6-one alkaloids isolated from *Picrasma quassioides* show cytotoxic activity against nasopharynx carcinoma cells [12]. Some quassinoids isolated from seed of *S. glauca* gives invitro cytotoxic activity against KB cells (human oral epidermoid carcinoma), including glaucarubin, glaucarubinone, glaucarubol and glaucarubolone [13, 14]. One more study demonstrated that glaucarubinone is a phytochemical present in *S. glauca* is active against solid tumors (human and mouse cell lines), multi-drug-resistant mammary tumors in mice, and antileukemic activity against leukemia in mice [15]. The chloroform soluble extract of *S. glauca* shows good anticancerous activity against several human cancer cell lines [16, 17].

In present study, *in vitro* antioxidant potential was analyzed by DPPH assay and anticancer activity was evaluated on three leukemic cell lines viz. K-562, MOLT-3 and KG-1 by MTT assay from leaf extract of *Simarouba glauca*.

2. Materials and methods
2.1 Collection and extraction of plant sample
2.1.1 Plant collection and identification
Fresh leaves of *Simarouba glauca* were collected from Sardarkrushinagar Dantiwada Agricultural University, Dantiwada, Banaskantha, Gujarat, India, in March 2015. The plant sample was identified and authenticate by Research Scientist, Dr. R. R. Shakhela, Centre for Agroforestry forage crops & Green belts, Sardarkrushinagar Dantiwada Agricultural University, Dantiwada, Banaskantha, Gujarat, India. A specimen representing leaves of *Simarouba glauca* has been deposited in the herbarium of the department. The leaves of *Simarouba glauca* were separated, washed under the running tap water and dried at 45˚C in the oven. The dried

leaves were then homogenized to fine powder and stored in air tight container for further use.

2.1.2 Preparation of leaf extract of *Simarouba glauca*

For extraction, approximately 5 g of dried leaves powder were taken in 50 ml different solvents (chloroform, petroleum ether, ethyl acetate, *n*-butanol, methanol and aqueous) based on increasing polarity and kept under gentle and continuous shaking on an orbital shaker (Orbital Shaking Incubator - REMI) for 24 h. The suspensions so obtained was filtered using Whatman No.1 papers to obtain the crude extracts. The resulting extracts were concentrated in vacuum at 40°C using a rotary evaporator and freeze drying. The dry weight of the leaves extracts were obtained by the solvent evaporation and used to determine concentration. Dry extracts were then kept in sterile bottles, under refrigerated conditions, until further use.

The % yield was calculated for each dry extract using the following equation:

% yield of dry extract = Mass of extract (g)/Mass of plant materials (g) x 100

2.2 *In vitro* Antioxidant Assay (2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay)

The antioxidant activity of the methanolic leaf extract of *Simarouba glauca* was evaluated by DPPH free radical assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was done using the reported Yamaguchi and Takamura method [18]. The reaction mixture containing 1 mL of DPPH solution (0.1 mmol /L in 95% ethanol v/v) with different concentrations of leaf extract was shaken and incubated for 30 min at room temperature and the absorbance was read at 517 nm UV-Visible Spectrophotometer against a blank. Blank was prepared with the addition of DPPH and for control 0.2 ml of methanol (without plant extract) was added. Ascorbic acid was used as standard. The radical scavenging activity was measured as a decrease in the absorbance of DPPH. Percentage of DPPH scavenging activity determined as follows:-

$$\% \text{ DPPH radical-scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample})}{\text{Absorbance of control}} \times 100$$

2.3 *In vitro* assay for Cytotoxicity studies

2.3.1 Cell lines and culture

Three different leukemic cancer cell lines (Table 1) were selected for anticancer study of leaf extract of *Simarouba glauca*. All cell lines were purchased from the NCCS (National Centre for Cell Science) Pune.

Table 1: Cell lines used for anticancer study

Common name	Generic name
Human chronic myelogenous leukemia cells	K-562
Human acute lymphoblastic leukemia cells	MOLT-3
Human acute myeloid leukemia cells	KG-1

All cell lines were cultured in tissue culture flasks with supplemented advanced DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) and incubated at 37° C with 5% CO_2.

