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ORIGINAL ARTICLE

Pharmacognosy and Pharmacology of *Calotropis gigantea* for Discovery of Anticancer Therapeutics

Zahra Khosravi[1,2], Arun H. S. Kumar[2]

[1]School of Medicine and Medical Science, University College Dublin, [2]Veterinary Biosciences, School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland

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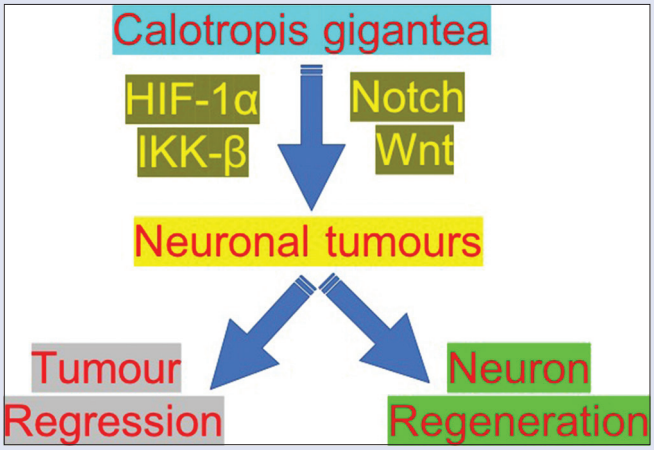
ABSTRACT

Background: *Calotropis gigantea* (CG) is a shrubby plant which is traditionally used for the treatment of diseases affecting the nervous system, digestive system, and skin. Several bioactive compounds from CG are isolated and investigated for their pharmacological properties. **Methods:** A systematic analysis of PubMed, Google Scholar, and PubChem database was performed, and the pharmacognosy and pharmacological properties of CG compounds were correlated. **Results:** Major phytochemicals such as those of the cardenolide, uscharin, and calotropin family were dominant in the CG plant. Phytochemicals found in the leaves, root, and latex of CG plant showed selective cytotoxicity, proapoptotic effects, and cell cycle arrest potential by modulating hypoxia-inducible factor 1 α, inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β), Notch, and Wnt signaling. **Conclusion:** The selective cytotoxicity against neuronal tumors, together with their neurogenesis/synaptogenesis potential and drug candidate like features merit the development of CG compounds as therapeutics for neuronal tumors.

Key words: Anti-cancer drugs, apoptosis, *Calotropis gigantea,* pharmacognosy, regeneration, synaptogenesis

SUMMARY

- In summary, this review highlights the potential of several compounds from the various parts of *Calotropis gigantea* (CG) in therapeutic drug development based on their correlation with established bioactivities. The major phytochemicals highlighted in this study were cardenolide, calotropin, and uscharin family of compounds which were present in the leaves and latex of CG. The CG compounds may be particularly useful in the development of anti-cancer therapeutics due their mechanistic targeting of several synergistic pathways involved in tumor growth. On account of the promising drug candidate like features of compounds in CG and their synergistic targeting of several collateral pathways of tumor proliferation, this study establishes the merit of studies evaluating their development for clinical use.



Abbreviations used: CG: *Calotropis gigantea*; ROS: Reactive oxygen species; IKK-β: inhibitor of nuclear factor kappa-B kinase subunit beta; MCF7: Michigan Cancer Foundation-7; HIF-1α: Hypoxia inducible factor- alpha; CBA: Cell based assay; DEHP: Di-(2-ethylhexyl) phthalate

Correspondence:

Dr. Arun H. S. Kumar,
School of Veterinary Medicine, University College Dublin, Belfield, Dublin-04, Ireland.
E-mail: arun.kumar@ucd.ie

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INTRODUCTION

Calotropis gigantea (CG) is latex-producing large shrubby plant of the *Apocynaceae* family which serves as host for variety of insects including butterflies and bees.[1] This shrub has clusters of lavender or white waxy flowers with pointed petals around a small crown. The flowers have a long self-life and hence are used for floral decorations. The latex of this plant is rich in calcium oxalate[2] which when in contact with the skin or eyes can lead to intense irritation, conjunctivitis, and corneal opacity. The ideal habitat of CG is in South-east Asia and Africa, wherein the tropical dry weather provides an ideal environment for its growth.[3]

