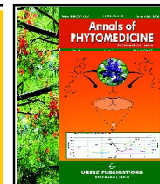
Annals of Phytomedicine 8(2): 121-126, 2019

DOI: http://dx.doi.org/10.21276/ap.2019.8.2.15

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original article

Preliminary assessment on phytochemical composition, cytotoxic and antitumor efficacy of *Simarouba glauca* DC. leaf methanolic extract

Shaji E. Mathew, Smitha K. Ramavarma, Thekkekara Devassy Babu, Balu T. Kuzhivelil* and Achuthan C. Raghavamenon◆

Department of Biochemistry, Amala Cancer Research Centre (Recognized Research Centre, University of Calicut), Amala Nagar, Thrissur-680 555, Kerala, India

**Applied Biochemistry and Biotechnology Laboratory, Department of Zoology, Christ College, Irinjalakuda, University of Calicut, Calicut-680 125, Kerala, India*

Received August 8, 2019: Revised September 30, 2019: Accepted October 5, 2019: Published online December 30, 2019

Abstract

Simarouba glauca DC. (Paradise tree) has gained much consideration in recent times in South India, as adjuvant medicine patients undertake different types of conventional cancer treatments. The current study is intended to disclose the reliability of medicinal properties of this plant. The study included the phytochemical analysis, short term cytotoxicity assessment, antiproliferative action, acute toxicity and antitumour effects of aqueous methanolic extract (ME) of *S. glauca* leaves in murine models. Preliminary phytochemical analysis of ME unravelled the presence of pharmacologically significant group of molecules like tannins, coumarins, phenols, saponins, alkaloids, flavonoids, glycosides terpenoids, and carbohydrates. Further, TLC analysis could confirm the presence of classes of compounds like flavonoids, triterpenoids, terpenoids, alkaloids and phenolics in ME. ME exhibited low cytotoxic potential towards cancer and normal cells (IC_{50}>100 µg/ml) in short term cytotoxicity assay. In antiproliferative assay cell viability were found high in all cell lines tested, *i.e.,* HepG2 (79.78 ± 0.02%), HeLa (81.42 ± 0.02%), HCT15 (89.61 ± 0.01%) and Vero (81.63 ± 0.64%)/100 µg/ml. The acute toxicity level of ME was above 2 mg/kg b. wt in female Wistar rats. In ascites tumour study, the mean survival days were found 20.17 ± 2.23 at 200 mg/kg b. wt drug administrated animals and 19.83 ± 2.64 at 400 mg/kg b. wt drug concentration in contrast with 18.17 ± 3.19 in control group. The study revealed that the ME extract of *S. glauca* did not have direct potential cytotoxicity and tumour reducing ability.

Key words: *Simarouba glauca* DC, Paradise tree, phytochemical screening, cytotoxicity and antitumour activity

1. Introduction

With more than 10 million new cases every year, cancer continues to be one of the most dreadful diseases in the world. According to World Health Organization, cancer has become the second major cause of death worldwide and became responsible for nearly 9.6 million deaths in 2018. Currently, it is estimated that in global scenario, about one in six deaths is due to cancer (WHO, 2018). This disease causes substantial challenges to the health-care systems in undeveloped and wealthy nations alike. In 2018, a total of 1.15 million new cancer cases were reported from India. According to International Agency for Research on Cancer (IARC), it is predicted that by 2040, the number of new cancer patients will be doubled. The rate of mortality due to cancer in India is also found high now (IARC, 2019). This is owing to the lack of adequate measures taken in the prevention, diagnosis and treatment of the disease.

Author for correspondence: Dr. Achuthan C. Raghavamenon
Associate Professor, Amala Cancer Research Centre, Amala Nagar, Thrissur-680555, Kerala, India
E-mail: raghav@amalaims.org; achuthanm@gmail.com
Tel.: +91-9567988570

Copyright © 2019 Ukaaz Publications. All rights reserved.
Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

WHO estimates that 80% of the world's inhabitants rely mainly on traditional medicines for health-care (Reddy *et al.*, 2016). Natural products and their derivatives have historically been exploited as a precious source of novel healing agents. Plant molecules, their semi-synthetic and synthetic derivatives are important sources of anticancer and antitumor drugs (Polu *et al.*, 2015). According to Cragg and Newman (2000), over 50% of the drugs in clinical experiments for anticancer activity are isolated from natural sources or synthetic derivatives of these.

