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Review Article

Therapeutic Potential of Phytochemicals Isolated from *Simarouba glauca* for Inhibiting Cancers: A Review

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ABSTRACT

Simarouba glauca DC (Family: Simaroubaceae, abbreviated as *S. glauca* / SG), commonly known as 'Laxmitaru' or 'Paradise tree' is an eco-friendly tree that grows in tropical areas of America, The West Indies and Brazil. Nutritionally, SG has been used as a potential source of edible oil. Medicinally, the decoction prepared from SG leaves has been demonstrated to exhibit antitumor, antimalarial, antiviral activities. Objectives: Address various existing gaps in the research on *Simarouba glauca* and provide future directions to promote address these grey areas. Methods: Literature pertaining to *Simarouba glauca* and various related plants was collected using PubMed search engine with key words *Simarouba glauca*, Simaroubaceae, Paradise tree, Laxmitaru. Articles pertaining to anti-cancer activity were filtered and information collected for preparing the review article. Although pharmacological potential of *Simarouba glauca* is well documented; not much is known about the mechanism(s) of action of the isolated phytoconstituents. In addition, many gaps pertaining to the efficacy of pharmacological agents for inhibiting cancers does exist. Therefore, future studies should focus on (a) screening and isolating key pharmacological agents that exhibit better safety and efficacy profiles for treating cancers; (b) evaluating the pharmacodynamic and pharmacokinetic properties of phytochemicals in experimental animal models; (c) testing the compounds in clinical trials; and (d) developing better strategies to effectively deliver the isolated compounds from SG. Studies are also warranted to determine whether isolated compounds of SG are better or the crude extracts that contain a combination of agents are better. Extensive research is required to address various unanswered questions. Therefore, future studies should focus on providing answers to fill the existing gaps about the utility of *Simarouba glauca* phytochemicals for treating cancers.

Key words: Laxmitaru, Paradise tree, Quassinoids, *Simarouba glauca.*

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INTRODUCTION

Simarouba glauca (S.*glauca, abbreviated as SG*), commonly known as 'Laxmitaru' or 'Paradise tree' belongs to the family Simaroubaceae. SG has been utilized in traditional system of medicine as anticancer, antimicrobial, antiviral and antihelminthic agent, especially in regions covering Southern Florida, the West Indies and Brazil.[1] SG is a rich source of quassinoids (a group of degraded triterpene lactones),[2,3] such as glaucarubin, glaucarubolone and glaucarubinone.[4–7] The health promoting oil prepared using SG extract contains oleic acid and devoid of bad cholesterol.[1] Nutrapotent DS, a herbal formulation made up of *S. glauca* extract, has been shown to contain anticancer agents and used for the treatment of cancers.[8] Studies have demonstrated that the water extract of SG promote skin keratinocyte differentiation[1] and improve skin hydration and moisturisation.[7] Products of SG are currently in the market in the form of skin lotion and dry leaf powder / dried seeds to treat skin disorders. Traditionally, the bark has been used for the treatment of malaria. SG extract has also been used as an effective natural remedy by the Brazilian tribes for managing chronic and acute dysentery.[1] In the present report, we have thoroughly reviewed the literature on the anticancer agents isolated from SG and mentioned about future studies requiring better understanding of the phytochemicals was also discussed.