Several bioactive compounds are reported from the various parts of CG, which are traditionally been used to treat dermatological, digestive, respiratory, neurological, and circulatory complications.[3-5] The extract of CG flowers and leaves is reported to have hypoglycemic effects.[1,2,5] The stem extract of CG is reported to be used as an expectorant and in the treatment of pneumonia and splenomegaly. The latex of CG also contains several fatty acids and cardiac glycosides due to which it is traditionally used in the treatment of heart failure.[2,5,6] The anti-inflammatory pharmacological properties of CG plant parts has lead to its use in the treatment of rheumatism and certain tumors. The leaves and flowers of CG contain many cardenolides, and hence, infusions of leaves or flowers are used for the treatment of burns.[1,5] Some of the glycosides isolated from the latex of the CG are reported to have inhibitory effects on sodium-potassium ATPase pump.[7] In Ayurveda, the CG root and leaf extracts are used for the treatment

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of asthma, bacterial infections (syphilis), while the bark is used for improving several liver and spleen ailments. Extracts of CG have also shown efficacy against several cancers, including lung, liver, and colon cancers by increasing caspase activity and reducing the expression of antiapoptotic proteins.[8,9] The selective cytotoxicity of CG extract against endothelial cells compared to the epithelial cells together with it analgesic properties,[10] perhaps merits understanding its pharmacology for the development of anti-cancer therapeutics.

Although the ethno-medical use of CG plant extract is widely documented in Ayurveda and traditional medicinal practices, the pharmacological rationale behind such therapeutic effects is not exclusively established. Therefore, this study was designed to review the literature on CG and document its pharmacognosy and correlate it with potential pharmacological effects. Such a correlation of pharmacognosy with pharmacological effects will be a first step in the drug discovery and development process to facilitate validation of potential therapeutics.

APPROACH TO REVIEWING PHARMACOGNOSY AND PHARMACOLOGY OF *CALOTROPIS GIGANTEA*

A systematic review of the literature on CG was conducted using the following two databases i.e., (1) PubMed (http://www.ncbi.nlm.nih.gov.ucd.idm.oclc.org/pubmed) and (2) Google Scholar (https://scholar.google.com) with no set timeline restriction on the date of the reported publications. "CG" was used as the search keyword and articles published from 1970 onwards to 2020 were reviewed. A third database, PubChem (https://pubchem.ncbi.nlm.nih.gov), was then used to search for the chemical structure and properties of the compounds reported to be isolated from CG plant. Only the articles published in the English language were reviewed in this study.

The articles published in PubMed or Google scholar were screened for any compounds isolated from CG, which were then recorded based on whether the compounds isolated were present in leaves, stems, roots, flowers, fruits, and/or other parts of the plant. The reported bioactivity (if any) of the compound was also recorded and the product that exhibited this was then subclassified into either the bioactivity was reported for crude extract or pure compound. The test system used to study the bioactivity and the species in which the bioactivity was assessed (cells, animals, and humans) was also recorded. Each of the isolated compound was then searched in the PubChem database and its PubChem ID, molecular weight and Log *P* value was documented (when reported).

PHYTOCHEMICALS IN *CALOTROPIS GIGANTEA*

PubMed yielded 452 articles of which 25 were included in this report because they explicitly and specifically reported compounds isolated from the different parts of the plant. Google Scholar yielded 8960 articles, of which 16 were included in this review after being assessed against the criteria mentioned above.