Simarouba glauca DC. (Paradise tree) commonly called 'Laxmitaru' belonging to the family Simaroubaceae, has gained much importance due to its wide spectrum of biological properties. *S. glauca* is a medium-sized tree that grows up to 20 m high, with a trunk of maximum 80 cm in diameter. The tree produces bright green leaves 20 to 50 cm in length, white flowers, and red fruits. *S. glauca* is a native of Central and South America and found under a broad range of conditions and at different elevations from Southern Florida to Costa Rica, Jamaica, Caribbean islands, Bahamas, Cuba, Hispaniola, Puerto Rico, Mexico, Nicaragua, EI Salvador, *etc.* (Lakshmi *et al.*, 2014). This plant was brought in India by National Bureau of Plant Genetic Resources (NBPGR) to their research station at Amravati, Bangalore in 1986, (Patel *et al.*, 1997).The plant is unique, that all its

parts are useful in any way. Edible oil is produced from its seeds. At present, the cultivation of *S. glauca* extends to semiarid dry and saline land areas of Indian states like Gujarat, Tamil Nadu, Maharashtra, Karnataka and Andhra Pradesh. This plant has the potential ability to grow well even in wasteland and dry land with degraded oil (Govindaraju *et al.*, 2009).

Simaroubaceae comprises 32 genera and over 170 species (Fo *et al.*, 1992). Several constituents have been isolated from these plants mainly, alkaloids with high cytotoxicity (Jiang and Zhou, 2011) and quassinoids with prominent antifungal characteristics (Li *et al.*, 1993). Bark and leaves of Simarouba contain triterpenes, useful in curing amoebiasis, diarrhoea and malaria (Patil and Gaikwad, 2011). Quassinoids, the bitter principles of the plant family Simaroubaceae, have exhibited promising anticancer activity (Devkota *et al.*, 2014).

Paradise tree is becoming a 'tree of solace' for many cancer patients in Kerala. Several incidents on the cure of cancer of various types by the consumption of decoction of *S. glauca* have got wide publicity in recent time in this state over media. Many patients vouch that the decoction made out of the leaves of *S. glauca* can cure cancer at its primary and secondary stages and also alleviate chemotherapy-induced side effects. Because of this, people have started cultivating the plant in various states of South India, particularly in Kerala. However, there is no scientific basis are available on the magical anticancer and antitumour properties of *S. glauca*. Whether, *S.glauca* really cure cancers is an important questions. To address this question, the present study is focused on methanolic extract of *S. glauca* leaves in terms of its cytotoxic and antitumour properties.

2. Materials and Methods

2.1 Chemicals

Trypan blue, Rosewell Park Memorial Institute medium (RPMI-1640), Dulbecco's Modified Eagle's Medium (DMEM), and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemicals (St. Louis, MO, USA). Foetal bovine serum was purchased from Gibco (Grand Island, NY, USA). All other chemicals and reagents used in this study were of analytical grade and acquired from reputed Indian manufacturers.

2.2 Collection of plant samples

Leaves of *S. glauca* were collected from herbal medicine garden, maintained by Amala Ayurvedic Hospital, Thrissur and was identified by a taxonomist, Dr Sunil Kumar, Associate Professor and HOD, SNM College, Maliankara, Ernakulam, Kerala.

2.3 Preparation of extract

Mature plant leaves were used for the study. Collected leaves were washed and air-dried and powdered. Then the powder was extracted (1:10 w/v) using methanol (70%) for getting aqueous methanol extract (ME). The supernatant was collected by centrifugation (3000 rpm, 15 min), also filtered by using Whatman no. 1 filter paper and kept for evaporation in hot air oven at 40°C. The average yield was 23.15 ± 0.06%. The total residue was stored in an airtight bottle at 4°C for further studies.

2.4 Cell lines

Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites (EAC) cell lines were obtained from Amala Cancer Research Centre, Thrissur. These cells were maintained in mice by intraperitoneal inoculation. Human cervical cancer cell line (HeLa), human colorectal cancer cell line (HCT 15), human hepatocellular carcinoma cell line (HepG2) and Vero (normal kidney epithelial cells) procured from National Centre for Cell Science (NCCS), Pune were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with FBS (10% v/v), Streptomycin (100 µg/ml) and Penicillin (100 U/ml) at 37°C in an incubator with 5% CO_2.

2.5 Phytochemical analysis of extracts

The solvent-free extract obtained was dissolved in methanol and was used to test for the presence of various phytochemicals based on standard procedures of Harborne (1973), Trease and Evans (1989), Sofowara (1993) and Modi *et al.* (2018).

2.6 Animals

Female Wistar rats (130-150 g) and male Swiss albino mice (25-28 g) were purchased from Small Animal Breeding Station (SABS), Kerala Veterinary and Animal Science University, Mannuthy, Thrissur. The animals were kept under standard conditions in the animal house of Amala Cancer Research Centre (24-28°C, 60-70% relative humidity, 12 h dark/light cycle) and fed with standard rat feed bought from Sai Durga Feeds, Bangalore, India and water *ad libitum*. All the experiments in the present study were carried out with the prior permission of the Institutional Animal Ethics Committee (IACE). The experiments were conducted strictly according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted by Ministry of Environment and Forest, Government of India (Approval No. ACRC/IAEC/16-06/11).

