E-ISSN: 2278-3229

IJGHC, June 2020 –August 2020 Sec.B; Vol.9, No.3, 292-309.
DOI: 10.24214/IJGHC/HC/9/3/29209.

International Journal of Green and Herbal Chemistry

An International Peer Review E-3 Journal of Sciences

Available online at www.ijghc.com

Section B: Herbal Chemistry

Research Article

CODEN (USA): IJGHAY

Phytochemistry and anticancer activity of anthraquinones from *Ceratotheca triloba* (Bernh.) E. Mey. ex Hook.f: a review

V. Mohanlall

Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, P. O Box 1334, Durban, 4000, South Africa

Received: 08 April 2020**; Revised:** 11 June 2020; **Accepted:** 15 June 2020

Abstract: *Ceratotheca triloba* (Bernh.) E. Mey. ex Hook.f is native to tropical and subtropical African countries and is used in traditional medicine to treat painful menstruation, stomach cramps, nausea, fever and diarrhoea. Anecdotal reports of its effects in cancer treatment and prevention, with many successful cases, have warranted that these pharmacological properties be scientifically validated. A bibliographic search was conducted using the key words "Ceratotheca", "anticancer", and "antitumor" along with cross-referencing. No clinical or animal cancer studies were identified and only two in vitro cell-culture-based studies were reported; these indicate that extracts of *C. triloba* may alter the growth of several types of cancer cell lines. This review summarizes the results of anthraquinones and emphasizes the aspects that warrant future research to explore the anthraquinones in *C. triloba* for their anticancer activities.

Keywords: *C. triloba*, antioxidant, mucilaginous, anthraquinones, Pedaliaceae

INTRODUCTION

C. triloba is a South African annual plant that is found in the summer rainfall areas of South Africa, mainly the grasslands. The plant is commonly called South African Foxglove, Wild foxglove,

Vingerhoedblom, Ludvonca (Swazi), Udonqa (Swazi, Zulu), Undoncalwabathwa or Udonqabathwa (Zulu). There are only four known species of Ceratotheca that are found in Southern Africa [1]. The genus name ―Ceratotheca‖ means a horned capsule which is derived from the Greek words *kerato* (horned) and *theke* (a case). The species name *triloba* is derived from Latin, meaning three-lobed, alluding to leaves [2]. The plant was first named *Sporledera triloba* Bernh. in 1842 and it has undergone several name changes since then **(Table 1).**

According to the current classification system **(Table 2)** it belongs to the family Pedaliaceae [3]**.** This family of plants is characterized by having mucilaginous hairs which give the stems and leaves a slimy or clammy feel. The fruits have hooks or horns. *C. triloba* germinates optimally in disturbed areas like roadsides, where they grow, flower and seed before winter. The height of the plant depends on the water uptake during summer. The leaves are soft, green and about 50 mm long; they are divided into three lobes with a bluntly serrated margin. The plant has pink flowers with red stems or white flowers with yellow-green stems **(Figure 1A).**

The flowers are 50 mm long with 5 lobes. The bottom lobe is longer than the others and has streaks of delicate lines running down the throat of the flower. The small seeds are black and are located in 30 mm long fruits which have very sharp horns at the tips (Figure 1B). The green fruits turn brown and dry and split open to release the flat pear-shaped seeds. The stems and leaves of the plant are covered with fine white hairs. *C. triloba* is slightly sticky and when crushed it produces a strong unpleasant smell [4].

C. triloba is used in many traditional cultures. Some people soak the whole plant in water and use it as a substitute for soap or shampoo. The plant is also used in traditional medicine to treat painful menstruation, stomach cramps, nausea, fever and diarrhea [5]. Other traditional uses include the preparation of infusions of the leaves which are administrated as an abortifacient and its use for the treatment of diarrhea and gastro-intestinal cramps [6-9]. Studies show that *C. triloba* serves as a good source of energy and magnesium [10,].

Literature on the biological activities of *C. triloba* show that the plant has no angiotensin 1-converting enzymes [11]. Extracts of the plant can be used to control diabetes as it inhibits α-amylases and also has antioxidant activity [12]. In addition, the plant has been shown to inhibit lipo-oxygenase, thus it can be used as an anti-inflammatory agent. Root extracts of *C. triloba* contain three anthraquinones, namely: 9,10-Anthracenedione, 1-Hydroxy-4-methylanthraquinone and 5,8-Dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione, and one steroid, Androst-5-ene-3, 17, 19-triol [13].

Reports have shown that these types of compounds possess anticancer activity [14-17]. Hairy root cultures of *C. triloba* were induced by *Agrobacterium rhizogenes* transformation to produce a higher yield of anthraquinones than cell suspension cultures and the roots of the field plant [18].

Also, the following compounds from the hairy root extract were identified: one acridone derivative (5-methoxy-2-nitro-10H-acridin-9-one), one naphthoquinone derivative (2H-naphto[2,3-b]pyran-5,10-dione,3,4-dihydro-2,2-dimethyl-) and seven anthraquinone derivatives (5,8-dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione; 2-methyl-9,10-anthracenedione; 1-hydroxy-4-methyl anthraquinone; 2-ethyl-9,10-anthracenedione; 1,5-diamino anthraquinone; 3,6-dimethoxy-9-methyl-phenanthrene; 1,4- dimethyl-9,10-anthracenedione. The anthraquinones have shown to inhibit the human topoisomerase II enzyme which transforms supercoiled DNA to linear DNA. This mechanism is currently the basis of many anticancer compounds such as mitoxantrone.

Table 1: History of *C. triloba* (Bernh.) E. Mey. ex Hook.f

Synonym	*Full Citation*
Ceratotheca lamiifolia (Engl.) Engl.	*Ceratotheca lamiifolia (Engler) Engler, Bot. Jahrb. Syst. 19: 156. 1894. BASIONYM: Sesamum lamiifolium Engler 1888.*
Sesamum lamiifolium Engl.	*Sesamum lamiifolium Engler, Bot. Jahrb. Syst. 10: 256, t. 8. 1888. Type: South Africa:*
Sporledera kraussiana Bernh.	*Sporledera kraussiana Bernhardi, Linnaea 16: 41. 1842. TYPE: South Africa:*
Sporledera triloba Bernh.	*Sporledera triloba Bernhardi, Linnaea 16: 42. 1842.Type: South Africa:*
Volkameria lamiifolia (Engl.) Kuntze	*Volkameria lamiifolia (Engler) Kuntze, Revis. Gen. Pl. 2: 482. 1891. BASIONYM: Sesamum lamiifolium Engler 1888.*

Table 2: Classification for Kingdom Plantae [19]

Kingdom Plantae –Plants
Subkingdom Tracheobionta -Vascular plants
Superdivision Spermatophyta -Seed plants
Division Magnoliophyta -Flowering plants
Class Magnoliopsida –Dicotyledons
Subclass Asteridae -Daisy superorder
Order Scrophulariales -figwort order
Family Pedaliaceae - Sesame family
Genus Ceratotheca Endl. - ceratotheca P
Species - *Ceratotheca triloba* (Bernh.) E. Mey. ex Hook. f.

Figure 1: Wild Foxglove - *C. triloba* plant (A) and seed pods (B) [20].

Secondary Plant Metabolites

Plants contain two classes of compounds; primary metabolites that are required for the livelihood of the plant i.e. the plants machinery, and secondary metabolites that are not a necessity for the plants survival but are produced to protect the plant against fungi, bacteria, insects, and viruses. These have been used as food flavourants, colour dyes, poisons, perfumes, industrial products and prescription drugs [21]. These secondary metabolites are the biggest source of pharmaceutical drugs and they serve as templates for many medicinal derivatives [22] currently used. The major groups of secondary metabolites and their physiological use and effects are summarized in Table 2 are: (i) Flavonoids and Allied Phenolics; (ii) Alkaloids and (iii) Terpenoids. Only anthraquinones are discussed in detail as they form a major part of this review.