MAJOR PHYTOCHEMICALS PRESENT IN THE LEAVES OF *CALOTROPIS GIGANTEA*

Most of the phytochemicals of CG reported in the literature were present in the leaves of this plant. A total of 62 compounds were identified in the leaf of CG, which are summarized in Table 1. The majority of compounds are hydroxy and cholesterol containing compounds such as (24R)-24-ethylcholest-4-en-3-one, (E)-3-(4-meth oxyphenyl-2-O-beta-D-4C1-glucopyranoside)-methyl propenoate and 12b-hydroxycoroglaucigenin.[11,12] A lot of these enantiomers had log *P* values >8, which could pose considerable challenge when developing them for therapeutic applications.[13]

The compounds containing hydroxyl groups had lower log *P* values. For example, 15 β-hydroxycalotropin has a log *P* value of 0, while 5-Hydroxymethylfurfural has a log *P* = 0.6.[4,6] Major cardenolides such as 18,20-epoxycardenolide and 19-Nor-epoxycardenolide which are both cytotoxic, were also found in the leaves of the plant.[2] A majority of the compounds found in the leaf had cytotoxic bioactivity, which was predominantly established using cell-based assays [Table 1]. Some of the compounds did not have any known bioactivity associated with them. A few compounds showed free radical scavenging activity, which was demonstrated using both cell-based assays and in animal models. Some compounds showed pro-apoptotic effects which were potentially mediated by modulation of Hypoxia-inducible factor 1 (HIF-1) α or Notch signaling. Stigmasterol and Gamphoside were reported to have neurogenesis and synaptogenesis potential, a feature which can potentially find the application of these compounds for the regeneration of neurons and therapy of Alzheimer's disease.

MAJOR PHYTOCHEMICALS PRESENT IN THE FLOWERS OF *CALOTROPIS GIGANTEA*

Literature review of compounds isolated from CG flowers, showed 13 compounds, which are summarized in Table 2. Compounds in the flowers of CG contain molecules with benzene and phenol rings such as Folic acid, 9,11-dehydroergosterol peroxide, and 2-Methoxy-4-vinylphenol. Some of these compounds are reported to aid in the green synthesis of copper nanoparticles, suggesting that the plant products can be helpful for its mechanical properties as well.[14] The compounds ergosterol peroxide and 9,11-dehydroergosterol peroxide are epidioxysterols that are exclusively found in the flowers of CG, which inhibit the nuclear factor inhibitor IKK-β. They have log *P* values of 6.7 and 6.1 and molecular weights of 426.6 and 428.6 Dalton, respectively.[15] Overall, the compounds found in the flowers of *CG* had higher molecular weights than those in the leaves. Predominant number of compounds in the flower of CG showed cytotoxic and free radical generation bioactivity in both cell-based assays and in animal models, suggesting a greater potential of these compounds in the development of anticancer therapeutics.

MAJOR PHYTOCHEMICALS PRESENT IN THE FRUITS OF *CALOTROPIS GIGANTEA*

There were 23 compounds identified from fruits of CG [Table 3]. Most of the bioactivity of the compounds from CG fruits was performed in cells-based assays. Many of the compounds in the fruits of CG contain hydroxyl groups, namely those of the uscharin family. These include compounds such as 15 β-hydroxyuscharin, 16α-hydroxyuscharin, and 19-deoxy-15 β-hydroxyuscharin,[16] all of which showed HIF-1α inhibition potential. Considering the widely reported role of HIF-1α in ischemic reperfusion injuries, these compounds may be potentially useful in the development of therapeutics for myocardial infarction and stroke. Further analysis revealed that the beta configurations of these compounds at the C-2' and C-3' positions were relatively effective in suppressing transcriptional activity of HIF-1α.[16]

Uscharin compounds and their stereoisomers are reported to contain thiazoline rings and aldehyde groups, which sets them apart from the other chemicals isolated and paraphs account for their HIF-1α inhibition potential. The log *P* values of these compounds were in the ranges of 0.9–1.8,[17,18] supporting the potential of these compounds for therapeutic development. Besides HIF-1α inhibition, compounds present in the fruits of CG also showed antimicrobial and cytotoxic bioactivity