2.7 Thin-layer chromatography of ME of *S. glauca*

Leaf extract (10 µl of 10 mg/ml) was applied on the TLC plate and developed with the solvent system chloroform: ethyl acetate: formic acid (8:2:0.2, V/V/V). The separated compounds were visualized under visible light, UV (254 nm) and iodine vapour. The Rf value was calculated for each band.

2.8 Short term *in vitro* cytotoxicity analysis

The cancer cell lines, EAC and DLA were used to test the short term toxicity of the ME of *S. glauca* and were aspirated from the peritoneal cavity of mice after 15 days of inoculation. The cells were washed with PBS and centrifuged at 1,500 rpm for 3 min for the removal of debris and the pellet was resuspended in PBS. A stock cell suspension was made by using PBS ($1x10^7$ cells/ml).

Spleenocytes from male Sprague Dawley rat were used as normal cells. The rats weighing 200-250 g were anaesthetized and spleen was excised out following dissection. The spleen was washed in PBS and kept in a sterile nylon sieve over a Petri dish, which was half-filled with PBS. The spleen was gently pressed through the sieve using a plunger. The disaggregated spleenocytes were transferred to a test tube and centrifuged at 2000 rpm for 5 min. The cell pellet was resuspended in PBS and count was adjusted to $1x10^7$ cells/ml.

The toxicity of ME towards the cells was checked using the trypan blue exclusion method (Talwar, 1974).The assay system contained $1x10^6$ cells and different concentrations of ME of *S. glauca* in a total volume of 1 ml of PBS and incubated at 37°C for 3 h. Control tubes

were maintained without any additive. At the end of incubation, 0.1 ml of 1% trypan blue was added and kept for 2-3 min and loaded on a haemocytometer to determine the number of dead and live cells.

2.9 Antiproliferative study

The long term cytotoxicity of the ME of *S. glauca* was carried out by MTT assay (Mosmann, 1983). Four different cell lines including neoplastic (HepG2, HeLa, and HCT-15) and normal cells (Vero) were used for the study. Approximately, 2.5 x 10^4 cells/ml of each cell line were seeded in 12 well plates and incubated at 37°C. At 80% confluency, cells were incubated with different concentrations of ME (10-100 µg/ml) at 37°C for the next 48 h. After the incubation period, the spent medium was removed and fresh medium containing 100 µl of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was added to each well and incubated further for 4 h. The dark blue formazan crystals were dissolved in 1 ml solubilisation solution (10% Triton X-100, 0.1 N HCl in 125 ml isopropanol) by continuous aspiration and resuspension. The absorbance of the coloured product was measured at 570 nm in a spectrophotometer (80+, PG Instruments). The cytotoxicity was determined by comparing the percentage loss of viability of treated cell population with the untreated control, indicated by their respective absorbance assessed with the MTT assay.

2.10 Acute toxicity studies using female Wistar rats

Aqueous methanolic extract of *S. glauca* (2000 mg/kg b. wt) was administrated to three female Wistar rats (130-150 g) orally as per OECD guidelines (OECD, 2001). The animals were monitored for 14 successive days for any visible changes in behaviour, body weight, water and food intake, hair loss, *etc.* At the end of experimental period animals were sacrificed and the internal organs were examined for any sign of change by conducting necropsy. The experiment was repeated with another three animals.

2.11 Determination of the antitumour effect of ME of *S. glauca* on ascites tumour bearing animals

The effect of ME of *S. glauca* on ascites tumour bearing Swiss albino mice was conducted in two different ways to determine the antitumor effects. The ME of *S. glauca* was administrated orally to the experimental animals in both experimental setups.

2.11.1 Administration of ME of *S. glauca* along with induction of ascites tumour

Ascites tumour was induced by injecting EAC cells (1 x 10^6 cells/ animals) in the peritoneal cavity of male Swiss albino mice (30 number).They were divided into five groups (six animals/group). Animals in the first group (Group I) received no treatment and served as the control group. Animals in Group II were given propylene glycol orally (200 µl, 1%), was the vehicle control. Animals in Groups III were administrated cyclophosphamide (15 mg/kg b. wt) orally. Groups IV and V animals have administrated ME of *S. glauca* at 200 and 400 mg/kg b. wt. for ten successive days orally following inoculation.