Table 3: Major groups of secondary metabolites isolated from plants. [23]

Class of compounds	Subclass of compounds	Example Compounds	Some Effects and Uses
ALKALOIDS	Monoterpenes	Nicotine, cocaine theobromine	interfere with neurotransmission, block enzyme action
	Monoterpenes	Menthol, linalool	interfere with neurotransmission, block ion transport, anesthetic
	Sesquiterpenes	parthenolid	contact dermatitis
TERPENOIDS	Diterpenes	gossypol	block phosphorylation, toxic
	Triterpenes, cardiac glycosides	digitogenin	stimulate heart muscle, alter ion transport
	Sterols	spinasterol	interfere with animal hormones
	Phenolic acids	caffeic, chlorogenic	cause oxidative damage, browning in fruits and wine
	Coumarins	umbelliferone	cross-link DNA, block cell division
	Lignans	Podophyllin, urushiol	cathartic, vomiting, allergic dermatitis
PHENOLIC COMPOUNDS	Flavonoids	anthocyanin, catechin	flower, leaf color; inhibit enzymes, anti- and pro-oxidants, estrogenic
	Tannins	gallotannin, condensed tannin	bind to proteins, enzymes, block digestion, antioxidants
	Lignin	lignin	structure, toughness, fiber

PHYTOCHEMISTRY OF ANTHRAQUINONES FROM *CERATOTHECA TRILOBA*

Anthraquinones: Anthraquinones are a class of natural compounds that consists of several hundreds of compounds that differ in the nature and positions of substituent groups [24]. This class of compounds contains derivatives that consist of the basic structure of 9.10-anthraquinone [25]. Anthraquinones can be divided into alizarin and emodin types based on two main biosynthetic pathways. The alizarin types are formed via chorismate/δ-succinylbenzoic acid pathway and only have one of the rings unsubstituted [26,27]. These anthraquinones are found in the family of plants known as Rubiacea *(Rubia, Morinda, Galium, Cinchona)* [28] . The emodin types are formed via the polyketide pathway (acetate-malonate pathway) and have both rings substituted. These anthraquinones are present in the following plant families: Fabaceae *(Cassia, Araroba),* Rhamnaceae *(Rhamnus, Frangula)* and Polygonaceae *(Rheum, Rumex, Fagopyrum)* [25] . Anthraquinones are widely applied in medicine, food chemistry and the dye industry. In the pharmaceutical industry, the natural and synthetic derivatives of 9.10-anthraquinone are beneficial to mammals and humans as they can display antibacterial, antitrypanosomal and antineoplastic activities.

Anthracycline antibiotics are also 9.10- anthraquinones and are key substances that have been known to be used for therapy of several cancers [29]. Anthraquinone glycosides are used to produce Pyralvex to treat gingivitis, stomatitis, mouth ulcers, inflammatory oral mucosa and periodontal conditions. Senna is another member of the anthraquinone class which is used in the preparation of stimulant laxative drug, Senokot.

This drug is used to treat constipation or bowel evacuation prior to abdominal radiological procedures [30,31].The quinones are compounds with either a 1,4-diketocyclohexa-2,5-denoid or a 1,2-diketocyclohexa-3,5-denoid. The former types are p-quinones and the latter o-quinones. They may occur with one, two or three rings or as larger polycyclic quinones [32]. Hydroxylation and glycosylation reactions are responsible for the formation of a large variety of quinine structures.

Biosynthetic Pathways for the Production of Anthraquinones: A striking feature of quinones is that they can be derived from several biosynthetic routes, including a number of alternative pathways in plants. Leistner[33] identified at least six possible biosynthetic routes to benzoquinones. For naphthoquinones, two main routes have been characterized, involving contribution of a phenolic ring from the shikimate pathway and formation of the quinone ring with a mechanism involving either a-ketoglutarate or isopentenyl diphosphate derivatives.

At least two biosynthetic routes have also been suggested for the anthraquinones, either starting from a similar pathway to that for naphthoquinones or arising from acetyl-CoA and malonyl-CoA in the polyketide pathway [34].

The polyketide pathway that is common in fungi may also operate in Leguminosae, Rhamnaceae and Polygonaceae plant species. It involves one acetyl-CoA unit being extended by seven malonyl-CoA units, by undefined enzyme systems. The formation of anthraquinones via the chorismate pathway is well understood. The A- and B-rings of anthraquinones are formed by joining of isochorismate with a-ketoglutarate in the presence of thiamine diphosphate to form an o-succinylbenzoic acid intermediate, catalysed by the enzyme o-succinylbenzoic acid synthase.

Isochorismate is formed from chorismate by the enzyme isochorismate synthase (ICS). Elicitation of anthraquinone formation in cell cultures causes a marked increase in isochorismate synthase activity. In *M citrifolia* cultures this increase in isochorismate synthase activity was not accompanied by induction of other enzymes of the shikimate pathway that direct chorismate into other biosynthetic pathways, specifically deoxy-D-arabinoheptulosante 7-phosphate synthase and chorismate mutase [35] .

The biosynthetic pathway for the production of anthraquinones via the shikimic acid is regulated by the isochorismate synthase enzyme, thus providing a key regulatory target. The theory of isochorismate synthase being a key regulatory target is further supported by transgenic experiments using bacterial isochorismate synthase gene [36] . The recent isolation of the isochorismate synthase gene from *M. citrifolia* [37] should allow genetic approaches to test the role of the endogenous isochorismate synthase genes in controlling the rate of anthraquinone biosynthesis.

O-succinylbenzoic acid (OSB) is activated at the aliphatic carboxyl group to produce o-succinylbenzoic acid-Co-enzyme A ester (OSB CoA), a reaction carried out by the enzyme o-succinylbenzoic acid:Co-enzyme A -ligase[38]. Ring closure then produces the A- and B-rings as the intermediate 1,4- dihydroxynaphthalene-2-caroxylic acid (DHNA). Subsequent prenylation of DHNA yields a prenylated naphthoquinone intermediate and finally the formation of the C-ring (**Fig. 2).**