Table 1: Phytochemicals present in the leaves of *Calotropis gigantea*

Phytochemicals	Bioactivity/system studied
Calotropin, Afroside, Calatropagenin	Induced apoptosis/CBA
(+)-Pinoresinol, (+)-pinoresinol 4-O-[6″-O-vanilloyl]-β-d-glucopyranosi de, (+)-Syringaresinol, (24R)-3beta-hydroxy-24-ethylcholest-5-en-7-one, 12b-hydroxycoroglaucigenin, 14α-hydroxypregna-4,6-diene-3,20-dione, 15β-hydroxycalotropin, 16a-hydroxycalotropagenin, 18,20-Epoxycalotropin, 18,20-epoxycardenolide, 19-nor-10-hydrocalactic acid methyl ester, 19-Nor-epoxycardenolide, 2-Methylpyridin-3-ol, 6β-hydroxy-24-ethylc holest-4,22-dien-3-one, 6-O-(E-4-hydroxycinnamoyl) desglucouzarin, 9,12,13-trihydroxyoctadeca-10(E),15(Z)-dienoic acid, 9′-methoxypinoresinol, Calofurfuralside A, calotoxin, Calotropone, Carofurfuralside B, Drummondol, Isoliquiritigenin, Loliolide, Medioresinol, Pinoresinol, pregna-4,6-diene-3,20-dione, R-mevalonolactone, Salicifoliol, Uzarigenin, β-sitosterol	Cytotoxic/CBA
3,3’-dimethoxyquercetin, 3,6,7,3’,4’-pentamethoxyquercetin, Azaleatin, Quercetin, isorhamnetin	Free-radical scavenging activity/CBA and animals
5-Hydroxymethylfurfural, isorhamnetin 3-O-β-D-rutinoside	Cytotoxic, ROS generation/CBA and animals
Calactin, Uscharin	Induced apoptosis or HIF-1α inhibition/CBA
Carboxylcalactic acid methyl ester	Notch signal inhibition/CBA
Diterpene, linoleic acid, triterpene, palmitic acid	Antimicrobial/CBA
Gamphoside, stigmasterol	Induces apoptosis, neurogenesis, synaptogenesis/CBA and animals
(24S)-24-ethylcholest-4,22-dien-3-one	No activity reported/not reported
(E)-3-(4-methoxyphenyl-2-O-beta-D-4C1 -glucopyranoside)-methyl propenoate, (24R)-24-ethylcholest-4-en-3-one, 2a ,15b -Dihydroxy-19-oxo-uzarigenin, 3,5,8-trihydroxy-24-methylcholest-6,22-diene, caffeic acid, isoquercitrin, Isorhamnetin-3-O-glucopyranoside, Isorhamnetin-3-O-rutinoside, methyl caffeate, quercetagetin-6-methyl ether 3-O-beta-D-4C1-galacturonopyranoside, taraxasteryl acetate, isorhamnetin 3-O-β-D-robinoside	No activity reported/CBA and animals

CBA: Cell based assay; ROS: Reactive oxygen species

Table 2: Major phytochemicals present in the flowers of *Calotropis gigantea*

Phytochemicals	Bioactivity/system studied
2-Methoxy-4-vinylphenol, 3, 7, 11-trimethyl-1-dodecanol, 5-Hydroxymethylfurfural,	Cytotoxic
6-Acetyl-Beta-d-mannose, Tetraacetyl-d-xylonic nitrile	ROS generation/animals
9,11-dehydroergosterol peroxide, ergosterol peroxide	IKK-β inhibition/CBA
Anhydrosophoradiol-3-acetate	Antibacterial, cytotoxic, antifungal/CBA and animals
DEHP	Cytotoxic, antimicrobial/CBA and animals
Folic acid	Cytoprotectant antioxidant/animals
Isorhamnetin-3-O-glucopyranoside, isorhamnetin-3-O-rutinoside, taraxasteryl acetate	No activity reported/CBA

CBA: Cell based assay; ROS: Reactive oxygen species; DEHP: Di-(2-ethylhexyl) phthalate