2.11.2 Administration of ME of *S. glauca* before inducing ascites tumour

Eighteen animals were divided into three groups (six animals/group). Animals in group VI were administrated with 200 µl, 1 % propylene glycol (vehicle control) five days before the inoculation of EAC. Similarly, animals in groups VII and VIII were given ME of *S. glauca* 200 and 400 mg/kg b.wt. solubilised in propylene glycol. Ascites tumour was then induced by injecting EAC cells (1 x 10^6 cells/ animals) in the peritoneal cavity of all of these animals.

2.12 Statistical analysis

Each *in vitro* assay in duplicate was performed at least thrice and the data were expressed as mean ± SD. For acute toxicity studies, three animals were used per group and rest all animal experiments had six animals per group and data are mean ± SD of six animals.

3. Results

3.1 Phytochemical analysis

The different chemical tests conducted, unravelled the presence of phytochemicals like tannins, coumarins, terpenoids, phenols, saponins, alkaloids, flavonoids and carbohydrates in ME of *S. glauca*. Organic molecules like proteins and free amino acids, steroids, sterols, anthraquinones, leucoanthocyanins, emodins and phlobatamins showed negative reaction for their specific identification tests (Table 1).

Table 1: Phytochemical screening of ME of *S. glauca* (+ indicates the presence and – indicates absence)

Phytochemicals	ME
Carbohydrates	+
Tannins	+
Coumarins	+
Terpenoids	+
Phenols	+
Saponins	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Resins	-
Proteins & amino acids	-
Steroids	-
Sterols	-
Anthraquinones	-
Leucoanthocyanins	-
Emoidns	-
Phlobatamins	-

3.2 Thin-layer chromatography

Total of twenty-eight bands were developed while the ME of *S. glauca* was separated by TLC. The number of bands observed under visible light was eleven. But, number of bands could visualize under visible light after spraying iodine vapour were nine and under UV light (254 nm), eight bands were noticed (Figure 1). Three bands with similar Rf values (0.83, 0.92 and 0.94) were noted under visible light and iodine vapour. Band with Rf value 0.98 was observed as a common one under iodine vapour and UV light (254 nm). Another band with Rf value 0.23 was obtained under visible light and UV light (254 nm). Band with high Rf value (0.92) was present under all the conditions are taken for visualizing the bands (Table 2).

3.3 Short term *in vitro* cytotoxicity analysis

The short term cytotoxicity of ME of *S. glauca* at various concentrations (5-100 µg/ml) is depicted in Figure 2. The maximum percentage of cytotoxicity (35.32 ± 2.35) could be observed only at 100 g/ml concentration of ME when added into EAC cell

suspension. At the same concentration, the cytotoxicity obtained in DLA cells was 43.34 ± 1.71%. Towards spleen cells, the action of ME (100 g/ml) was found to be feeble (16.26 ± 1.71% inhibition). The IC_{50} value of ME extract in these cells were supposed to be >100 g/ml.

Table 2: Rf values of fractions obtained in TLC of ME of *S. glauca* viewed under visible light, iodine vapour and UV (254 nm)

Sl. No.	Band	Rf value		
		Normal light	Iodine vapour	UV (254 nm)
1	Band - I	0.23	0.19	0.15
2	Band - II	0.48	0.21	0.23
3	Band - III	0.55	0.36	0.38
4	Band - IV	0.72	0.75	0.61
5	Band - V	0.78	0.83	0.76
6	Band - VI	0.83	0.91	0.82
7	Band - VII	0.90	0.92	0.92
8	Band - VIII	0.92	0.94	0.98
9	Band - IX	0.94	0.98	-
10	Band - X	0.95	-	-
11	Band - XI	0.97	-	-

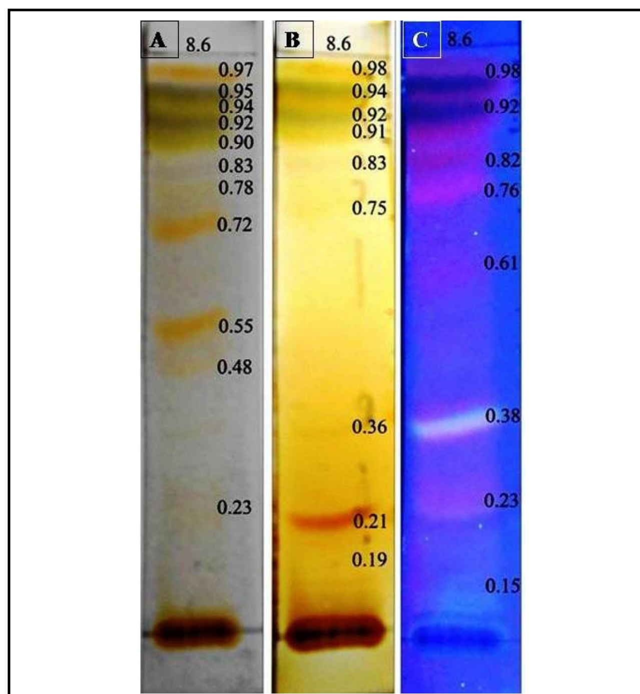


Figure 1: TLC analysis of ME of *S. glauca*: Approximately 10 µl of 10 mg/ml ME was applied on precoated thin layer chromatographic aluminium plate and developed using mobile phase, chloroform: ethyl acetate: formic acid (8:2:0.2, v/v/v) and observed under under A) Visible light, B) Iodine vapour and C) UV light: (254 nm).