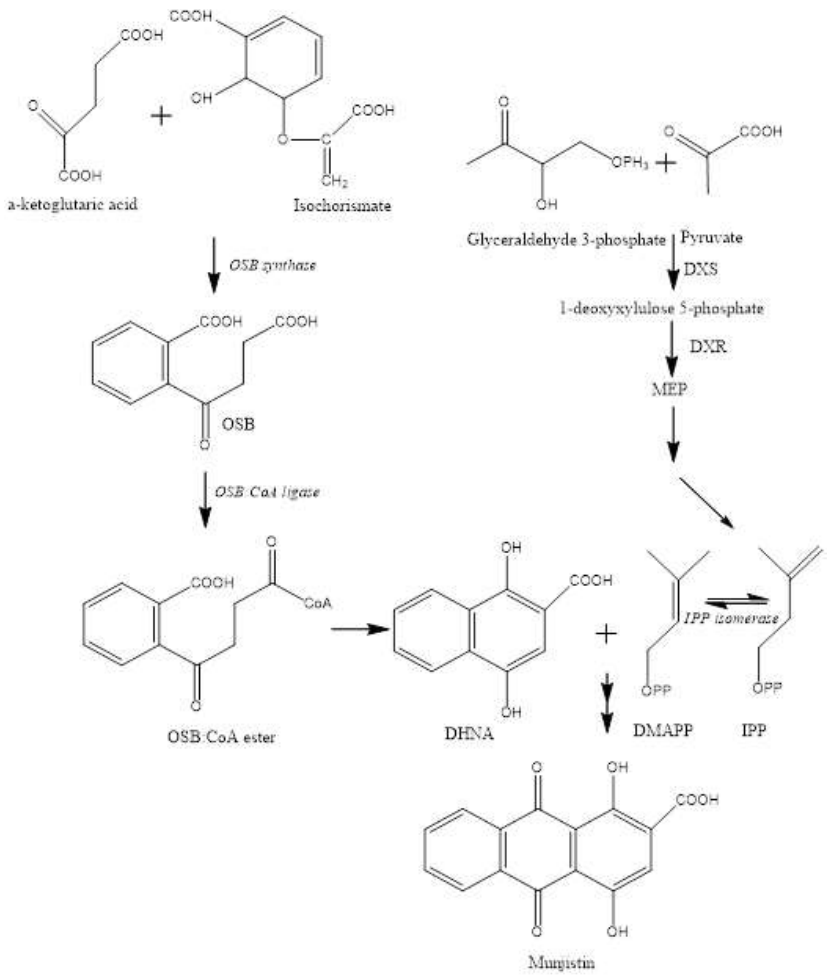

Figure 2: Proposed biosynthetic pathway for the anthraquinone pigments of the Rubiaceae. DXS (1-deoxy-D-xylulose 5-phosphate synthase), DXR (1-deoxy-D-xylulose 5-phosphate reductase).

The source of the prenyl groups is either isopenthyl diphosphate (IPP) or 3,3-dimethylallyl diphosphate (DMAPP), which are interconverted by the enzyme isopenthyl diphosphate isomerase [39]. Changes in isopenthyl diphosphate isomerase activity accompany induction of coloured anthraquinone production in Rubiaceae cell cultures [40] [41]. Induction of isopenthyl diphosphate isomerase activity at the same time as a reduction in the activity of the enzyme farnesyl diphosphate synthase (which converts IPP and DMAPP into farnesyl diphosphate) may assist in channeling more isopenthyl diphosphate into anthraquinone biosynthesis.

Biosynthesis of anthraquinones via the Acetate pathway: A number of natural anthraquinone derivatives are also excellent examples of acetate-derived structures. Endocrocin (Fig 3), found in species of *Penicillium* and *Aspergillus* fungi are formed by folding a polyketide containing eight C_2 units to form the periphery of the carbon skeleton.

Three aldol-type condensations would give a hypothetical intermediate 1, and, except for a crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reactions. Emodin, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction. A dehydration reaction, two oxidations, and a decarboxylation are necessary to attain the islandicin structure. In chrysophanol,

aloe-emodin, and rhein, the same oxygen function is lost by reduction as in islandicin, and decarboxylation also occurs.

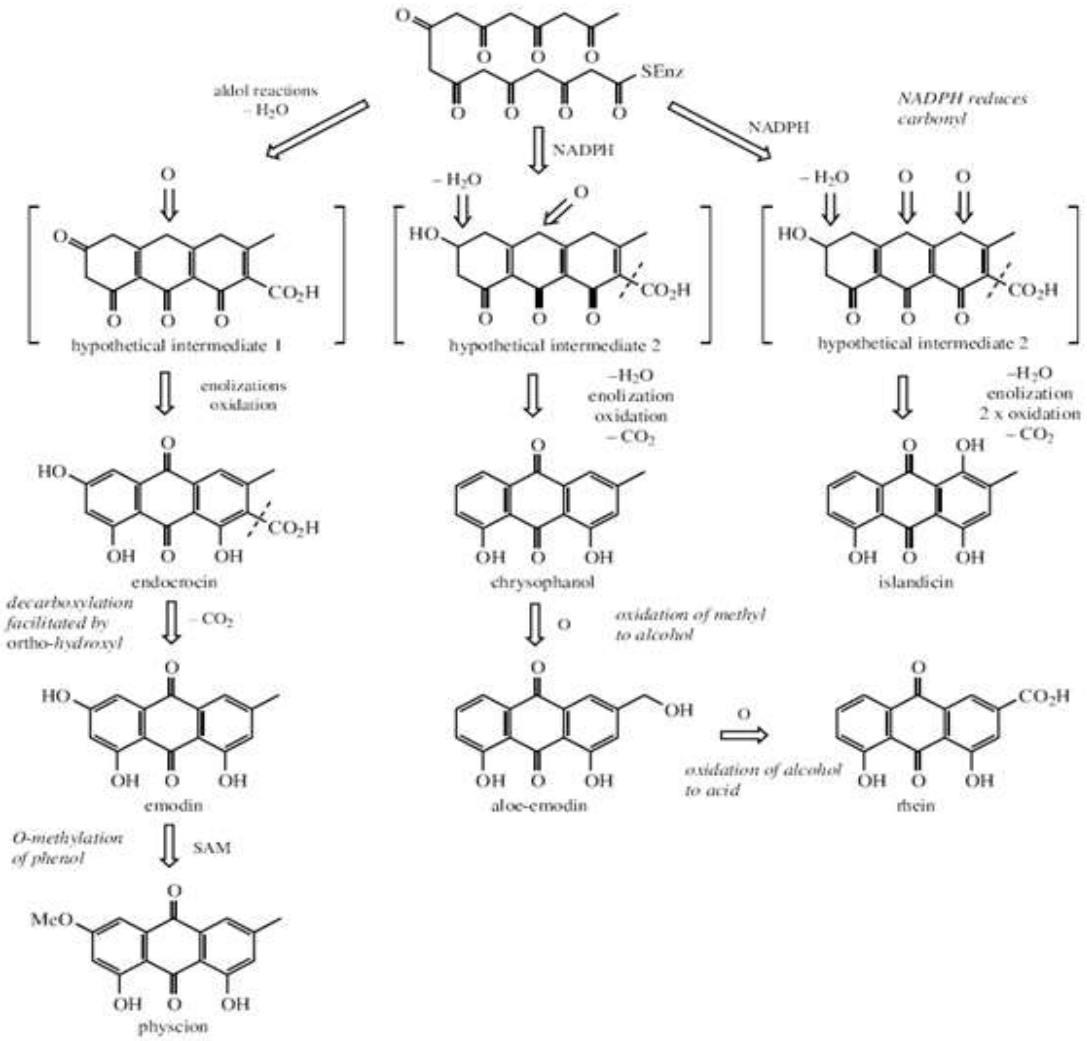

Figure 3: Speculative acetate pathways for the production of polyketides

The three compounds are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloe-emodin, and a carboxyl in rhein. The pathway outlined for the biosynthesis of endocrocin and emodin is shown in Fig 3. The only difference between the speculative pathway (Fig 2) and mechanistically correct pathway is the alteration of the sequence of reactions. Decarboxylation appears to take place before aromatization of the last-formed ring system, and tetrahydroanthracene intermediates such as atrochrysone carboxylic acid and atrochrysone are involved. These dehydrate to the anthrones, emodin and endocrocine, prior to introduction of the extra carbonyl oxygen as a last transformation in the production of anthraquinones.

Many other natural anthraquinone structures are not formed via the acetate pathway, but by a more elaborate sequence involving shikimate and an isoprene unit. Such structures do not contain the characteristic *meta* oxygenation pattern, and often have oxygenation in only one aromatic ring.

