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REVIEW ARTICLE

Eurycoma longifolia, A Potential Phytomedicine for the Treatment of Cancer: Evidence of p53-mediated Apoptosis in Cancerous Cells

Hnin Ei Thu[1], Zahid Hussain[2], Isa Naina Mohamed[1] and Ahmad Nazrun Shuid[1,*]

[1]*Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia (The National Universiti of Malaysia), Jalan Yaacob Latif 56000, Cheras, Malaysia;* [2]*Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam 42300, Selangor, Malaysia*

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Abstract: ***Background*:** *Eurycoma longifolia* is a well-documented herbal medicine that has gained widespread recognition due to its versatile pharmacological activities including anticancer, antimalarial, antimicrobial, antioxidant, aphrodisiac, anti-inflammatory, anxiolytic, anti-diabetic, anti-rheumatism and anti-ulcer. Plethora of *in vitro* and *in vivo* studies evidenced their excellent anti-proliferative and anticancer efficacy against various types of human cancers.

***Objective*:** This review was aimed to critically analyze the therapeutic viability and anticancer efficacy of *Eurycoma longifolia* in the treatment of cancer and also to propose its molecular and translational mechanism of cytotoxicity against cancerous cells.

***Results*:** Among a range of medicinally active compounds isolated from various parts (roots, stem, bark and leaves) of *Eurycoma longifolia*, 16 compounds have shown promising anti-proliferative and anticancer efficacies. Eurycomanone, one of the most active medicinal compounds of *Eurycoma longifolia*, displayed a strong dose-dependent anticancer efficacy against lung carcinoma (A-549 cells) and breast cancer (MCF-7 cells); however, showed moderate efficacy against gastric (MGC-803 cells) and intestinal carcinomas (HT-29 cells). The prime mode of cytotoxicity of *Eurycoma longifolia* and its medicinal compounds is the induction of apoptosis (programmed cell death) *via* the up-regulation of the expression of p53 (tumor suppressor protein) and pro-apoptotic protein (Bax) and down-regulation of the expression of anti-apoptotic protein (Bcl-2). A remarkable alleviation in the mRNA expression of various cancer-associated biomarkers including heterogeneous nuclear ribonucleoprotein (hnRNP), prohibitin (PHB), annexin-1 (ANX1) and endoplasmic reticulum protein-28 (ERp28) has also been evidenced.

***Conclusion*:** *Eurycoma longifolia* and its medicinal constituents exhibit promising anticancer efficacy and thus can be considered as potential complementary therapy for the treatment of various types of human cancers.

Keywords: *Eurycoma longifolia*, eurycomanone, anti-proliferative activity, anticancer efficacy, p-53 mediated apoptosis, *in vitro* and *in vivo* studies.

1. INTRODUCTION

Cancer, also known as malignancy, is a group of diseases involving an abnormal growth of cells with the potential to invade other organs or parts of the body. The signs and symptoms of cancer depend on the type of cancer, where it is located, and/or where the cancer cells have spread. For example, breast cancer may present as a lump in the breast or as nipple discharge while metastatic breast cancer may present with symptoms of pain (if spread to bones), extreme fatigue (if spread to lungs), or seizures (if involve brain). Most common signs and symptoms associated with cancer include unexplained severe pain or ache, unusual breast changes, extreme fatigue, blood in urine, blood in cough, abnormal bleeding, heavy night sweat, unexplained weight loss, unusual lump or swelling in any region of body, persistent ulcers/ulcerate that are not healing, prolonged cough, and a change in bowel movements. There are more than 100 types of cancer, including lung cancer, ovarian cancer, malignant melanoma, breast cancer, prostate cancer, colorectal cancer, cervical cancer, skin cancer, leukemia, and lymphoma.