3.4 Antiproliferative analysis

Addition of various concentrations (20-100 µg/ml) of ME did not achieve an appreciable inhibition on the cell viability of different cancerous and non-cancerous cells in culture. Even at the highest concentration of ME used (100 µg/ml), there was 89.61 ± 0.01% viable cells were found in HCT culture and 79.78 ± 0.02, 81.42 ± 0.02 and 81.63 ± 0.64% cell viability were noted in HepG2, HeLa and Vero cells, respectively (Figure 3).

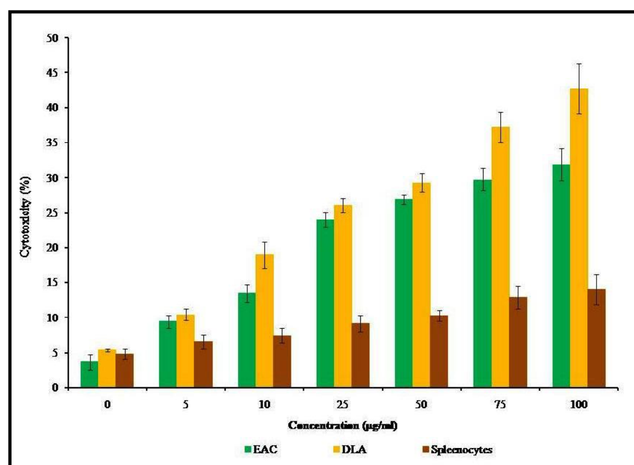


Figure 2: Cytotoxicity of ME of *S. glauca* on EAC, DLA & Spleenocytes: Cells ($1x10^6$) in 1 ml PBS (spleenocytes in RPMI 1640 medium) were exposed to various concentration of ME as indicated for 3 h and the cytotoxicity was evaluated by Trypan blue method. Values are expressed as mean ± SD of at least three experiments done in duplicate tubes.

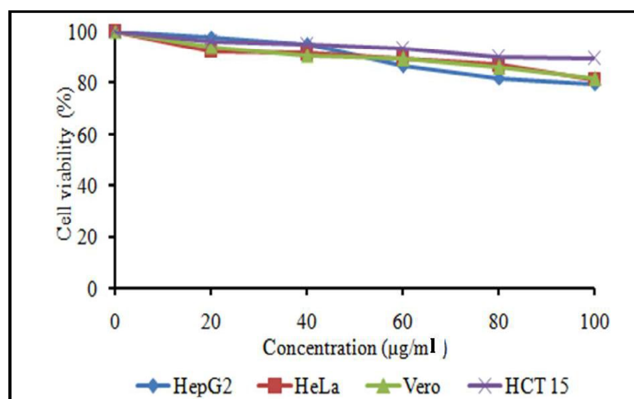


Figure 3: Effect of ME of *S. glauca* on different cell lines: Approximately $2.5x10^4$ cells/ml (HepG2, HeLa, Vero and HCT 15) were seeded in 12 well plates and incubated at 37°C. At 80% confluency, cells were exposed to different concentrations of ME as indicated at 37°C for the next 48 hr. The cell viability was then measured using MTT assay.

3.5 Acute toxicity studies using female Wistar rats

Female Wistar rats administrated with 2000 mg/kg b.wt. the concentration of ME was observed for 14 consecutive days, showed no abnormal changes in its behaviour, consumption of water and food, hair loss and finally gain of body weight. Study of internal organs after sacrificing the experimental animals at the end of the experimental period, revealed no change in colour and texture of internal organs.

3.6 Determination of the antitumour effect of ME of *S. glauca* on ascites tumour bearing animals

Significant antitumor activity was not obtained for ME administered ascites tumour bearing male Swiss albino mice when compared to the standard cyclophosphamide administered animals (Table 3). Animals in Group I (control group) had a mean survival rate of an increase in life span of 30.28%. Animals in Groups IV and V, which

were administrated with ME at 200 and 400 mg/kg b. wt. observed to have mean survival rate of 20.17 ± 2.23 (11.01% increase in life span) and 19.83 ± 2.64 days (9.17% increase in life span), respectively.