Emodin, physcion, chrysophanol, aloe-emodin, and rhein form the basis of a range of purgative anthraquinone derivatives found in long-established laxatives such as Senna, Cascara, Frangula, Rhubarb, and Aloes. The free anthraquinones themselves have little therapeutic activity and need to be in the form of water-soluble glycosides to exert their action.

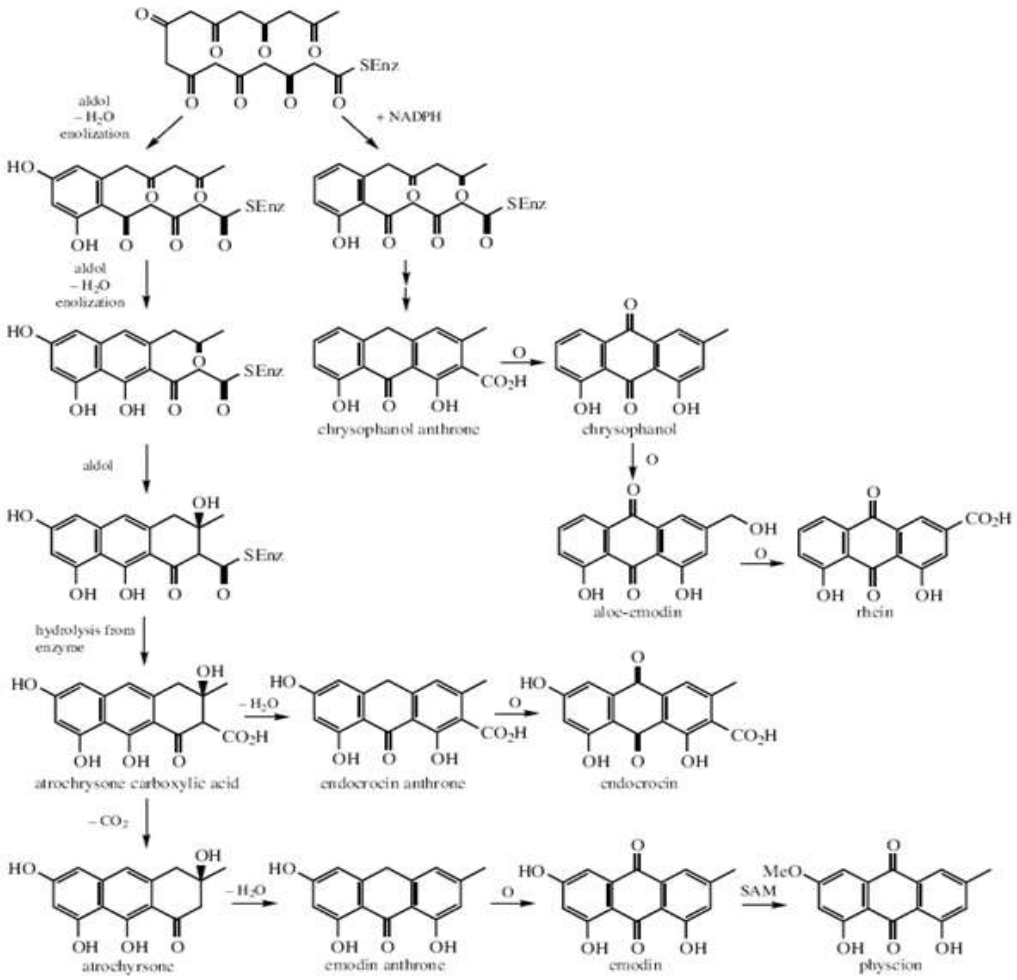

Figure 4: Biosynthesis of endocrocin and emodin via the acetate pathway.

Gene Regulation: Little information regarding the genes encoding the biosynthetic enzymes, or pathway regulatory factors has been published. Furthermore, it is not known which signals regulate the anthraquinone biosynthetic genes, although fungal elicitors, methyl jasmonate, salicylic acid and the protein phosphatase inhibitor cantharidin, all induce anthraquinone accumulation in transgenic cell cultures. On the other hand, light generally inhibits anthraquinone accumulation and the impact of auxins is variable [37,42-44] . These gaps in the knowledge of anthraquinone biosynthesis may start to be filled as data emerges from proteomic and gene studies [37].

The production of anticancer compounds in hairy root cultures: Cancer is a leading cause of death worldwide, accounting for 9.6 million deaths. [45]. Several chemo-preventive agents have been developed and are used for the treatment of cancer but the toxicity of these agents limits their use [46] . Thus there is a great interest to research new and better treatments. The plant kingdom is one of the most attractive sources of novel anticancer compounds. Various potent anticancer compounds from higher plants have been identified by the National Cancer Institute in the United States of America through conducting an intensive screening program[47] . However, there are certain limitations towards using plants; the concentration of the active compounds present in the plants is generally low, the growth rate of plants is slow and geographical or environmental conditions affects the accumulation of the active compounds [48] . Thus, the economical production of the active compounds by extraction of the intact plant is a difficult task. Furthermore, many anticancer compounds isolated from higher plants are

secondary metabolites and have complex structures which are difficult to chemically synthesize [49] . Although plant tissue culture technology is not a very cost-effective option it is undoubtedly one of the most appropriate approaches to solve the above problems if the active compounds could not be manufactured by extraction or chemical processes. Therefore, over the last decade research studies have focused on applying plant tissue culture technology for the possible commercial production of anticancer drugs such as Taxol, Vinblastine, Vincristine, Camptothecin derivatives and Podophyllotoxin (Table 7).

Table 4: Anticancer compounds produced by hairy root cultures.

Plant	Compound/s	Treatment	Reference/s
Taxus cuspidata	Taxol	Ovarian, breast, non-small cell lung	Rowinsky *et al*,[50] ; Kim *et al*., [51]
Catharanthus roseus L. (G.) Don	Vinblastine [Velban®], vincristine [Oncovin®]	Acute leukaemia, small-cell lung cancer, cervical, breast, head and neck cancer	Ataei-Azimi *et al*. [52], Schmelzer and Gurib-Fakim, [53]
Ophiorrhiza pumila	Camptothecin derivatives (irinotecan [Campto®], topotecan [Hycamtin®])	Colorectal and ovarian cancers	Mathijssen *et al*., [54] Sudo *et al*., [55]
Podophyllum hexandrum	Podophyllotoxin (used as a precursor for production of etoposide [VP-16-213] and teniposide [VM-26])	Lung cancer, testicular cancer, a variety of leukemias and other solid tumours	Holthius, [56] Giri and Narasu[57],

Anthraquinones as anticancer compounds: Anthraquinones are widely applied in the pharmaceutical, food and dye industry. In the pharmaceutical industry, natural and synthetic derivatives of 9,10- anthracenedione are beneficial to mammals and humans as they can display antibacterial, antitrypanosomal and antineoplastic activities [58]. In addition, several commercial pharmaceutical products that have been developed contain anthraquinone derivatives. Anthraquinone glycosides are used to produce Pyralvex which is used to treat gingivitis, stomatitis, mouth ulcers, inflammatory oral mucosa and periodontal conditions. Senna is another member of the anthraquinone class which is used in the preparation of the stimulant laxative drug, Senokot. This drug is used to treat constipation or bowel evacuation prior to abdominal radiological procedures [30,31]. Anthracycline antibiotics are also anthraquinone derivatives and are key substances that have been known to be used for therapy of several cancers [58] .