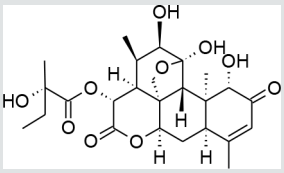
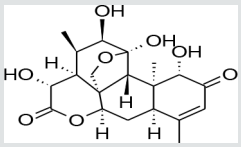
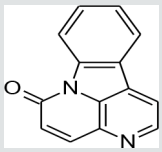
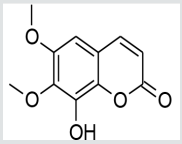
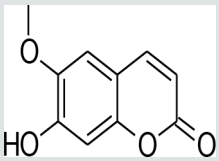
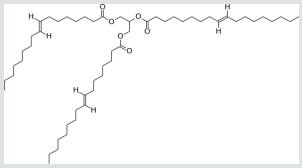
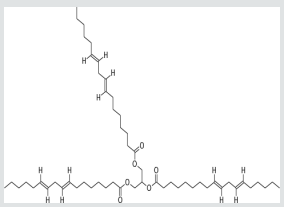
A search using the words "*Simarouba glauca*", "Paradise Tree", "Tricaproin", "Laxmitaru" was conducted in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Google scholar (https://scholar.google.co.in/scholar) and the articles found were selected based on their relevance to the theme of the review. Priority has been given to those that are directly related and recent. Data collected from the literature search was grouped into a) Phytochemicals isolated b) *In vitro* anticancer studies c) Molecular mechanisms d) Toxicity evaluation and e) Animal models tested.

PHYTOCHEMICALS ISOLATED FROM *SIMAROUBA GLAUCA*

Although several phytochemicals isolated from SG have been patented, very few of them are currently being tested in clinical trials (Table 1). Glaucarubin is one of the most extensively studied compounds isolated from SG seeds by Ham E.A., in 1954.[4] The presence of this crystalline compound was confirmatively reported in 1978-87.[5,9,10] The seeds of the plant contain quassinoids such as glaucarubol, glaucarubolone and the two esters of glaucarubolone, ailanthinone and glaucarubinone.[6,11–16] Compounds such as scopoletin, canthin-6-one and a canthin-6-one dimethoxy derivative were also isolated from the wood extract of the plant.[17] Very recently, J. Fausto Rivero-Cruz has isolated and purified several compounds namely canthine alkaloids- canthin-6-one, 2-methoxycanthin-6-one, 9-methoxycanthin-6-one, 2-hydroxycanthin-6-one, 4,5-dimethoxycanthin-6-one and 4,5-dihydroxycanthin-6-one, a limonoid-melianodiol, an acyclic squalene type triterpenoid- 14-deacetyleurylene, two coumarins- scopoletin and fraxidin; and two triglycerides - triolein and trilinolein.[18] Likewise, Jose *et al.* have isolated tricaproin from the leaves of SG and elucidated the anti-cancer properties *in vitro.*[19]

Quassinoids are the major group of phytochemicals present in the family of Simaroubaceae.[2,3] To date approximately 200 quassinoids were isolated and structures elucidated. For instance triterpene degradation products derived from the euphol/tirucalol series, (+)-polyandrol, eurylactones A and B, ailanquassins A and B, 6-dehydroxylongilactone have been

Table 1: Phytochemicals isolated and patented from *Simarouba glauca*.

Common Name and Structure	Uses	Patent Number	References
GLAUCARUBINONE 	Inflammatory Ailments	US2016051553	PubChem CID:441796 [12,13]
	Psoriasis	US9095606	
	Microbicidal and antiparasitic	US8865235	
	Skin Condition	US8734859	
	Antineoplastic, antiviral and herbistatic activity	US2014205685	
		US2003158088	
	Cancer	US6573296	
		US8734859	
		US6573296	
		WO8807372	
GLAUCARUBOLONE 	Antineoplastic, antiviral and herbistatic activity	US6573296	PubChem CID: 441797 [14]
CANTHIN-6-ONE 	Inflammatory Ailments	US2016051553	PubChem CID: 97176 [17]
	Psoriasis	US9095606	
	Mycobacteria-linked pathologies	US2011059977	
	Trypanosomiases	US7705013	
	Extensive cancer	US7705013	
FRAXIDIN 	Antileishmaniasis	US2014287030	PubChem CID: 3083616 [18]
	Treating pathologies	US2002002139	
	Anti-infective activity	WO9426282	
	Urinary tract infections	US2010028469	
SCOPOLETIN 	Balloon surface coating	US2016082159	PubChem CID: 5280460 [18]
	Promoter control elements	US2016076046	
	carrying therapeutic substances into cells	US2016067338	
	Tissue specific reduction of lignin	US2016017355	
	Nanoparticle formulations for delivering Multiple therapeutic agents	US2015342896	
	Treatment of tobacco material	US2015296870	
	Psychotic disorders	US2015299227	
	Balloon surface coating for valvuloplasty	US2015231362	
	Analgesic	US2014349969	
TRIOLEIN 	Post-operative chronic pain	US2016089335	PubChem CID: 5497163 [18]
	Diabetes mellitus	US2016089409	
	Raynaud's disease	US2016081954	
	Myelination diseases	US2016081955	
	X-linked adrenoleukodystrophy	US2016074369	
	Acute pancreatitis	US2016051637	
	Lysosomal acid lipase deficiency	US2016051638	
TRILINOLEIN 	Myocardial infarction	US2016038147	PubChem CID: 5322095 [18]
	Vestibular schwannoma	US2015359851	
	Cardioprotection and cardioregeneration	US2015343023	
	Immunomodulatory agent	US2015216955	