American Cancer Society (ACS) recommended that there are seven warning signs or symptoms that need serious medical attention. These cautionary signs and symptoms include; 1) persistent chronic sore throat that is not healing,

*Address correspondence to this author at the Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif 56000, Cheras, Malaysia; Tel: +6(0)391459567; Fax: +6(0)291459547; E-mail: anazrun@yahoo.com

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2) change in bowel or bladder habits, 3) unusual heavy bleeding or fluid discharge (*e.g.*, nipple discharge, chronic sore that is not healing and oozes transudate or exudates), 4) unusual lump in breast, testicles, or elsewhere in the body, 5) indigestion (usually chronic) or difficulty in swallowing, 6) apparent change in the color, size, shape, or thickness of a wart or mole, and 7) worse hoarseness or nagging cough. Though these conditions may arise from non-cancerous cause, anyone experiencing these signs or symptoms should consult to medical practitioners. Among various non-pharmacological and pharmacological interventions, the most common cancer therapeutic modalities include chemotherapy, radiation, and/or surgery.

Traditional herbal medicines have been well-documented as complementary and alternative medicines (CAMs) for the managment, prevention or cure of a wide range of human diseases including cancer [1, 2], gastrointestinal diseases [3, 4], hepatitis [5], skin inflammatory disorders [6-8], heart diseases [9], blood disorders, joint problems, Parkinson's diseases [10], respiratory issues, urinary tract infections, epilepsy [11] and muscle and bone disorders [12]. CAM has been growing throughout the healthcare industry, spurred by patient empowerment among the patient and doctor populations. Historically, allopathic medicines were considered as the alternative form of treatment because practitioners who dealt primarily with herbal interventions were providing healthcare to most of the patients. The switch from alternative herbal-based medicine to the present allopathic regimens has only occurred in the past century. Presently, according to the World Health Organization (WHO), only 10 to 30% of the healthcare is being delivered by allopathic practitioners; whereas, the remaining 70 to 90% of healthcare is still being provided by alternative healthcare providers. These alternative modalities range from self-care according to folk principles to care given in an organized health care system based on a traditional or common practice. Among the CAMs, *Eurycoma longifolia* Jack is well-documented herbal medicine in Southeast Asia. Different parts of this plant (roots, stem, leaves, bark *etc*.,) are currently being used for the treatment of various diseases in many countries in Asia. Besides this, recently *Eurycoma longifolia* has contributed a prominent role as CAM in herbal therapies, in the West.

Eurycoma longifolia, a potent medicinal herb in the family of Simaroubaceae, is known locally as Tongkat Ali in Malaysia, Tung saw in Thailand, Pasak bumi in Indonesia and cay ba bihn in Vietnam [13]. *Eurycoma longifolia* has gained remarkable recognition among various ethnic groups in Malaysia, China and South Africa due to its excellent pharmacological activities [14-16]. *Eurycoma longifolia* has also shown strong antiproliferative and anticancer activities against various types of human cancers including hepatocellular carcinoma, malignant melanoma, cervical cancer, ovarian carcinoma, breast cancer, colorectal cancer and lung cancer [17-21]. Besides its anticancer potential, *Eurycoma longifolia* has also been well-recognised due to its other pharmacological activities including aphrodisiac [22-26], anti-malarial [27-29], antibacterial [30, 31], anti-inflammatory [32, 33], anxiolytic [34], anti-diabetic [35, 36], anti-ulcer [37], anti-rheumatism [32, 38] antitumor [39, 40] and anti-osteoporotic activities [41, 42].

Numerous *in vitro* and *in vivo* studies indicated promising potential of *Eurycoma longifolia* for the treatment of various types of human cancers. The current review was therefore aimed to summarize the convincing evidence for the pharmacological and therapeutic viability of *Eurycoma longifolia* in the treatment of human cancer. The potential molecular and translation mechanism for the anticancer ability of *Eurycoma longifolia* has also been critically discussed.