There are several anthraquinone derivatives that have been useful for treating different kinds of cancers; these include Ametantrone, Mitoxantrone, Doxorubicin, Daunorubicin and Carminomycin [17] (Fig. 5). Mitoxantrone has been studied since the 1980‘s for the treatment of multiple sclerosis [59]. This compound is currently used clinically on its own or in combination with other chemotherapeutic agents for the treatment of a variety of human cancers such as breast and lung cancer, leukemia, melanoma and lymphoma. It is also used to treat Hodgkin‘s disease. [60]. Doxorubicin is an antibiotic that is highly effective in the treatment of tumors of the mammary gland and gynecological and hematological malignancies [17]. Many studies have reported the anticancer activity of other anthracenedione derivatives on different types of cancer cell lines which are shown in **Table 5**.

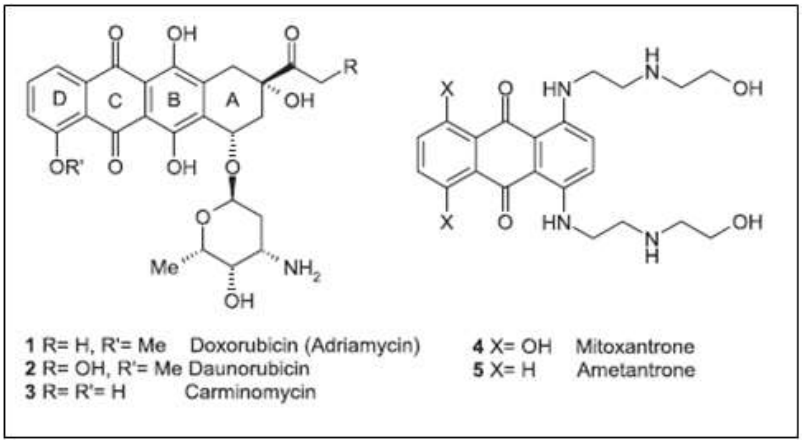

Figure 5: Anthracenedione derivatives used in the treatment of cancer [17].

Table 5: Anthraquinone derivatives that have anticancer potential.

Source	Anthraquinones	Cancer line	Reference
Daylies (*Hemerocallis spp.*)	Kwanzoquinone C, kwanzoquinone E, 2-hydroxychrysophanol, rhein	MCF-7 (breast cancer), SF-268 (CNS cancer), HCT (colon cancer), NCI (lung cancer)	Smith *et al.* [61],
Hedyotis diffusa	2-hydroxy-3-methylanthraquinone, 1- methoxy-2-hydroxyanthraquinone	SPC-1-A, Bcap37,HepG2	Shi *et al.* [62],
Chemically synthesized (Sigma Chemical Co., USA)	Emodin (1,3,8-trihydroxy-6- methyl-anthraquinone)	HepG2/C3A, PLC/PRF/5, and SK-HEP-1(hepatoma cell lines)	Shieh *et al.* [63],
Rheum palmatum L.	Emodin (1,3,8-trihydroxy-6- methyl-anthraquinone)	HL-60 (leukemia cell line)	Chen *et al.* [64]
Chemically synthesized	(S,S)1,4-bis[2-(4-methylsulfanyl-butyrylamino) ethylamino]-5,8-dihydroxyanthracene-9,10- dione	A549 (non-small cell lung cancer), DU145 (androgen-independent prostate cancer), HT-29 (colorectal cancer), MCF- 7 (breast cancer).	Hsiao *et al.* [65]

MECHANISM OF ACTION OF ANTICANCER COMPOUNDS

The Cell Cycle: In order to understand the mechanism of action of anticancer compounds, it is important to be familiar with the human cell cycle. The cell cycle can be divided into five phases (Figure 10). The S-phase is when the chromosomal DNA replicates. This phase is followed by the M (mitosis) phase where the segregation of the chromosomes into two daughter cells occurs [66]. In between the S and M phase are the two gap phases, G_1 and G_2. The G_1 phase proceeds after the M phase, during this time the cell becomes responsive to negative and positive growth signals. The G_2 phase proceeds after the S phase where the cell prepares for entry into the M phase. The final phase is the G_0 phase where the cell exits from the G_1 into the G_0 phase if it is deprived of the appropriate growth promoting signals [67].

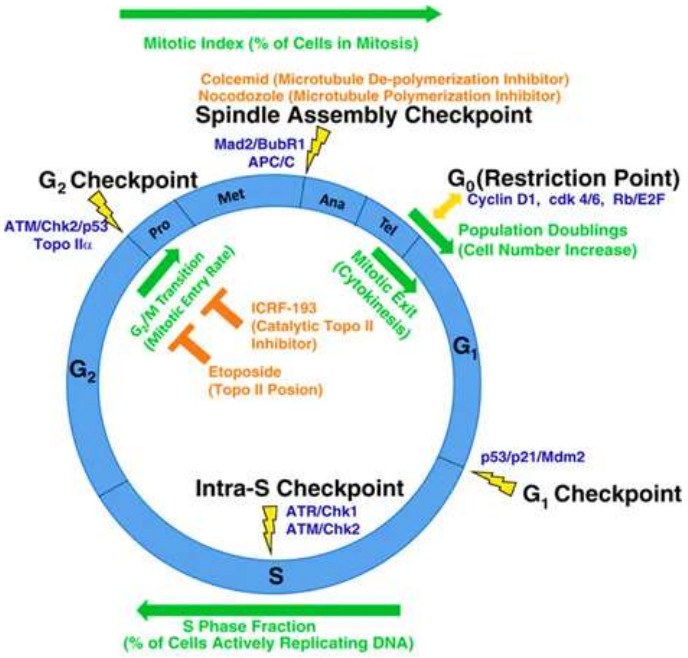

Figure 6: Diagram of cell cycle regulation. Phases of the cell cycle are shown inside the blue circle in the center of the figure (G_0, G_1, S, G_2, and mitosis which consists of several sub-phases: prophase (Pro), metaphase (Met), anaphase (Ana), and telophase (Tel). Drugs that inhibit cell cycle progression are shown in orange with their targets and mechanisms of action designated in subsequent parentheses. Components of major regulatory pathways triggering each checkpoint are listed in dark blue font near the checkpoint in which they play a role [68],

The progression of the normal cell cycle is controlled by a family of kinases called cyclin dependent kinases (cdks) and regulated by positions within the cell cycle called the check points [69,70]. Hartwell and Weinert [70], first defined the cell cycle check points as a mechanism that maintains the observed order of events of each cell cycle. This means that there are a number of key genes that participate in the checkpoints which function to see if the integrity of the genome is retained throughout the normal cell cycle[67]. Normal cells protect themselves against the exposure to growth- limiting conditions or toxic agents by using the checkpoint control mechanism [71]. For example, if normal cells undergo DNA damage by UV radiation, they arrest in the G1 phase in order to repair the DNA prior to replication. However, if any of the genes involved in the check points are mutated, the integrity of the cell‘s genome will be at stake and the cancer cell cycle may proceed. Cancer cells exhibit poor check points and therefore they are more susceptible to the cytotoxic effects of drugs such as anti-topoisomerases [71].

Anti-topoisomerase drugs: Anti-topoisomerase drugs cause DNA damage to cancer cells by inhibiting enzymes known as DNA topoisomerases [72]. These enzymes function to open transient, protein-bridged, single- or double-stranded DNA breaks. DNA is passed through these breaks so that topological problems can be solved and accumulated torsion stress can be relieved during cellular transactions of the DNA molecule. Topoisomerases have been classified as type I and II according to their ability to cleave single- or double- stranded DNA molecules, respectively [73]. In cancer therapy anti-topoisomerase drugs inhibit DNA topoisomerase II (Figure 11) and prevent the disentangling of the cell‘s DNA and subsequently causes cell death via apoptosis [72].