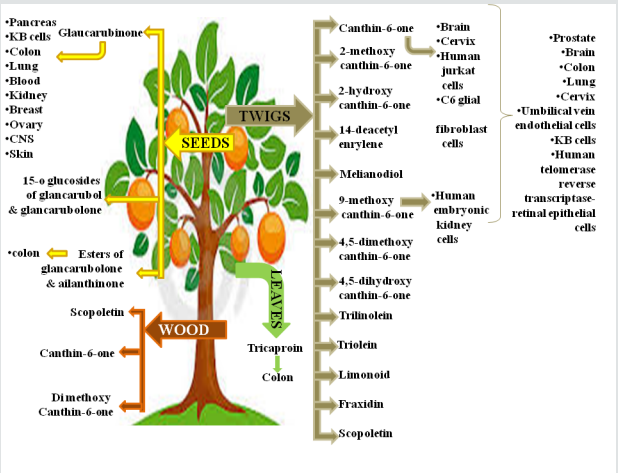


Figure 1: The phytochemicals isolated and characterized from various parts of *Simarouba glauca* are shown. The pure compounds were screened for the cytotoxicity against various cancer cell lines and identified tricaproin, glaucarubinone and canthin-6-one as potent anticancer agents.

purified and characterized.[20] Structurally, majority of isolated quassinoids have a twenty carbon skeleton.[21–23] Glucoside forms of quassinoids such as 15-O-β-D-glucopyranosyl glaucarubolone and glaucarubol have also been reported in SG.[16] β-carboline alkaloids, canthin alkaloids, triglycerides, coumarins, squalene type triterpenoids and fatty acids are the other category phytoprinciples purified from SG.[18,24] The phytochemicals isolated and studied are shown in Figure 1

NUTRITIVE VALUE OF *SIMAROUBA GLAUCA*

S. glauca is a rich source of nutrients that include lipids, fatty acids, carbohydrates and proteins[1]. While seeds contain oil (up to 60% weight/weight), the kernals provide edible fat consisting of palmitic (12.5%), oleic (56%) and stearic (27%) acids.[24–27] In addition, the kernal has an average protein content of 51.8g /100g.[25] Kernal proteins are rich in essential amino acids namely leucine (7.76g/100g protein), lysine (5.62g/100g protein) and valine (6.12g/100g protein). The plant meal contain calcium (143mg/100g), sodium (79mg/100g), saponins with triterpenoid aglycone (3.7g/100g), alkaloids (1.01g/100g), phenolics (0.95g/100g) and phytic acid (0.73g/100g).[7] The leaves contain flavonoids (0.14 to 0.18%), phenolics (250-400µg/mg) and tannin substances (67-200µg/mg), which help in combating various diseases such as cancer and diabetes.[28]