2. *EURYCOMA LONGIFOLIA* AND ITS BIOACTIVE COMPOUNDS

2.1. Bioactive Compounds Having Anti-proliferative and Anticancer Efficacy

Among various compounds isolated from Eurycoma longifolia, 16 compounds have shown strong anti-proliferative and anti-cancer effects against various human cancer cell lines. These bioactive compounds include eurycomanone, eurycomanol, 13β, 21-dihydroxyeurycomanone, 14-hydroxyglaucarubol, eurycomalactone, eurycomadilactone, 5-iso-eurycomadilactone, 13-epi-eurycomadilactone, longilactone, 6-dehydroxylongilactone, canthin-6-one, 9-methoxycanthin-6-one, canthin-6-one 9-*O*-β-glucopyranoside, 14,15β-dihydroxyklaineanone, pasakbumin B, and pasakbumin C. The chemical structures of all these compounds having cytotoxic activities against various human cancers are presented in (Fig. **1**).

Their specificity against various human cancers is variable. Kuo *et al.* [20] demonstrated that eurycomalactone, longilactone, 14,15β-dihydroxyklaineanone, eurycomanone, and 13,21-dihydroeurycomanone showed strong anti-proliferative and anticancer activities against lung cancer (A-549 cells). They have also tested the anti-proliferative activity and cytotoxicity of these compounds against MCF-7 cells and demonstrated that compounds such as eurycomalactone, 6-dehydroxylongilactone, 9-methoxycanthin-6-one, 14,15β-dihydroxyklaineanone, pasakbumin B and pasakbumin C have shown promising cytotoxicity against MCF-7 cells [1, 20].

Cytotoxicity of eurycomanol, 13β,21-dihydroxyeurycomanone, 14-hydroxyglaucarubol, eurycomadilactone, 5-iso-eurycomadilactone and 13-epi-eurycomadilactone has also been evaluated against many types of human cancers such as cervical carcinoma (HeLa cells), liver carcinoma (HepG2 cells), gastric carcinoma (MGC-803 and BGC-823 cells), intestinal cancers (HT-29 and LOVO cells), lung carcinoma (A-549 cells) and breast cancer (MCF-7 cells) using fluorouracil as a control [2]. The results demonstrated that the tested compounds exhibited a strong cytotoxicity against breast cancer (MCF-7 cells) and gastric carcinoma (MGC-803 cells); however, showed a moderate efficacy against other types of human cancers.

2.2. Analytical Tools

Generally, unknown compounds or chemical entities were verified by a collective approach using infrared spectroscopy, UV/visible spectroscopy, mass spectrometry, and X-ray analysis followed by ^{1}H- and ^{13}C-NMR spectral techniques. However, these highly sensitive techniques require high purity of the test compound/chemical entity. Unfortunately, herbal extracts or isolates of medicinal plants are not