There are a diverse group of natural and synthetic compounds that target topoisomerase IIα and IIβ [74-76], some of which are described in Table 9. These compounds are potent *in vitro* and in human cells. They are widely used as some of the most successful chemotherapeutic drugs for the treatment of

human cancer and malignancies. Studies have shown that the cytotoxic effect of anthraquinones on cancer cells is due to their ability to inhibit the DNA topoisomerase II enzyme which in turn leads to the activation of apoptotic pathways. For example, Hsiao *et al.* [65] showed that (S,S)1,4-bis[2-(4-methylsulfanyl-butyryl amino) ethyl amino]-5,8- dihydroxy anthracene-9,10-dione inhibited the topoisomerase II enzyme and induced chromosomal DNA breaks which led to S and G2 phase arrest of the cell cycle and activation of apoptotic pathways in prostate cancer cells.

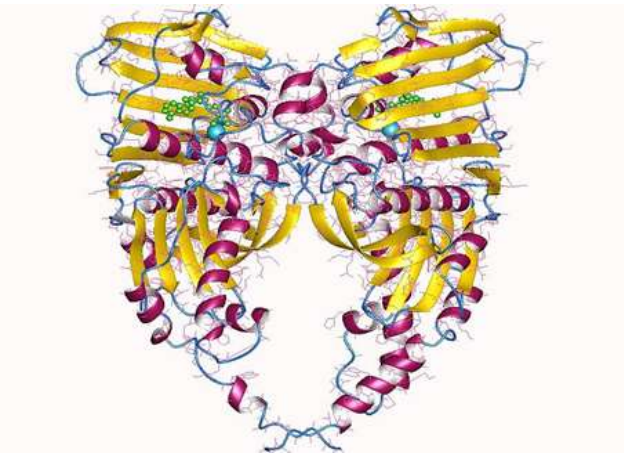

Figure 7 :High resolution 3-D crystallography image of the binding and cleavage core of type II topoisomerase from *Saccharomyces cerevisae.* The central core of the protein contains a Toprim fold and a DNA-binding core that contains a winged helix domain (WHD), often referred to as a CAP domain, since it was first identified to resemble the WHD of catabolite activator protein.

Table 6: Natural and synthetic compounds that target topoisomerase I and II.

Drug class	Example	Topoisomerase Inhibited	Effects	References
Acridines	Amsacrine (m-AMSA)	II	Stabilize cleavable complex	Louie and Issel, [77]
Anthracenediones	Mitoxantrone	II	stabilize cleavable complex	Harker *et al.*, [78]
Anthracyclines	Doxorubicin	II	stabilize cleavable complex	Au *et al.*, [79]
Isoflavonoids	Genistein	II	PTK inhibitor and cleavable-complex blocker	Markovits *et al.*, [80]
Alkaloid	Camptothecin and its derivatives	I	stabilize cleavable complex	Hsiang *et al.* [81],
bis-piperazinediones	ICRF-159, 193	II	inhibits DNA relaxation and cleavable complex	Jensen *et al.* [82],
Anthracenyl peptides	Merbarone	II	inhibits cleavable complex formation	Khelifa and Beck, [83]

CONCLUSION

The battle against cancer requires a joint effort between the different platforms such as computational medicinal chemistry and pharmacology. These technologies will reveal the detailed regulation mechanisms, epigenetic and genetic information and signaling network for the growth, proliferation and metastasis of cancer cells. This will reveal novel strategies to eradicate cancer cells from the body, overcome tumor resistance and discover "magic" drugs that kills cancer cells only without affecting the normal cells. Cancer in humans proliferates via three main stages which include initiation, promotion and progression. The main target for novel anticancer drugs is the promotion stage. Phytochemicals are mainly present in fruits and vegetables and their regular intake would be effective to cancer prevention. New technologies that have been developed to understand the biological behaviors of cancer growth and metastasis include systems biology. Systems biology includes the integration of expertise from diverse fields including cancer biology, oncology, genetics, mathematics, bioinformatics, imaging, physics and computer science. Systems biology could provide a more holistic understanding of the complete metastatic process and proper application of such knowledge in anticancer drug development would result in novel drugs that can cure cancer in a similar manner to common infectious diseases.

ACKNOWLEDGEMENT

The author would like to thank the Durban University of Technology and the Department of Higher Education and Training (DoHET) for providing facilities and the National Research Foundation (NRF) for funding.

REFERENCES

1. S.J.Smithies, Pedaliaceae. In: OA. Leistner, (Ed.) Seed plants of southern Africa: families and genera. Pretoria, National Botanical Institute, 2000, 436-437.
2. Hutchings A. ed., *Zulu Medicinal Plants, an Inventory*. Pietermaritzburg: University of Natal Press. 1996,104.
3. R.P.Wunderlin and B.F. Hansen, Atlas of Florida vascular plants, Available at http://www.plantatlas.usf.edu/. [Online] University of South Florida, Tampa, 2002.
4. L. Van der Walt, *Ceratotheca triloba* (Bernh.) Hook.f. [Online] 2001, Available at http://www.plantzafrica.com/plantcd/ceratothectrilob.htm. [Accessed: 20 February 2005]
5. M.H.Tredgold , ed., Food of plants of Zimbabwe. Gweru: Mambo Press, 1986.
6. J.M.Watt and M.G. Breyer-Brandwijk, The medicinal and poisonous plants of Southern and Eastern Africa. Livingstone, London.1962.
7. M. Roberts, Indigenous Healing Plants. Halfway House: Southern Book Publishers, 1990.
8. E. Pooley E. ed., A field guide to wildflowers Kwazulu-Natal and the East Region. Southern Africa, Durban: Natal Flora Publications Trust.1998.
9. B.E.Van Wyk and N. Gericke N. eds., People's plants. A guide to useful plants of Southern Africa. Southern Africa, Pretoria: Briza Publications, 2000.
10. B. Odhav, S. Beekrum , U.S. Akula and H. Baijnath, Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. Journal of Food Composition and Analysis, 2007, 20: 430-435.
11. S. Ramesar, H. Baijnath , T. Govender and I. Mackraj , Angiotensin I-Converting Enzyme Inhibitor Activity of Nutritive Plants in KwaZulu-Natal. Journal of Medicinal Food, 2008, 11:

331-336.