IN VITRO STUDIES DEMONSTRATING THE ANTICANCER POTENTIAL OF ISOLATED PHYTOCHEMICALS

Since SG is known to contain phytochemicals that inhibit cancer growth, an attempt was made to review the literature highlighting the isolation, characterization, safety and efficacy properties of these compounds.[4,16–18] List of the isolated phytochemicals exhibiting *in vitro* anticancer activities is shown in Table 2. The butanol fraction of the wood has been shown to inhibit 80 % of murine embryonic stem cells, human prostate carcinoma cells PC-3 and human embryonal carcinoma cells NT-2 at 50µg/ml concentration.[17] The methanolic extract has been shown to exhibit cytotoxic effect on squamous cell carcinoma cell line SCC9.[28] Similarly, another compound isolated from methanolic extract ie, laucarubol, has retarded the growth of renal carcinoma cell line RXF 393 (PubChem CID: 441794). Studies have also explored the possibility of targeting cancers by using combination strategies. For example, a combination of glaucarubinone and gemcitabine synergistically inhibited murine pancreatic cancer cell lines PANC-1, MiaPaCa-2 and PAN02 *in vitro.*[29,30] Cytotoxicity of glaucarubinone against human oral epidermoid carcinoma cell (KB cells), human prostate cancer cells (DU145), human lung cancer celsl (A549), human vincristine-resistant nasopharyngeal cells (KBvin) and human promyelocytic leukemia cells (HL60) has been studied.[31] Studies on the efficacy of glaucarubinone for inhibiting cell lines representing carcinomas of kidney (ACHN, TK10, UO-31,SN12C,RXF 393), breast (MCF7, MDA-N, BT549, T47D, MDAMB231, HS578T), ovaries (IGROVI, SKOV3, OVCAR-3, OVICAR-4, OVICAR-5, OVICAR-8), central nervous system (SF295, SF268, SF539, SNB-75, SNB-78, SNB-19, U251, XF498), lung (NCI-H23, DMS114, DMS273, HOP-92, NCI-H522, LXFL529, EKVX, NCI-H23, NCI-H226, NCI-H460, HOP-62, HOP-18), colon (HT29, Colo205, DLD-1, HCT15, KM12), skin (SK-MEL-28, SK-MEL-2, SK-MEL5, UACC-62, LOX IMVI, M14, M19-MEL, MALME-3M, UACC-257) and prostate (PC-3, DU-145) have also been reported.[32]

Canthin-6-one is another potent anti-cancer agent tested using various *in vitro* and *in vivo* models.[33,34] Canthin-6-one has been shown to be effective against human Jurkat cells, PC3 (prostate cancer cell line) and HT29 (colorectal carcinoma cell line) and cell lines representing carcinomas of cervix a brain and mouse NIH/3T3 cells and C6 glial fibroblast cells of rat.[34] Canthin-6-one, 9-methoxy canthin-6-one, 2-methoxy canthin-6-one, 2-hydroxy canthin-6-one, melianodiol, 14-decetyl eurylene have shown cytotoxicity towards human colon cancer cell line (Col2), umbilical vein endothelial cells (HUVEC), KB cells, prostate cancer cells (LCNaP), lung cancer cell line (Lu1) and human telomerase reverse transcriptase-retinal pigment epithelial cell line (hTERT-RPE) in nano molar concentration.[18] The anticancer activity of 9-methoxy canthin-6-one was shown to be due to the inhibition of NF-kappaB activity in TNF-alpha stimulated human HEK-293/NF-kB-luc cells.[35] Scopoletin inhibits human PANC1,[36] SW480 colon adenocarcinoma cells,[37] LoVo, HL60, human epithelial connective tissue (HT1080), doxorubicin-resistant LoVo cells[38] and human breast cancer cells MCF-7 and SK-BR-3. A short chain fatty acid containing tricaproin has shown potent cytotoxicity against human colorectal carcinoma cell lines HCT-116 and HCT-15 at lower concentrations.[19]

MOLECULAR MECHANISMS LEADING TO CANCER CELL DEATH BY PHYTOCHEMICALS ISOLATED FROM *SIMAROUBA GLAUCA*

Induction of cancer cell death by the isolated phytochemicals is mediated by (a) upregulation of apoptosis; (b) arrest of cells in cell cycle stages; (c) halting of proliferation through inhibition of cyclins with simultaneous induction of p21 and p27 inhibitors (d) modulation of autophagy and (e) downregulation of oncogenes and upregulation of tumor suppressors.[30,39–41] The isolated compounds has also been studied extensively not only for their safety and efficacy but also explored in depth for determining the mode of action (Table 3).