Table 3: Major Phytochemicals present in the fruits of *Calotropis gigantea*

Phytochemicals	Bioactivity/system studied
15β-hydroxycalotropin	Cytotoxic/CBA
15β-hydroxyuscharin, 19-deoxy-15β-hydroxyuscharin, 2″-oxovoruscharin	HIF-1α inhibition Cytotoxic/CBA
16α-hydroxyuscharin	Notch signal inhibition/CBA
2′-epi-uscharin	HIF-1α inhibition, angiogenic nanocarrier, cytotoxic/CBA
Afroside	Induces apoptosis/CBA
Anhydrosophoradiol-3-acetate	Antitumoric, Antibacterial, cytotoxic, antifungal/CBA
Asclepin	HIF-1α inhibition, cytotoxic, notch signal inhibition, Wnt signalling inhibition/ CBA
Calactin	Cytotoxic, ROS generation Retinoic-acid-related orphan receptor antagonization, T cell differentiation inhibition, HIF-1α inhibition/CBA
Calactinic acid methyl ester	Notch signal inhibition/CBA
Calotoxin	Cytotoxic/CBA
Calotropin, gomphoside, uscharin	HIF-1α inhibition, cytotoxic, induces apoptosis/CBA
DEHP	Antibacterial, cytotoxic, antifungal/animals
Uscharidin	Notch signal inhibition β-catenin inhibition Wnt-signalling inhibition/CBA
Isorhamnetin-3-O-glucopyranoside, Isorhamnetin-3-O-rutinoside, taraxasteryl acetate, 15β-hydroxycalactin, 3′-epi-afroside, 3′-epi-gomphoside	No activity reported

CBA: Cell based assay; ROS: Reactive oxygen species; DEHP: Di-(2-ethylhexyl) phthalate

supporting their role in development of anticancer therapeutics. Some of the compounds were also observed to inhibit Notch and Wnt signaling, which could have potential application in regenerative therapeutics.

MAJOR PHYTOCHEMICALS PRESENT IN THE STEM OF *CALOTROPIS GIGANTEA*

The stem of CG was observed to contain 16 compounds, which are summarized in Table 4. Similar to the fruits, the compounds in the stem showed HIF-1α inhibition, although most of these studies were performed using cell-based assays. Cardenolides such as coroglaucigenin, corotoxigenin, and digitoxigenin[19] were predominantly present in the stem extracts. The cardenolide compounds are steroid constituents which contain a cis ring, tertiary hydroxyl group, and a butenolide substituent.[19] The cardenolide compounds showed cytotoxic and reactive oxygen species (ROS) generation potential,[19] which are often valuable traits of anti-cancer therapeutics. The logP values of these compounds ranged from 1.3 to 2.6 and these compounds were selectively cytotoxic against cancer cells but not healthy epithelial cells.[19] Thus, these compounds may be suitable for the development of anti-cancer therapeutics.

Two compounds exclusive present in the stem of CG plant were (19s)-3 β,19-epoxy-2α,3 β,14 β-trihydroxy-19-methoxy-5α-c ard-20 (22)-enolide and 6'-O-(E-3,5-dimethoxy-4-hydroxycinnamoy l) desglucouzarin, which are recently identified. We did not find any record of these compounds in the PubChem database. However, their pure extracts have been reported to have cytotoxic and ROS generating potential.[19] Isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside and taraxasteryl acetate were found in multiple parts (stem, leaves, fruits, and flowers) of the CG plant. These compounds present in multiple parts of the plant are flavonol glycosides with log *P* values of 1 and 9.7[20] and exhibited cytotoxic bioactivity. Triterpenoid was one of the compounds observed in the stem extract which showed anti-angiogenic, anti-inflammatory, antioxidative, and hepatoprotective bioactivity in animal models. This collective feature can find application in several therapeutic strategies.