12. B. Odhav, T. Kandasamy, N. Khumalo and H. Baijnath, Screening of African traditional vegetables for their alpha-amylase inhibitory effect. Journal of Medicinal Plants Research, 2010 4: 1502-1507.
13. V.Mohanlall, P.A.Steenkamp and B. Odhav, Isolation and characterization of anthraquinone derivatives from *Ceratotheca triloba* (Bernh.) Hook.f. Journal of Medicinal Plants Research, 20111,.5: 3132-3141.
14. B.C.Baguley, DNA intercalating anti-tumour agents. Anticancer Drug Design, 1991, 6: 1-35.
15. N. Dhingra, T.R.Bhardwaj, N. Mehta, T.Mukhopadhyay, A.Kumar and M. Kumar, Synthesis, antiproliferative activity, acute toxicity and assessment of the antiandrogenic activities of new androstane derivatives. Archives of Pharmacological Research, 2011, 34, 1055-1063.
16. C.Monneret, Recent developments in the field of antitumour anthracyclines. European Journal of Medicinal Chemistry, 2001, 36, 483-493.
17. M.N.Preobrazhenskaya, A.E.Shchekotikhin, A.A.Shtil, and H.S.Huang, Antitumor anthraquinone analogues for multidrug resistant tumor cells. Journal of Medical Sciences, 2006, 26, 1-4.
18. L.Naicker , Genetic transformation of *Ceratotheca triloba* for the production of anthraquinones from hairy root cultures. Masters of Technology Thesis. Durban University of Technology,2012.
19. R.P.Wunderlin and B.F.Hansen, Atlas of Florida vascular plants [Online]. Tampa: University of South Florida.2002, Available: http://www.plantatlas.usf.edu/. [Accessed: 23 January 2020]
20. L. Van der Walt, *Ceratotheca triloba* (Bernh.) Hook.f. [Online]. Kirstenbosch: South African National Biodiversity Institute, 2001. Available:http://www.plantzafrica.com /plantcd/ ceratothectrilob.htm. [Accessed: 23 January 2020]
21. M.Wink, Interference of alkaloids with neuroreceptors and ion channels. Bioactive Natural Products.2000, 21, 3–129.
22. J.A.Saunders and N.R.O'Neill, The characterization of defense response to fungal infection in alfalfa. BioControl, 2004, 49: 715–728.
23. G.A.Rosenthal, The biochemical basis for the deleterious effects of L-canavanine. Phytochemistry, 1991, 30: 1055-1058.
24. J. Schripsema , A. Ramos-Valdivia and R. Verpoorte, Robustaquinones novel anthraquinones from elicited *Cinchona robusta* suspension culture. Phytochemistry, 1999, 51, 55-60.
25. Y.P.S.Bajaj ed. Biotechnology in Agriculture and Forestry. Medicinal and Aromatic plant. Berlin: Springer-Verlag, 1999, 159.
26. H. Koblitz, Anthraquinones. In: Constabel F and Vasil IK. (Eds.) Cell culture and somatic cell genetics of plants. Orlando: Academic Press, 1988,113
27. A.J.J.Van der Berg and R.P.Labadie, Quinones. In: Dey M. and Harborne JB. (Eds.) Methods in Plant Biochemistry. London: Academic Press, 1989, 413-491.
28. K. Korunaglo, M. Gbeassor, O. Nikabu, C. De Souza and W.Werner, Effects of three compounds extracted from *Morinda lucida* on *Plasmodium falciparum*. Planta Med.1992, 58: 533-534.
29. K. Dzierzbicka and A.M.Kolodziejczyk, Anthracenedione analogues – synthesis and biological activity. Polish Journal of. Chem.2005, 79: 1-29.
30. Drug Information for Malaysia and Singapore (DIMS), Medical Information for Malaysia and

Singapore (MIMS), 1992, 21: 204.

31. British National Formulary. 1994. British National Formulary, London: BMA and Royal Pharmaceutical Society of Great Britain.

32. R.H.Thomson, Quinones, Nature, Distribution and Biosynthesis, in Chemistry and Biochemistry of plant pigments, 2nd edition, 1976a, Vol 1, ed. TW Goodwin. 527. London, New York and San Francisco: Academic Press.

33. E.Leistner, Second pathway leading to anthraquinones in higher plants. Phytochemistry ,1980,10, 3015-3020.

34. E.Leistner, Biosynthesis of plant quinones. In: Conn EE (ed) The biochemistry of plants, Vol VII. Academic Press, London New York, 1981, 403–423.

35. M. Stalman, A.M. Koskamp, R.Luderer, J.H.J.Vernooy, J.C.Wind, G.J.Wullems and A.F.Croes, Regulation of anthraquinone biosynthesis in cell cultures of *Morinda citrifolia*. Journal of Plant Physiology, 2003, 160, 607–614.

36. A.H.Lodhi, R.J.M.Bongaerts, R. Verpoorte, S.A.Coomber, and B.V.Charlwood , Expression of bacterial isochorismate synthase (EC 5.4.99.6) in transgenic root cultures of *Rubia peregrina*. Plant Cell Reports, 1996. 16, 54-57.

37. Y.Han , R.Heijden and R.Verpoorte, Biosynthesis of anthraquinones in cell cultures of the Rubiaceae. Plant Cell Tissue and Organ Culture.2001, 67. 201-220.

38. M. Simantiras and E. Leistner, Formation of o-succinylbenzoic acid from iso-chorismic acid in protein extracts from anthraquinone-producing plant cell suspension cultures. Phytochemistry, 1989, 28. 1381-1382.

39. A.C.Ramos-Valdivia, R.van der Heijden and R. Verpoorte, Isopentenyl diphosphate isomerase: a core enzyme in isoprenoid biosynthesis. A review of its biochemistry and function. Natural Product Reports, 1997a. 14, 591–603.

40. A.C.Ramos-Valdivia, R.van der Heijden, R.Verpoorte and B.Camara, Purification and characterization of two isoforms of isopentenyl diphosphate isomerase from elicitor-treated *Cinchona robusta* cells. European Journal of Biochemistry, 1997b, 249, 161–170.

41. A.C.Ramos-Valdivia, R.Heijden and R.Verpoorte, Isopentenyl diphosphate isomerase and prenyltransferase activities in rubiaceous and Apocynaceous cultures. Phytochemistry,1998, 48. 961-969.

42. V.P.Bulgakov, G.K.Tchernoded, N.P.Mischenko, M.V.Khodakovskaya, V.P. Glazunov, S.V.Radchenko, E.V.Zvereva, S.A.Fedoreyev, and Y.N.Zhuravlev, Effect of salicylic acid, methyl jasmonate, ethephon and cantharidin on anthraquinone production by *Rubia cordifolia* callus cultures transformed with the rolB and rolC genes. Journal of Biotechnology,2002, 97: 213-21.

43. V.P. Bulgakov, G.K.Tchernoded, N.P.Mischenko, Y.N.Shkryl, V.P.Glazunov , S.A.Fedoreyev and Y.N.Zhuravlev, Effects of Ca^{2+} channel blockers and protein kinase/phosphatase inhibitors on growth and anthraquinone production in *Rubia cordifolia* cultures transformed by the rolB and rolC genes. Planta, 2003, 217: 349-55.

44. O.V.Mantrova, M.V.Dunaeva, I.N.Kuzovkina, B.Schneider and F.Müller Uri, Effect of methyl jasmonate on anthraquinone biosynthesis in transformed madder roots. Russian Journal of Plant Physiology,1999, 46: 276–279

45. J.Ferlay, M. Colombet, I.Soerjomataram , C.Mathers, D.M.Parkin, M.Piñeros , A.Znaor and F.

Bray, Global and Regional Estimates of the Incidence and Mortality for 38 Cancers: GLOBOCAN 2018. Lyon: International Agency for Research on Cancer/World Health Organization, 2018.