Quassinoids that exhibit cytotoxic properties have been shown to act through the inhibition of protein- and nucleic acid synthesis via interference at the peptidyltransferase site or through the down-modulation of phosphoribosyl pyrophosphate aminotransferase[42–48] (Figure 2). Quassinoids and triterpenoids helps to induce cell differentiation, downregulate c-Myc and promote apoptosis in cancer cells through a wide array of mechanisms.[49] Analysis of structure-activity relationship has revealed

Table 2: The anticancer activity studies of *Simarouba glauca* crude extracts and the isolated phytochemicals.

Study performed	Name of the compound/ extracts	Cell line/species used	IC50 / ED50 / potency / zone of inhibition	Author and Year of Publication
Cell proliferation assay	Butanol fraction	Murine embryonic stem cells	2.50 µg/ml	Reynertson *et.al* 2011[17]
Cytotoxicity	Methanolic extract	SCC9	312.20 µg/ml	Umesh *et.al* 2014[28]
Nrf2 qHTS screen	Glaucarubol	A549	2.91 µM	PubChem CID:225484
Nrf2 ARE-Fluc Confir- mation Assay for hit validation	Glaucarubinone	A549	5.17 µM	PubChem CID:441796
Cytotoxicity	Glaucarubinone	KB	0.44 µM	Usami *et al*. 2010[31]
		DU145	0.47 µM	
		A549	0.88 µM	
		KBVIN	1.24 µM	
		HL60	1.00 µM	
3H-thymidine-incorporation assay	Glaucarubinone	PANC-1	300.00 nM	Mata-Greenwood *et al*. 2001[58]
		MiaPaCa-2	58.00 nM	
		PAN02	960.00 nM	
		HL60	0.50 nM	
Boyden Chamber assay	Glaucarubinone	PANC-1	210.00 nM	Yeo *et al*. 2014[30]
		MiaPaCa-2 PAN02	44.00 nM	
		PAN02	220.00 nM	
Inhibition of AP-1 by FRET assay	Glaucarubinone	Jurkat	15.00 µM	Yeo *et al*. 2014[30]
Antiproliferative activity	Canthin-6-One	PC3	15.00 µM	Dejos *et al*. 2014[34]
		HT29	15.00 µM	
		HeLa	15.00 µM	
		mouse NIH/3T3	15.00 µM	
		Rat C6	15.00 µM	
		Col2	389.00nM	Rivero-Cruz *et al*. 2005[18]
		HUVEC	431.00 nM	
		KB	347.00 nM	
		LCNaP	>1000 nM	
		Lu1	510.00 nM	
		hTERT-RPE1	210.00 nM	
Inhibition of NF-kappaB activity by luciferase reporter gene assay	9-Methoxy canthin-6-one	HEK-293/NF-kB-luc	7.40µM	Tran *et al*. 2014[35]
Cytotoxicity	9-Methoxy canthin-6-one	Col2	609.00 nM	Rivero-Cruz *et al*. 2005[18]
		HUVEC	609.00 nM	
		KB	674.00 nM	
		LCNaP	564.00 nM	
		Lu1	478.00 nM	
		hTERT-RPE1	509.00 nM	
Cytotoxicity	2-Methoxy canthin-6-one	Col2	543.00nM	Rivero-Cruz *et al*. 2005[18]
		HUVEC	503.00 nM	
		KB	642.00 nM	
		LCNaP	725.00 nM	
		Lu1	654.00 nM	
		hTERT-RPE1	583.00 nM	

Table 2: The anticancer activity studies of *Simarouba glauca* crude extracts and the isolated phytochemicals.