2.3.2 MTT cell viability assay

The anticancer study of leaf extract of *Simarouba glauca* against three leukemic cell lines viz., K-562, MOLT-3 and KG-1 were determined by the MTT assay [23-26]. Cells were seeded in 96-well plates (1.0×10^4 to 3.5×10^4 cells/well) in 150 µL of medium and incubated at 37° C with 5 % CO_2 for 24 hr. After 80 % confluence, the cells were treated with different concentrations of leaves extract of *Simarouba glauca* (2 to 64 µg/ml) for a further 24 hr. Following 24 hr incubation, the supernatant was removed, the cells were washed with phosphate-buffered saline (PBS), pH 7.4 and a mixture of 150 µL of supplemented advanced DMEM (without serum) and 50 µL of MTT in PBS solution (5.0 mg/mL) was added to each well. Following incubation for 2 hrs at 37°C with 5 % CO_2 a purple formazan product was produced. DMSO was then added (125 µL) followed by incubating the plates for another 45 min at room temperature to dissolve the formazan. The absorbance was measured with a spectrophotometric microplate reader (Epoch Elisa reader) at a wavelength of 560 nm. The optical density was a measure of the density of live cells. The assays were performed in triplicates. The percentage of cytotoxic activity was calculated using the following formula:

% of cytotoxic activity = 100 – (O.D. of test sample/ O.D. of negative control x 100)

The negative control contains all components including solvent (0.75 % DMSO) of the test sample without test compounds. Whereas the positive control contains all components including 25 % DMSO without test compounds.

2.4 Statistical analysis

Data were analysed using GraphPad Prism 7 (Version 7.01, GraphPad Software, Inc., USA). Results are expressed as the mean±Standard deviation of three independent experiments. The data were analysed for statistical significance by multiple t- test, $P < 0.05$ was considered to be significant.

3. Results and Discussions

3.1. Extraction yields of *Simarouba glauca*

For the anticancer activity study of leaf extract of *Simarouba glauca*, the dried powder was successively extracted in chloroform, petroleum ether, ethyl acetate, *n*-butanol, methanol and aqueous. The extraction yields of *Simarouba glauca* are summarized in Table 2. On average, the lowest extraction yield was obtained with chloroform then petroleum ether, ethyl acetate, *n*-butanol, water and methanol (Table 2). It can be seen that the polar solvents (*n*-butanol, methanol and water) gave much higher % yields in the extractions than the non-polar solvents (chloroform, petroleum ether, ethyl acetate) indicating that the leaf extract of *Simarouba glauca* have a greater abundance of polar over non-polar compounds. Moreover, highest extraction yield was obtained in methanol as compared to other solvents (petroleum ether, ethyl acetate, n-butanol and water) as shown in Figure 1. Thus, methanolic extract was used for the anticancer activity of *Simarouba glauca*.

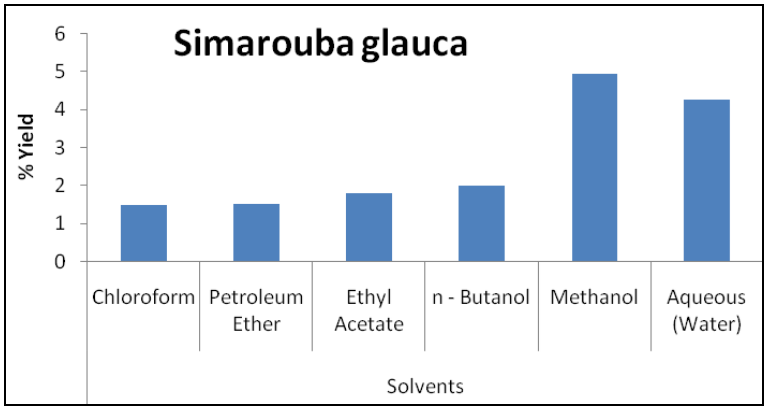

Fig 1: Graphical representation of % yield of *Simarouba glauca*

Table 2: Extraction yields of *Simarouba glauca*

Name of the Plant		Solvents					
		Chloroform	**Petroleu Ether**	**Ethyl Acetate**	**n-Butanol**	**Methanol**	**Aqueous (Water)**
Simarouba glauca	Mass of Extract (g)	0.075	0.077	0.09	0.1	0.247	1.07
	(% of yield)	1.5	1.54	1.8	2	4.94	4.28

3.2 Antioxidant property of *Simarouba glauca* against DPPH free radicals

Antioxidant potential of *Simarouba glauca* was carried out against DPPH molecules in comparisons to ascorbic acid. Leaf extracts of *Simarouba glauca* exhibited good radical scavenging capacity against DPPH having IC_{50} values 28.78 µg/ml. *Simarouba glauca* reflected dose dependence of the antioxidant potentials as there was increase in their concentration (2 to 64 µg/ml), antioxidant potential was also increased (Figure 2).