In the pre-treated animals included groups, Group VI which was administrated with propylene glycol, five days before the inoculation of EAC survived for 18.17 ± 2.93 days. The mean survival rate of animals in Groups VII and VIII, those who were given ME at 200 and 400 mg/kg b. wt., respectively for five consecutive days before the induction of EAC cells was 20.67 ± 2.07 and 21.67 ± 2.07 days (Figure 4).

Table 3: Effect of ME of *S. glauca* on the survival rate and life span of ascites tumour bearing animals

Group	Treatment	Mean survival days	% increase in lifespan
I	Control	18.17 ± 3.19	--
II	Vehicle control	18.33 ± 2.80	0.92
III	EAC + Cyclophosphamide (15 mg/kg b. wt) - Standard	23.67 ± 3.14	30.28
IV	EAC cells + ME (200 mg/kg b. wt)	20.17 ± 2.23	11.01
V	EAC cells + ME (400 mg/kg b. wt)	19.83 ± 2.64	9.17
	Pre treatment groups		
VI	Vehicle control	18.17 ± 2.93	0
VII	EAC cells + ME (200 mg/kg b. wt)	20.67 ± 2.07	13.76
VIII	EAC cells + ME (400 mg/kg b. wt)	21.67 ± 2.07	19.27

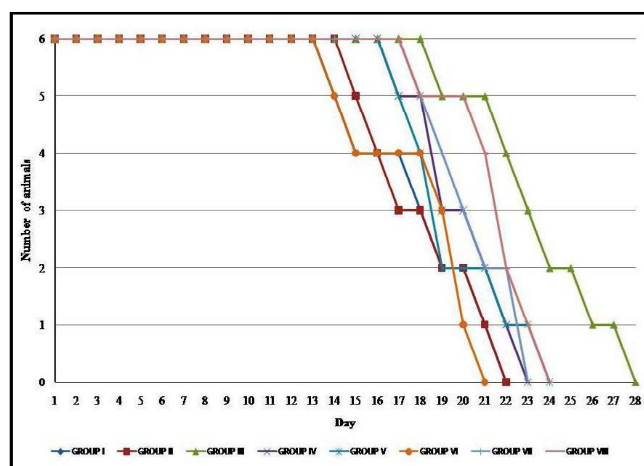


Figure 4: Effect of administration of ME of *S. glauca* on the mean survival rate of ascites bearing animals: Animals were orally administered with propylene glycol (Group II), cyclophosphamide (15 mg/kg b. wt; Group III), and different doses of ME were administered (200 and 400 mg/kg b. wt Groups IV and V) 24 h after tumour inoculation for ten consecutive days. Group II remained untreated control. Groups VI, VII and VIII animals were pre-treated with 1% propylene glycol, ME (200 and 400 mg/kg b. wt), respectively for five consecutive days before the inoculation of EAC and followed with 10 consecutive days of administration. The survival of animals in days were documented. Values are ± SD of 6 animals per group.

4. Discussion

Currently, the search for phytochemicals possessing antioxidant, anti-inflammatory, and antimicrobial activities have been on the rise because of their possible use in the therapy of various chronic and infectious diseases (Lakshmi *et al.*, 2014). Joshi and Joshi (2002) speculated that the chemicals present in leaf, fruit, pulp and seed of *S. glauca* possess medicinal properties such as analgesic, antiviral, antimicrobial, astringent, stomachic, emmenagogue, tonic and vermifuge. The study done by Valdés *et al.* (2008) revealed a strong inhibitory activity of ethanol extracts of *S. glauca* against protozoan like *Trypanosoma brucei, T. cruzi, Leishmania infantum* and *Plasmodium falciparum*, and also proved significant cytotoxicity. Lakshmi *et al.* (2014) has reported excellent antimicrobial, antioxidant, haemolytic and thrombolytic activities of methanolic extract of *S. glauca* leaves.

Phytochemical analysis of the ME of *S. glauca* conducted in the present study, well coincides with the study conducted by Lakshmi *et al.* (2014). In their study, alkaloids, flavonoids, carbohydrates, phenolic compounds, and tannins in methanolic extract of leaves of *S. glauca* is reported to be present. In addition to these compounds, we have detected terpenes, saponins and coumarins in the ME of *S. glauca*. Govindaraju *et al.* (2009) have detected the presence of saponins in ME of oilseed meal of *S. glauca*. The presence of coumarins has not been attempted by these authors. These molecules are found separated in the TLC profiling.

The different phytochemicals identified in the present study especially flavonoids and tannins (Fresco *et al.*, 2006; Ruch *et al.*, 1989) are remarkable in cancer prevention. According to Garg *et al.* (2007), terpenoids detected in the ME of *S. glauca* is an eminent class of antitumour chemicals.