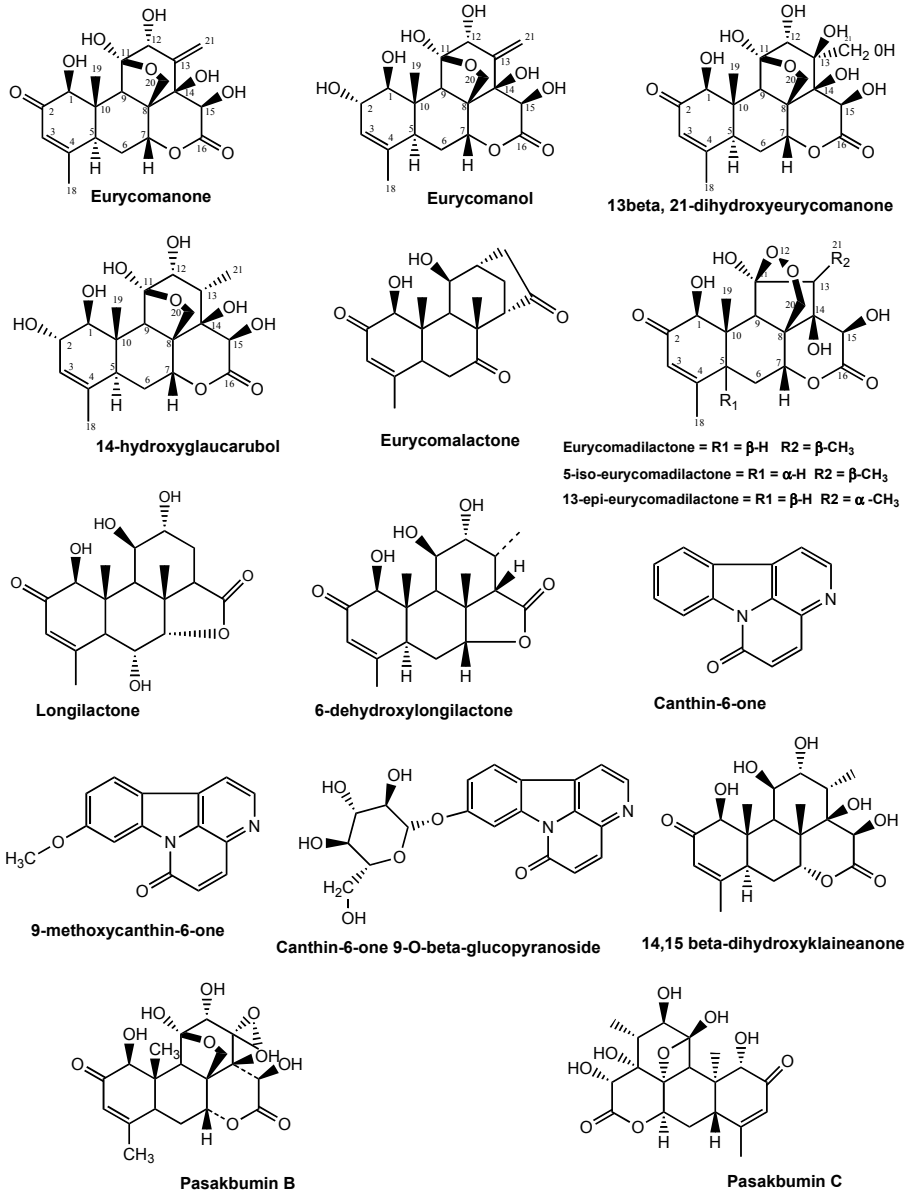

Fig. (1). Chemical structures of 16 bioactive compounds isolated/extracted from *Eurycoma longifolia* having anti-proliferative and anticancer activities.

highly pure which make it difficult to use these techniques to generate highly confident data. Mass spectrometry is nowadays the most versatile analytic method for the detection of unknown chemical constituents from polyherbal formulations or plant extracts [43, 44]. For further identification and quantification, liquid chromatography furnished with mass spectrometry (LC-MS) is recognized as most reliable and powerful tool [45-47].

Quassinoids are the most active and major constituents isolated/extracted from various species of Simaroubaceae family [48]. The analysis of chemical constituents of quassinoids is numerously performed using liquid chromatography, photodiode array or fluorescence and U.V/visible spectroscopic analysis. However, these methods were unable to detect and quantify non-chromophoric constituents of *Eurycoma longifolia*, such as eurycomanol [49, 50]. Hence, mass spectrometry can be the most reliable and sensitive approach to analyze most of the chemical constituents and secondary metabolites from *Eurycoma longifolia* [51, 52]. Recently, Han *et al.* [53] have analyzed the percentage purity of six major quassinoids including, 13(21)-epoxyeurycomanone, eurycomanone, 13,21-dihydroeurycomanone, longilactone, 14,15-dihydroxyklaineanone, and eurycomalactone of *Eurycoma longifolia* from dietary supplements tablets and capsules using LC-MS [53]. Near infrared (NIR) spectral database can be also be utilized for rapid screening of the test sample to verify their contents as labeled on the herbal products [54].

3. EVIDENCE-BASED OVERVIEW OF ANTI-PROLIFERATIVE AND ANTICANCER ACTIVITIES OF *EURYCOMA LONGIFOLIA* AND ITS MEDICINALLY ACTIVE COMPOUNDS

Investigations on the cytotoxicity of a newly synthesized, isolated, purified drug entity or traditional herbal medicines are very crucial before further screening of their pharmacol-

ogical activities. After establishing cytotoxicity, their anti-proliferative and anticancer efficacies are screened using various *in vitro* cell culture and *in vivo* (murine and other animals) models. Numerous compounds/metabolites isolated from *Eurycoma longifolia* Jack have been investigated for anti-proliferative effects, cytotoxicity and anticancer efficacy. Some of these compounds have shown promising anti-proliferative and anticancer efficacies against various types of murine or human cancers.