46. K. Kandasamy, N. Boopathy and S.Kavitha, Coastal vegetation - an underexplored source of anticancer drugs. Indian Journal of Natural Products and Resources, 2006, 5. 115-119.
47. M.Suffness and J. Douros , Current status of the NCI plant and animal product program. Journal of Natural Products, 1982, 45: 1–14.
48. O. Yesil-Celiktas, A.Gurel and F.Vardar-Sukan, Large scale cultivation of plant cell and tissue culture in bioreactors. Transworld Research Network, Kerala2010,
49. K.M.Oksman-Caldentey, D.Inzé, Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. Trends in Plant Science.2004, 9:433–440
50. E.K.Rowinsky, L.A.Cazenave and R.C.Donehower, Taxol: a novel investigational antimicrotubule agent. Journal of the National Cancer Institute, 1990, 82: 1247– 1259.
51. H. Kim, M.Rafiuddin-Shah, H.C.Tu, J.R.Jeffers, G.P. Zambetti, J.J.Hsieh and E.H.Cheng, Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nature Cell Biology, 2006, 8:1348 –1358.
52. A.Ataei-Azimi, B.Delnavaz, H.Ebrahimzadeh, and A.Majd , High in vitro production of ant-canceric indole alkaloids from periwinkle (*Catharanthus roseus*) tissue culture. African Journal of Biotechnology, 2008, 7.
53. G.H.Schmelzer and A.Gurib-Fakim, Plant Resource of Tropical Africa. Medicinal Plants 1. PROTA Foundation, Wageningen, Netherlands/ Backhuys Publishers, Leiden, Netherlands, 2008.
54. I. MathijssenI,H. Leeuwen, C.Vermeij-Keers and J.Vaandrager, FGF4 or FGF2 Administration Induces Apoptosis, Collagen Type I Expression, and Mineralization in the Developing Coronal Suture. Journal of Craniofacial Surgery, 2001, 12: 399-400.
55. T.Sudo, Molecular-targeted therapies for ovarian cancer: prospects for the future. International Journal of Clinical Oncology, 2012, 17: 424–429.
56. J.J.M.Holthius, Etoposide and teniposide: Bioanalysis, metabolism and clinical pharmokinetics. Pharmaceutisch Weekblad,1988, 10: 101-116
57. A.Giri and M.Narasu, Transgenic hairy roots. Recent trends and applications. Biotechnology Advances, 2000, 18. 1-22.
58. K. Dzierzbicka and A. Kolodziejczyk , Anthracenedione Analogues. Synthesis and Biological Activity. Cheminform, 2005, 36.
59. M.N.Levine and A.Eisen , Anthracycline Adjuvant Chemotherapy: How Much Is Enough? Journal of Clinical Oncology, 2001, 19:3, 599-601.
60. H.Escobar-Morreale, J. Serrano-Gotarredona and C.Varela, Isolated adrenocorticotropic hormone deficiency due to probable lymphocytic hypophysitis in a man. Journal of Endocrinological Investigation, 1994, 17:127–131.
61. F.Smith, D.Cichewicz, Z.Martin and S.Welch, The enhancement of morphine antinociception in mice by Δ9-tetrahydrocannabinol. Pharmacology, biochemistry, and behavior, 1998, 60: 559-566.
62. Y. Shi, C.H.Wang and X.G.Gong, Apoptosis-inducing effects of two anthraquinones from *Hedyotis diffusa* Wild. Biological and Pharmaceutical Bulletin, 2008, 31:1075–1078.
63. D.E.Shieh, Y.Y.Chen and M.H.Yen , Emodin-induced apoptosis through p53-dependent pathway

in human hepatoma cells. Life Science, 2004, 74:2279-90.

64. Y.C.Chen, S.C.Shen, W.R.Lee, F.L.Hsu, H.Y.Lin, C.H.Ko and S.W.Tseng, Emodin induces apoptosis in human promyeloleukemic HL-60 cells accompanied by activation of caspase 3 cascade but independent of reactive oxygen species production. Biochemical Pharmacology,2002, 64: 1713–1724.

65. C.J.Hsiao, T.K.Li, Y.L.Chan, L.W.Hsin, C.H.Liao, C.H.Lee , P.C.Lyu and J.H.Guh , WRC-213, an L-methionine-conjugated mitoxantrone derivative, displays anticancer activity with reduced cardiotoxicity and drug resistance: Identification of topoisomerase II inhibition and apoptotic machinery in prostate cancers. Biochemical Pharmacology, 2008, 75: 847-856.

66. A.Giordano, and M.M.Kasten, pRb and the Cdks in apoptosis and the cell cycle. Cell Death and Differentiation, 1998, 5: 132-140.

67. M.D.Garrett, Cell cycle control and cancer. Current Science, 2001, 81: 515-522.

68. J.J.Bower, L.D.Vance, M.Psioda, S.L.Smith-Roe, D.A.Simpson, J.G.Ibrahim, K.A.Hoadley, C.A.Perou and W.K.Kaufmann, Patterns of cell cycle checkpoint deregulation associated with intrinsic molecular subtypes of human breast cancer cells. NPJ Breast Cancer. 2017:3-9.

69. T.K.MacLachlan, N. Sang and A.Giordano, Cyclins, cyclin-dependent kinases and cdk inhibitors: implications in cell cycle control and cancer. Critical Reviews in Eukaryotic Gene Expression, 1995, 5: 127-156.

70. L.H.Hartwell and T.A.Weinert, Checkpoints: controls that ensure the order of cell cycle events. Science.1989, 246: 629-634.

71. D.W.Kufe , R.E.Pollock, R.R.Weichselbaum, R.C.Bast, T.S.Gansler, J.F.Holland and E.Frei , eds. Holland-Frei Cancer Medicine. Hamilton (ON), Canada: BC Decker, 2003.

72. A.K.McClendon and N. Osheroff , DNA topoisomerase II, genotoxicity, and cancer. Mutation Research, 2007, 623: 83-97.

73. P.R.Caron and J.C.Wang, Appendix II: Alignment of primary sequences of DNA topoisomerases. Advances in Pharmacology. 1994, 29B: 271-297.

74. J.M.Fortune and N. Osheroff , Topoisomerase II as a target for anti-cancer drugs: when enzymes stop being nice. Progress in Nucleic Acid Research & Molecular Biology, 2000, 64: 221-253.

75. A.M.Wilstermann and N.Osheroff, Stabilization of eukaryotic topoisomerase IIDNA cleavage complexes, Current Topics in Medicinal Chemistry, 2003, 3: 1349-1364.

76. R. Velez-Cruz and N. Osheroff, DNA topoisomerases: type II, in: Encyclopedia of Biological Chemistry, Elsevier Inc.2004, 806-811.

77. A.C.Louie and B.F.Issel, Amsacrine (AMSA): a clinical review. Journal of Clinical Oncology, 1985, 3: 569-599.

78. W.G.Harker, D.L.Slade, F.H.Drake and R.L.Parr, Mitoxantrone resistance in HL-60 leukemia cells: reduced nuclear topoisomerase II catalytic activity and drug induced DNA cleavage in association with reduced expression of the topoisomerase II beta isoform. Biochemistry, 1991, 30: 9953-9961.

79. W.W.Au , M.A.Butler, T.S.Matney and T.L.Loo, Comparative structure genotoxicity study of three aminoanthraquinone drugs and doxorubicin. Cancer Research, 1981, 41: 376-379.

80. Y.Markovits, C.Linassier, P.Fosse, J.Couprie, J. Pierre, A.Jacquemin-Sablon , J.M.Saucier, J.B.Le

Pecq and A.K.Larsen, Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Research, 1989, 49: 5111-5117.

81. Y.H.Hsiang, R. Hertzberg, S. Hecht and L.F.Liu , Camptothecin induces protein linked DNA breaks via mammalian DNA topoisomerase. Journal of Biological Chemistry, 1985, 260: 14873-14878.

82. L.H.Jensen , K.C.Nitiss, A.Rose, J.Dong, J. Zhou, T. Hu , N. Osheroff , P.B.Jensen , M.Sehested and J.L.Nitiss , A novel mechanism of cell killing by antitopoisomerase II bisdioxopiperazines. Journal of Biological Chemistry, 2000, 275: 2137- 2146.

83. T. Khelifa and W.T.Beck, Merbarone, a catalytic inhibitor of DNA topoisomerase II, induces apoptosis in CEM cells through activation of ICE/CED-3-like protease. Molecular Pharmacology, 1999, 55: 548-556.

***Corresponding Author: V. Mohanlall**

Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, P. O Box 1334, Durban, 4000, South Africa