MAJOR PHYTOCHEMICALS IN THE ROOTS OF *CALOTROPIS GIGANTEA*

The root extract of CG showed 33 compounds, which are summarized in Table 5. The roots of CG were found to have most of the calotropsides and their related compounds. Calotroposides A-E are glycoside triptopenoids that can be further classified into oxypregnane-oligoglycosides because they all contain an oxygen moiety.[6,12] Out of all the compounds isolated so far, these are the only ones which have molecular weights higher than 1000 dalton, which could pose a challenge for their therapeutic development, despite their reported cytotoxic potential against colon cancer cells through induction of apoptosis. Several compounds in the roots did not have any reported bioactivity associated with them, which highlights the need for more basic scientific observation of these structures to understand their therapeutic potential. Another dominant family of compounds found specifically in the roots of CG plant belongs to the gigantursenol family. Gigantursenol are pentacyclic triterpenic esters with log *P* values ranging from 6.8 to 8.8. Lupeol acetate B is another pentacyclic triterpenic ester found in the roots of CG with a high log *P* value of 10.4 but no known bioactivity was associated with this compound.[21]

COMPOUNDS FOUND IN ALL OTHER PARTS OF *CALOTROPIS GIGANTEA*

A total of 36 compounds were isolated from CG plant parts other than leaves, flower, fruits, stem and roots, which are summarized in Table 6. Other parts of CG refer to its latex, sap, and fiber component. The major compound family in these parts of the plant belongs to the calotropin family. Calotropins DI and DII are proteases found in the latex which contain a single sulfhydryl group responsible for their caseinolytic enzymatic activity.[22] Calotropains FI and FII are also proteases present in the latex which show caseinolytic activity[2] and are reported to be cytotoxic in cell-based assay platforms. α-calotropeol and β-calotropeol are natural polymers from the latex of CG which shows encapsulation efficiency and yield biocompatible and biodegradable products for medicinal use.[18] Several cytotoxic compounds such as 15b-Hydroxycaltropin, 15 β-hydroxyuscharin, and uscharin were also found in leaves, flower, fruits, stem, and roots sharing similar bioactivity.

RELEVANCE OF PHYTOCHEMICALS IN *CALOTROPIS GIGANTEA* FOR DISCOVERY OF ANTICANCER THERAPEUTICS

Understanding the pharmacological properties of the phytochemicals from CG may pave way for therapeutic drug development. Conventionally, CG plant part extracts have been used in the treatment of various ailments and understanding the pharmacology of its phytoconstituents will help evidence-based recommendation of its use in mainstream therapeutics. The evidence based could then be further advanced through detailed mechanisms of the actions at specific target/s through the use of *in silico* or *in vitro* screening platforms for selecting most optimal molecules into drug development pipelines. The most widely reported pharmacological effects of the CG were the cytotoxicity against cancer cells through triggering apoptosis, HIF inhibition, cell-specific ROS generation, and cell cycle arrest, which supports

Table 4: Major Phytochemicals present in the stem of *Calotropis gigantea*

Phytochemicals	Bioactivity/system studied
(19s)-3β,19-epoxy-2α,3β,14β-trihydroxy-19-methoxy-5α-card-20 (22)-enolide, 6'-O-(E-3,5-dimethoxy-4-hydroxycinnamoyl) desglucouzarin, Coroglaucigenin, Uzarigenin	Cytotoxic, ROS generation/CBA
Afroside, Calatropagenin, Gamphoside	Induces apoptosis/CBA
Calactin	Induced apoptosis HIF-1α inhibition/CBA
Calotropin	HIF-1α inhibition, cytotoxic Induces apoptosis/CBA
Uscharin	HIF-1α inhibition/CBA
Triterpenoid	Antiangiogenic, Anti-inflammatory, antioxidative Hepatoprotective/animals
Isorhamnetin-3-O-glucopyranoside, Isorhamnetin-3-O-rutinoside, taraxasteryl acetate, Corotoxigenin, Digitoxingenin	No activity reported