Cancer, also known as malignancy, is a group of diseases involving an abnormal growth of cells with the potential to invade other organs or parts of the body. Most common signs and symptoms associated with cancer include severe pain, abnormal bleeding, unexplained weight loss, a characteristic lump, ulceration, prolonged cough, and a change in bowel movements. The signs and symptoms can vary with the types of human cancer. There are more than 100 types of cancer, including lung cancer, ovarian cancer, malignant melanoma, breast cancer, prostate cancer, colorectal cancer, cervical cancer, skin cancer, leukemia, and lymphoma. The most common cancer therapeutic modalities include chemotherapy, radiation, and/or surgery.

Numerous studies evidenced that the water, methanolic, ethanolic or butanolic extract of *Eurycoma longifolia* Jack and various bioactive compounds isolated from this medicinal plant have shown promising anti-proliferative and cytotoxic effects against various human cancer cell lines including HepG2, HM3KO, Hela, CaOV-3, A-2780, MCF-7, HT-29, and A-549 cells.

Eurycomanone, one of the most bioactive medicinal compounds isolated from the extract of *Eurycoma longifolia* has shown strong anticancer efficacy against various types of human cancers including HepG2, HM3KO, Hela, CaOV-3, A2780, MCF-7, HT-29 and A549 cells. Wong *et al.* [39] evaluated the anti-proliferative and anticancer efficacy of the purified eurycomanone on the expression of selected genes of human lung adenocarcinoma (A549 cells) at a concentration range of 5 to 20 µg/mL. Eurycomanone significantly inhibited the proliferation of human A549 lung adenocarcinoma cells in a dose-dependent manner with lowest cell growth observed at 20 µg/mL (Fig. **2A**). Further analysis showed that at 5.1 µg/mL concentration, eurycomanone inhibited 50% of the cell growth (GI_{50}). These results were in line with a previous study which was executed on cancerous liver cells [17]. The anti-proliferative potential of eurycomanone was also compared with cisplatin, a well-known chemotherapeutic agent for the treatment of lung cancer. Results showed that cisplatin also inhibited the proliferation of A549 cells in a dose-dependent manner at concentrations ranging from 0.2 to 15 µg/mL and the lowest cell growth was observed at 15 µg/mL [39]. Wong and co-workers explained that cisplatin (GI_{50} = 0.58 µg/mL) was found to be ten-folds more potent than eurycomanone (GI_{50} = 0.58 µg/mL) for the inhibition A-549 cells (Fig. **2A**). Interestingly, results showed that even after the removal of the eurycomanone treatment (after 72 h), the normal cell growth efficacy was not restored (>30% cell growth was still inhibited) (Fig. **2B**). Eurycomanone had also reduced the colony formation effi-