Study performed	Name of the compound/ extracts	Cell line/species used	IC50 / ED50 / potency / zone of inhibition	Author and Year of Publication
Cytotoxicity	2-hydroxy canthin-6-one	Col2	567.00 nM	Rivero-Cruz *et al.* 2005[18]
		HUVEC	506.00 nM	
		KB	568.00 nM	
		LCNaP	524.00 nM	
		Lu1	565.00 nM	
		hTERT-RPE1	506.00 nM	
Cytotoxicity	Tricaproin	HCT-116	76 µM	Jose A *et al.* 2018[19]
		HCT-15	253 µM	
Inhibition of Insulin regulated aminopeptidase	Scopoletin		0.348 µM	Cai *et al.* 2013[36]
Inhibition of Endoplasmic reticulum aminopeptidase 1	Scopoletin		0.680 µM	Cai *et al.* 2013[36]
Competitive Inhibitors of Caspase-1	Scopoletin		1.99µM	Berger *et al.* 2006[59]
Inhibition of HSD17B4	Scopoletin		3.16 µM	PubChem CID: 5280460
Inhibition of ALR	Scopoletin		4.40 µM	PubChem CID: 5280460
Inhibition of HADH2	Scopoletin		5.01 µM	PubChem CID: 5280460
Identifying the Cell-Membrane Permeable IMPase	Scopoletin		10.00 µM	PubChem CID: 5280460
Inhibitors Inhibitors of GCN5L2	Scopoletin		31.62 µM	PubChem CID: 5280460
Induction of DNA re-replication	Scopoletin	SW480 colon adenocarcinoma cells	14.58 µM	Truong *et al.* 2011[37]
Inhibition of kidney aldose reductase 2	Scopoletin		46.00 µM	Dunlop *et al.* 2000[60]
Cytotoxicity	Scopoletin	LoVo	7.30 µM	Dall'Acqua *et al.* 2004[38]
		HL60	9.50 µM	
		HT1080	12.50 µM	
		Doxorubicin-resistant LoVo	18.50 µM	
		MCF-7	9.10 µM	
		SK-BR-3	28.80 µM	

that quassinoids with an α, β-unsaturated ketone in ring A, an epoxy-methylene bond in ring C and ester functional groups at C-15 exhibit potent anti-cancer activities.[50] Further studies are warranted to develop cancer cell selective quassinoid derivatives.

Canthin alkaloids exhibit antiproliferative activity by decreasing BrdU incorporation into DNA and by inhibiting the formation of mitotic spindle. In addition, it arrests cells in G2/M phase of the cell cycle[34] (Figure 2). Canthin-6-one has shown to inhibits the phosphorylation of AKT in human TG1 and TG10 cells.[33] 9-methoxy canthin-6-one inhibits NF-kappaB activity in TNF-alpha stimulated human HEK-293/NF-kB-luc cells.[35] It also inhibits Wnt signalling (Table 3). The degradation of β-catenin by 9-hydroxycanthin-6-one was decreased by GSK3β-siRNA. But the molecule decreased β-catenin even in the presence of CK1α-siRNA, suggesting the action through Wnt signalling by the activation of GSK3β independently of CK1α.[51] Selected Canthin-6-one alkaloids were tested in an Nf1- and p53-defective mouse malignant glioma tumor cell and the results indicated that the majority of the canthin-6-one alkaloids inhibited cell growth and exhibited toxicity.[52] Canthin-6-one reduced the phospho- and total- AKT from the malignant glioma stems cells TG1 and TG10 at a concentration of 300µM.[53]

Glaucarubinone is another key anti-cancer agent isolated from SG.[54] Mechanistically, glaucarubinone inhibits cancer cells growth by suppressing HIF 1α and β-catenin *via* PAK1-dependent pathway[55] (Figure 2 and Table 3). In addition, glaucarubinone sensitize KB cells to paclitaxel and inhibit ABC transporters (ATP-binding cassette transporters) via ROS-dependent and p53-mediated activation of apoptotic signalling pathways (Table 3). The combined treatment of Glaucarubinone (200nM) and Paclitaxel (PTX) (23.42nM) potentiated the cytotoxicity of PTX in KB cells and increased amount of cells in S-phase, along with a pronounced arrest in G2/M phase. The combination treatment also act through the mitochondrial pathway of apoptosis by increased mRNA expression of Bax, p53 and Caspase-9 in resistant KB cells and decreased mRNA expression of Bcl-2.[56] Similarly, glaucarubinone and ailanthinone, a structural analogue of glaucarubinone, suppressed CRC

Table 3: Mode of action of anti-cancer agents isolated from *Simarouba glauca*.