CBA: Cell based assay; ROS: Reactive oxygen species

Table 5: Major phytochemicals in the roots of *Calotropis gigantea*

Phytochemicals	Bioactivity/system studied
4'-O-β-D-glucopyranosylfrugoside, 18, 20-epoxycardenolide, 19-dihydrocalactin, 19-Nor-epoxycardenolide, Calotropone, Gofruside	Cytotoxic/CBA
15β-hydroxyuscharin	HIF-1α inhibition Cytotoxic/CBA
Calotropin	HIF-1α inhibition, cytotoxic Induces apoptosis/CBA
Calotroposide A	Induces apoptosis, caspase 8 expression/CBA
Coroglaucigenin	Cytotoxic, ROS generation/CBA
Procesterol	Antiamoebic activity/CBA
Stigmasterol	Neuritogenesis, synaptogenesis, cytotoxic/CBA and animals
Lupeol acetate B, giganturseno l, giganturseno l A, Giganturseno ne, Giganturseny l acetate A, Giganturseny l acetate B, Calotroposide (B, C, E, F, G, D), Calotropis juiterpenol, calotropisesquiteerpenol, Calotropisesterterpenol, calotroplupenyl acetate, Calotropnaphthalene, 4′β, 15β-dihydroxycalactin, Beta-Sitosterol, Calotropbenzofuranone, 15β-hydroxycalactinic acid methyl ester	No activity reported/CBA

CBA: Cell based assay; ROS: Reactive oxygen species

Table 6: Compounds found in all other parts of *Calotropis gigantea*

Phytochemicals	Bioactivity/system studied
15b-Hydroxycaltropin, Afroside	Induces apoptosis/CBA
15β-hydroxycalotropin, calotoxin	Cytotoxic/CBA
15β-hydroxyuscharin, 19-deoxy-15β-hydroxyuscharin, 2″-oxovoruscharin	HIF-1α inhibition, cytotoxic/CBA
16α-hydroxyuscharin, calactinic acid methyl ester, Voruscharin	Notch signal inhibition/CBA
2′-epi-uscharin, Beta-amyrin, α-calotropeol, β-calotropeol	HIF-1α inhibition, cytotoxic, angiogenic, nanocarrier/CBA
9,10,13-Trihydroxyoctadex-11-enoic acid	ROS generation
Asclepin	HIF-1α inhibition, cytotoxic, notch signal inhibition, Wnt signaling inhibition/CBA
Calactin, Kaempferol-4'-O-Rutinoside	Induced apoptosis, HIF-1α inhibition/CBA
Calotropain-FII, calotropin- DI, calotropin-DII	Caseinolytic/CBA
Calotropin, gomphoside, and uscharin	HIF-1α inhibition, cytotoxic, induces apoptosis/CBA
Erycordin	ROS generation induces apoptosis, cell-cycle arrest/CBA
Lupeol	Angiogenic, nanocarrier, cytotoxic anti-inflammatory/CBA and humans
Nicotiflorin	Induces apoptosis, cell cycle arrest, ROS generation/CBA
Rutin, Frugoside	Anti-cancer/CBA
Triterpenoid	Collagen synthesis, angiogenic anti-inflammatory, ros generation/animals
Uscharidin	Notch signal inhibition, β-catenin inhibition, Wnt-signalling inhibition/CBA
ψ-taraxasterol, 15β-hydroxycalactin, 3′-methylbutanoates α-amyrin, 3′-epi-afroside, 3′-epi-gomphoside	No activity reported/CBA

CBA: Cell-based assay; ROS: Reactive oxygen species

the need for studies to evaluate the potential of its phytoconstituents in developing anticancer drugs. Besides this, some of the compounds from CG also showed potential application in tissue reperfusion injuries through their targeting of HIF, Notch, and Wnt signaling. Furthermore, of considerable interest was some CG compounds showing neurogenesis and synaptogenesis potential, both features can be beneficial in the therapeutics of nervous system disorders requiring tissue regeneration.