Short term and long term cytotoxicity studies conducted here with ME of *S. glauca* do not show a significant cytotoxic potential towards cancer cells. However, produce cell death in a dose-dependent manner. Even at higher concentration, the mortality of normal cells was found minuscule in the present study. IC_{50} value was unable to detect in any of the cell lines treated with ME, since 100 g/ml is the maximum dosage of drug administrated in the study as per the preference of American Cancer Society. These cytotoxic study results coincide with the reports published by Tong *et al.* (2015). They have found that the administration of standardized quassinoids composition (SQ 40) showed more downbeat action on LNCaP human prostate cancer cells when compared to human normal prostate cells.

In general, the present work indicates limited cytotoxic and antitumour activity of ME of leaves of *S. glauca*. There are many possible reasons for explaining these results. First and foremost, the drug used for the cytotoxicity and antitumour studies in this experiment is in a crude form. All currently available scientific literature proclaims promising results on the cytotoxic and antitumour activities of pure compounds; especially quassinoids isolated from *S. glauca* on different cancer cell lines. Quassinoids are a group of diterpenoids found in plants from the Simaroubaceae family (Tong *et al.*, 2015). Several quassinoids extracted from *S. glauca* seeds, including glaucarubin, glaucarubinone, glaucarubol and glaucarubolone have shown cytotoxic activity *in vitro* against KB cells (human oral epidermoid carcinoma) (Polonsky *et al.*, 1978; Valeriote *et al.*, 1998). The esters of glaucarubolone, ailanthinone and glaucarubinone, exhibited significant activity *in vivo* in the P388 lymphocytic leukaemia model (Monseur and Motte, 1983).

The percentage composition of potent biomolecules may be less in the crude extract. This assertion coincides with the findings of Govindaraju *et al.* (2009). According to them, saponins are one among the major toxic components present in Simarouba meal; which are not highly polar compounds but amphiphilic compounds, in which sugars are linked to a non-polar group called sapogenin, which may be either a sterol or a triterpene. Even though saponin gave a positive reaction in the present work, in ME more polar compounds can be expected rather than these kinds of potent non-polar and amphiphilic compounds. Like saponins quassinoids are the most possible cytotoxic and antitumor substance reported in *S. glauca*. Quassinoids are degraded triterpenoids and are believed to be synthesized in plants from Δ7-euphol or Δ7-tirucallol (Polonsky, 1983). Since these compounds are more leaned towards non-polar side, there expect a minor proportion of these molecules in the ME of *S. glauca*.

5. Conclusion

In the present study, the methanolic leaf extract of *S. glauca* did not show an appreciable antitumour efficacy. However, the presence of pharmacologically active phytochemicals is detected in this extract. Quassinoid compounds which are known for the antitumor efficacy of *S. glauca* in the previous *in vitro* experiments have not been assessed in the present study. It is, thus assumed that either due to the absence of quassinoids or its lesser content could be the reason for the lesser antitumor efficacy of the extract. Based on the results so far obtained in this study, the vouched cancer cure potential of *S. glauca* could not be supported. While considering the less toxicity *in vivo* and the presence of pharmacologically active class of phytochemicals, the methanolic leaf extract of *S. glauca* warrants further exploration towards its anticancer potential.

Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

References

Cragg, G. M. and Newman, D. J. (2000). Antineoplastic agents from natural sources: achievements and future directions. Expert Opin. Investig. Drugs, **9**:1-15.

Devkota, K. P.; Wilson, J. A.; Henrich, C. J.; McMahon, J. B.; Reilly, K. M. and Beutler, J.A. (2014). Compounds from *Simarouba berteroana* which inhibit proliferation of NF1-defective cancer cells. Phytochem. Lett., **7**:42-45.

Fo, E. R.; Fernandes, J. B.; Vieira, P. C. and Silva, M. F. G. (1992). Canthin-6-one alkaloids from *Picrolemma granatensis*. Phytochemistry, **31**: 2499-2501.

Fresco, P.; Borges, F.; Diniz, C. and Marques, M. P. M. (2006). New insights on the anticancer properties of dietary polyphenols. Med. Res. Rev., **26**:747-766.

Govindaraju, K.; Darukeshwara, J. and Srivastava, A. K. (2009). Studies on protein characteristics and toxic constituents of *Simarouba glauca* oil seed meal. Food Chem. Toxicol., **47**:1327-1332.

Garg, U. K.; Kaur, M. P.; Garc, V. K. and Sud, D. (2007). Removal of hexavalent chromium from aqueous solution by agricultural waste biomass. J. Hazard. Mater., **140**:60-68.