Compound	Processes Effected By Compound Treatment	Relevance in Cancer	Author and Year of Publication
Canthine-6-one	Cell proliferation	Halogenated nucleotide, Brdu is normally incorporated into the newly synthesized DNA during the S phase of the cell cycle. Canthine 6-one decreases the Brdu incorporation thereby suppresses the cell proliferation	Dejos *et al.* 2014[34]
	Cell cycle	Canthine-6-one interferes with the progression of cells through G2/M checkpoint thereby result in DNA damage. In addition, it interferes with mitotic spindle formation followed by G2/M arrest.	
	Cell survival	Survival of cancer cells is regulated by PI3K-Akt pathway. Canthine-6-one inhibits the phosphorylation of AKT, leading to the antiproliferative effect	Cebrián-Torrejón *et al.* 2012[53]
9-hydroxy canthin-6-one	Wnt signalling Pathway	Wnt signalling pathway is involved in the formation of benign and malignant tumors as evidenced by elevated levels of β-catenin. Furthermore, it inhibits the activation of GSK3β, a key regulator of β-catenin expression in cells	Dai *et al.* 2016[51]
Glaucarubinone	PAKs	PAKs have a major role in the cell proliferation, survival and motility. Glaucarubinone decreases the amount of active PAKs by down regulating β-catenin and HIF 1α	Huynh *et al.* 2015[55]
	ABC transporters	Glaucarubinone inhibits the export of drugs from the cells, thereby increase the drug concentration inside the cancer cell.	Karthikeyan *et al.* 2016[56]
	Apoptosis markers	Glaucarubinone activates pro-apoptotic markers Bax, Caspase-9 and p53, which leads to the induction of apoptosis and cell death.	

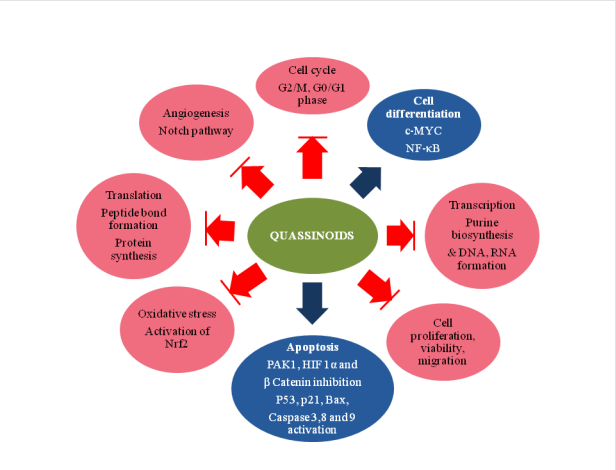


Figure 2: Mechanism of cell death induction by quassinoids is mediated by (a) down regulation of proliferation inducing proteins; cyclinD1 followed by arresting the cells in G0/G1 or G2/M phase; (b) induction of apoptosis proteins; p53, caspase-3 etc; (c) halting the angiogenesis through VEGF, MIC-1, IL-8 etc., (d) promoting cellular differentiation; (e) inhibiting the master regulator of oxidative stress controller Nrf2. Modulation of these processes leads to the inhibition of tumor cells growth and metastatic spread.

cell proliferation and migration. The molecule act as MDR modulator, which inhibit the MDR transport function thereby enhance the accumulation of Rh123 (indicator of ABCB1 function) inside the cells.