The majority of the compounds isolated from CG showed cytotoxic activity, which was either established in cell-based assay system or in animal models. Calotropin elucidated potent cytotoxic effects against many pure cancer cell lines such as MCF-7 cells (human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors)[16] through inhibiting HIF-1α. Calotropin and uscharin also inhibit Notch1 signaling, affecting neuronal differentiation which could merit its development for the treatment of neural tumors.[23] In one study, anhydrosophoradiol-3-acetate was reported to be lethal against brine shrimp.[24] Similarly, copper oxide nanoparticles isolated from compounds such as 5-Hydroxymethylfurfural was reported to be cytotoxic to zebrafish embryos[14] possibly by regulation of Notch and Wnt signaling. Overall, the cytotoxic effects of significant number of compounds isolated from CG plant parts have promising features of drug like candidates, which merit further studies in suitable preclinical models for their potential development for clinical use.

Many compounds such as erycordin, nicotiflorin, and narcissin showed potential to generate ROS in A549 and NCI-H1299 lung cancer cells[25] which could show that compounds isolated from CG have potential in treating tumors by synergistic mechanisms involving both direct cytotoxicity and ROS induced cell death. (19s)-3 β, 19-epoxy-2α, 3 β, 14 β-trihydroxy-19-methoxy-5α-card-20 (22)-enolide, uzarigenin, and coroglaucigenin are cardenolides that have shown the suppression of antioxidant molecule expression of cancer cells while not affecting normal and this mechanism may selectively induce cancer cell regression with minimal adverse effects. ROS generation and suppression of antioxidant molecules is widely reported to be effective in suppressing the growth of cancer cells, which can be helpful in treatment of both benign and metastatic tumors.

Some compounds isolated from CG were reported to have apoptotic effects on isolated cancerous cells. For example, crude extracts of 15b-Hydroxycaltropin, afroside and uscharin-induced apoptosis in non-small lung cancer cells and human breast carcinoma cells[2,25] by altering cell morphology. Compounds that are cytotoxic and generate ROS such as calotropin and calactin had superior apoptotic effects,

which validate the potential synergistic benefits from CG compounds by influencing multiple biochemical pathways.[2,25] Nevertheless, these crude extracts need to be purified and investigated to test if such synergistic effects are retained by purified compounds, which will add to the merit of their further development for the clinical use in the treatment of a variety of cancers. Furthermore, the cell cycle arrest potential of the CG compounds may further add to their synergistic potential by effectively being cytotoxic to cancer cells. Indeed, the crude extracts consisting of erycordin, narcissin, and nicotiflorin directly arrest the cell cycle through the stimulation of cyclin-dependent kinase inhibitor p27. This kinase controls cyclin D, which is involved in the cell cycle and specifically restricts the G1 phase, which can curtail unlimited cell growth[2,25] specifically observed in highly aggressive tumor types. Three compounds in CG were identified to have direct anti-cancer effects when tested against human cancer cell lines. Anhydrosophoradiol-3-acetate, frugoside, and rutin are anti-cancer compounds which decreased the viability of A549 and NCI-H1299 cells in a dose-dependent manner with efficacy similar to that of doxorubicin.[2,25] It will be interesting to see if such superior cell-based efficacy can be effectively translated for clinical application.

In summary, this review highlights the potential of several compounds from the various parts of CG in therapeutic drug development based on their correlation with established bioactivities. The major phytochemicals highlighted in this study were cardenolide, calotropin, and uscharin family of compounds which were present in the leaves and latex of CG. The CG compounds may be particularly useful in the development of anti-cancer therapeutics due their mechanistic targeting of several synergistic pathways involved in tumor growth. On account of the promising drug candidate like features of compounds in CG and their synergistic targeting of several collateral pathways of tumor proliferation, this study establishes the merit of studies evaluating their development for clinical use.

CONCLUSION

The selective cytotoxicity of phytochemicals from CG against neuronal tumors, together with their neurogenesis/synaptogenesis potential and drug candidate like features merit their development as therapeutics for neuronal tumors.

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Conflicts of interest

There are no conflicts of interest.

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