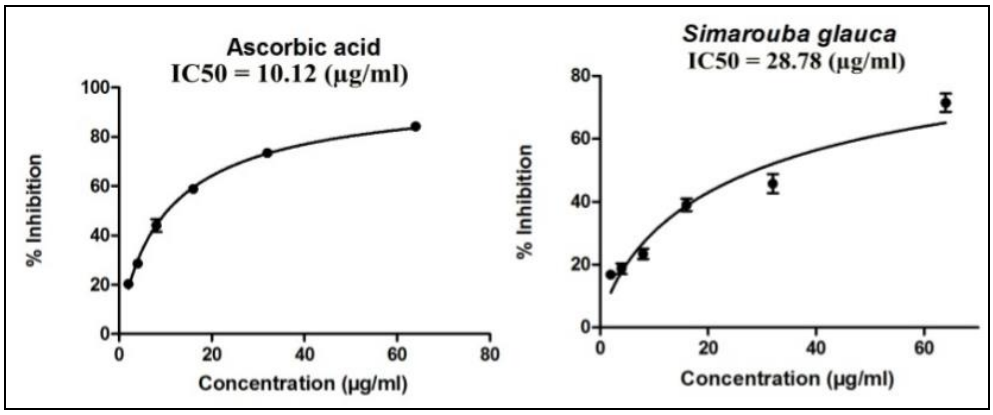

Fig 2: Antioxidant activity of ascorbic acid and different concentrations of leaf extract of *Simarouba glauca* by DPPH radicals.

3.3 Anticancer Activity by MTT assay

In the present study, anti-cancer property of leaf extract of *Simarouba glauca* was tested on three leukemic cell lines viz., K-562, MOLT-3 and KG-1. All three cell lines grow as suspension cells. MTT assay is a quantitative colorimetric assay for measuring cellular growth, cell survival and cell proliferation based on the ability of living cells. The assay was carried out using (3-(4, 5-dimethyl thiazol-2yl) - 2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity [19-22]. This assay was used to investigate the potential cytotoxic effects of *Simarouba glauca* leaf extract on three cell lines, after treatment with an increasing concentration from 2 µg/ml upto 64 µg/ml for two days incubation. The impact of the extract on cell growth is presented in Figure 3 and the cytotoxicity was recorded as IC_{50} (µg/ml) values as shown in Table 3.

Table 3: IC_{50} values of cell proliferation inhibition of *Simarouba glauca* extract

Common name	Generic name	IC_{50} (µg/ml)
Human chronic myelogenous leukemia cells	K-562	74.21
Human acute lymphoblastic leukemia cells	MOLT-3	69.69
Human acute myeloid leukemia cells	KG-1	131.1

The results showed that leaves extract of *Simarouba glauca* effectively inhibited the growth of K-562, MOLT-3 and KG-1 in a dose-dependent manner, with an IC_{50} range from 74.21, 69.69 and 131.1 µg/ml respectively. Leaves extract of *Simarouba glauca* show some inhibitory activities against all three cell lines. The inhibitory activities of *Simarouba glauca* leaves extract against MOLT-3 is highest as compared to other two cell lines (K-562 and KG-1). These data showed that *Simarouba glauca* leaf extract have the good anti-cancer effect towards MOLT-3 and K-562, however have limited activity against KG-1 cell line.

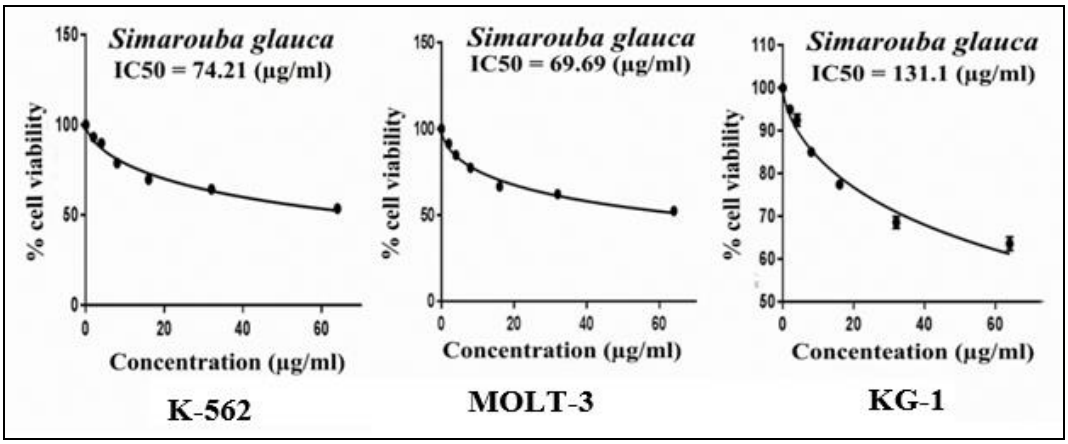

Fig 3: Graphical representation of different cells viability against different concentrations of *Simarouba glauca*

4. Conclusion

In the present study, *in vitro* anticancer activity of leaf extract of *Simarouba glauca* exhibited effective activity against three leukemic cell lines viz., K-562, MOLT-3 and KG-1. Phytochemicals present in leaves might have played a major role in anticancer activity. Hence leaves of *Simarouba glauca* can be recommended for cancer treatment especially for leukemic cancers. In pharmaceutical industries, isolation of pure compounds from leaves of *Simarouba glauca* is necessary to design the drug against cancer diseases.

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6. Conflict of interest

The authors report no conflict of interest.

7. References

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