Tricaproin (TCN) is another anticancer agent recently reported by Jose *et al.* 2018. TCN inhibited colorectal carcinoma cell lines *in vitro* by targeting Class-I HDACs. Mechanistically TCN retarded cancer cell proliferation through the induction of cell cycle arrest in G2/M phase followed by augmenting the apoptotic cascades mediated by caspase-3. TCN had minimal effect on normal lung epithelial cell lines, hence, could be considered for further evaluation in animals for treating colorectal carcinomas. Additional studies are also warranted to test its selectivity and efficacy against other cancer types.[19]

TOXICITY EVALUATION OF ISOLATED PHYTOCHEMICALS

Acute toxicity analysis of chloroform and ethanolic extracts showed better safety profile in mice.[18] Acute exposure of triolein into the ascending aorta of dogs resulted in the severe intravascular accumulation of fat in the brain, heart and kidneys. In addition, infusion of triolein emulsion in to the blood retinal barrier (BRB) caused the opening of the inner and outer BRB. Furthermore, non-specific reactions of lungs were observed when triolein was given intravenously.[57]

ANIMAL MODELS TESTED USING THE PHYTOCHEMICALS ISOLATED FROM *SIMAROUBA GLAUCA*

Preclinical evaluation of isolated phytochemicals is essential before considering for further development in drug discovery pipeline. Therefore, compounds isolated from SG have been tested *in vivo* using tumor models.[29,30] For instance, intra peritoneal administration of glaucarubinone (1mg/kg for first 1-week and 2mg/kg for subsequent 5 weeks) and gemcitabine (20mg/kg) synergistically reduced pancreatic xenografted (using MiaPaCa-2 and PANC-1 cell lines) tumors growth *in vivo* by downregulating PAK1 and PAK4. Compared to monotherapies, the combined treatment (glaucarubinone 2mg/kg i.p. every otherday and gemcitabine 20mg/kg weekly i.p.) significantly reduced the tumour volume from day 32 in PANC-1 cells. Similarly, analysis of the *in vivo* data using MiaPaCa cell xenografts showed a significant decrease in the tumor volume with glaucarubinone and gemcitabine alone beginning from day 17.[30] However, combined treatment with glaucarubinone and gemcitabine relatively more potently decreased the tumor size of MiaPaCa-2 xenografts. The combination data was significant compared

to gemcitabine, especially from day 28, but not with glaucarubinone. The authors have reported a tumor growth reduction to 28% and 44% for glaucarubinone and gemcitabine respectively. The combined treatment yielded a tumor growth reduction to 20% of the controls (80% growth inhibition). In another study the combination of glaucarubinone and gemcitabine reported to improve survival by two-fold compared to gemcitabine treatment alone, indicating the potential use of SG phytochemicals in reducing cancers.[29] Further studies evaluating the (a) molecular mechanisms of tumor growth inhibition; (b) pharmacokinetics and pharmacodynamics properties; (c) efficacy of compounds isolated from SG for treating drug resistant tumor types are required.

CONCLUSION

In addition to the ethnopharmacological uses, *Simarouba glauca* is of great importance for its chemical diversity due to the presence of quassinoids, alkaloids, terpenes, steroids, coumarins and saponins. Despite having potent anticancer properties, to date, no systematic research, using phytochemicals isolated from SG, has been carried out to explore the molecular mechanisms leading to cancer cell death. Therefore, future studies should focus on elucidating the molecular targets of these phytochemicals to improve the safety and potency

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

ATP: Adenosine triphosphate; **BAX:** Bcl2-associated X-protein; **CK1:** Casein kinase 1; **GSK3:** Glycogen synthase kinase 3*;* **DNA:** Deoxy ribonucleic acid; **HIF1α:** Hypoxia-inducible factor 1-alpha; **IMP:** Inosine monophosphate; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **p21:** Protein 21 (A cyclin dependant kinase inhibitor); **p53:** Protein 53 (A tumor suppressor); **PAK1:** P21-Activated kinase; **RNA:** Ribonucleic acid; ***S.glauca*/SG:** *Simarouba glauca;* **TCN:** Tricaproin; **WNT:** Wingless Integrated.

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