Harborne, J. B. (1973). Phytochemical methods. Capman and Hall Ltd, London. pp:148.

IARC, (2019). Cancer today. Retrieved from doi:https://gco.iarc.fr/

Jiang, M. X. and Zhou, Y. J. (2011). Canthin-6-one alkaloids from *Picrasma* quassinoids and their cytotoxic activity. J. Asian Nat. Prod. Res., **10**:1009-1012.

Joshi, S. and Joshi, S. (2002). Oil tree - Laxmitaru glauca. University of Agricultural sciences, Banglore and Indian council of Agricultural Research, New Delhi, India, pp:86.

Lakshmi, K. S.; Sangeetha, D.; Sivamani, D.; Tamilarasan, M.; Rajesh, T. P. and Anandraj, B. (2014). *In vitro* antibacterial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of *Simarouba glauca* leaves extracts. Int. J. Pharm. Sci. Res., **5**:432-437.

Li H-Y,; Koike, K. and Ohmoto, T. (1993). New alkaloids, picrasidines W, X and Y, from *Picrasma quassinoids* and X-ray crystallographic analysis of picrasidine. Chem. Pharm. Bull., **41**:1807-1811.

Modi, C. M.; Ladumor, V. C.; Patel, U. D.; Patel, H. B.; Solanki S. L. and Bhadarka. D. H. (2018). Phytochemical analysis and comparative study of *in vitro* free radical scavenging activity of different extracts of leaves of *Abrus precatorius* L. Ann. Phytomed., **7**:133-137.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays. J. Immunol. Methods, **65**:55-63.

Monseur, X. and Motte, J.C. (1983). Quantitative high performance liquid chromatographic analysis of the bitter quassinoid compounds from *Simarouba glauca* seeds. J. Chromatogr., **264**:469-473.

OECD, (2001). OECD guideline for testing of chemicals. Acute Oral Toxicity-Acute Toxic Class Method, pp:423.

Patel, D. P.; Verma, V. D.; Loknathan, T. R.; Bhatt, K. C. and Mishra, J. B. (1997). *Simarouba glauca*: A non-traditional oil source. Indian Farming, **47**:7-9.

Patil, M. S. and Gaikwad, D. K. (2011). A critical review on medicinally important oil yielding plant Laxmitaru (*Simarouba glauca* DC.). Journal of Pharmaceutical Science and Research, **3**:1195-1213.

Polonsky, J. (1983). Chemistry and biological activity of the quassinoids. In: The Chemistry and Chemical taxonomy of the Rutales (ed. Waterman, P. G. and Grundon, M. F.), Academic Press, New York, pp:247.

Polonsky, J.; Varon, Z.: Jacquemin, H, and Pettit, G. R. (1978). The isolation and structure of 13, 18dehydroglaucarubinone, a new antineoplastic quassinoid from *Simarouba amara*. Experientia, **34**:1122-1123.

Polu, P.; Nayanabhirama, U. and Khan, S. (2015). Herbal medicinal plants as anticancer agents. Ann. Phytomed., **4**:37-45.

Reddy, P. V.; Sreenivas, N. and Urooj, A. (2016). Acute toxicological studies of leaf extracts of *Morus indica* L. in rats. Ann. Phytomed., **5**:108-112.

Ruch, R. J.; Cheng, S. J. and Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogens, **10**:1003-1008.

Sofowara, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, pp:289.

Talwar, G. P. (1974). Hand book of practical immunology. National Book Trust, New Delhi. pp:336-339

Tong, K. L.; Chan, K. L.; Abu Bakar, S.; Low, B. S.; Man, H. Q. and Wong, P.F. (2015). The *in vitro* and *in vivo* anticancer activities of standardized quassinoids compostion from *Eurycoma longifolia* on LNCap human prostate cancer cells, PLoS ONE, **10**:e0121752.

Trease, G. E. and Evans, W. C. (1989). Pharmacognsy. Brailliar Tiridel Can Macmillan Publishers.

Valeriote, F. A.; Corbett, T. H.; Grieco, P. A.; Moher, E. D.; Collins, J. L. and Fleck, T.J. (1998). Anticancer activity of glaucarubinone analogues. Oncol. Res., **10**:201-208.

WHO. (2018). Cancer, Retrieved from doi:https://www.who.int/en/news-room/fact sheets/detail/cancer

Citation: Shaji E. Mathew, Smitha K. Ramavarma, Thekkekara Devassy Babu, Balu T. Kuzhivelil and Achuthan C. Raghavamenon (2019). Preliminary assessment on phytochemical composition, cytotoxic and antitumor efficacy of *Simarouba glauca* DC. leaf methanolic extract. Ann. Phytomed., 8(2):121-126.