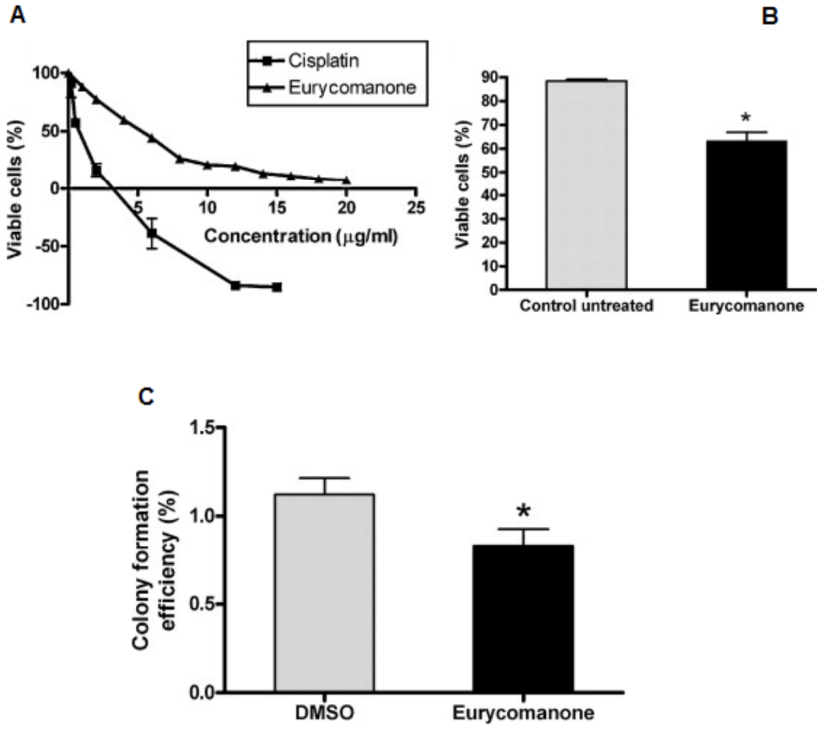

Fig. (2). The effects of eurycomanone on A-549 lung cancer cell proliferation. (**A**) Dose-dependent inhibition of A549 human lung cancer cell proliferation by eurycomanone and cisplatin. (**B**) The effects of removing eurycomanone following treatment of A549 cell. Cells were treated with eurycomanone for 72 h. Following that the treatment medium was removed and replaced with fresh growth medium. Cells were reseeded and cell proliferation was measured using CellTiter 96® Cell Proliferation Assay. (**C**) The effects of eurycomanone on A549 lung cancer cell anchorage-independent growth. Three independent experiments were performed [39]. Reprinted with permission from Elsevier GmbH (Copyright © 2011) through Copyright Clearance Centre.

ciency (anchorage-independent growth) of A549 cells significantly compared to the DMSO (Fig. **2C**) [39]. They have also noticed a remarkable reduction in the mRNA expression of lung carcinoma-related biomarkers including hnRNP, p53 tumor suppressor protein, PHB, ANX1, and ERp28 within 72 h of treatment. These results indicated a promising anti-proliferative and anti-clonogenic efficacy of eurycomanone against A-549 lung cancer cells [39].

Mahfudh *et al.* [55] evaluated the cytotoxicity of eurycomanone against Hela cells using methylene blue staining assay, Hoechst 33258 nuclear staining, flow cytometry and TUNEL assay. They further investigated the mechanism of cytotoxicity of eurycomanone by examining the protein expression of p53, Bax and Bcl-2 using Western blotting and immunostaining assays. Eurycomanone showed selective cytotoxicity against various cancerous cell lines (MCF-7, HeLa, CaOv-3, HM3KO, and HepG2) but was least toxic against normal human cells (MDBK and Vero cells). The cytotoxicity of eurycomanone is mainly attributed to its ability to induce apoptosis in cancerous cells by inducing chromatin condensation, appearance of apoptotic bodies, and DNA fragmentation in cancerous cells treated with this potent medicinal compound [55]. These results were in line with a previous study in which authors demonstrated that eurycomanone was found very efficacious against various types of human cancer cell lines [56].

Methanolic extract of *Eurycoma longifolia* has also been investigated against human leukemia cells (K-562) [57]. In this study, a wide range of *in vitro* experiments were performed including cell viability, clonogenic assay, annexin V-FITC/PI assay, Hoechst 33342 staining, cell cycle, and RT^2 profiler™ PCR array. The anticancer potential of *Eurycoma longifolia* was also evaluated by measuring the tumor volume, inhibition of tumor growth and histological examination using Balb/C nude mice. Their results showed a significant anti-proliferative and growth inhibition activity against K-562 cells treated with various fractions of *Eurycoma longifolia.* Potent cytotoxicity and anti-proliferative effects were observed after 48 h of treatment with IC_{50} value of 1963 and 661 mg/mL, respectively [57]. In this study, authors have also observed a dose- and time-dependent cytotoxicity of K-562 cells at different concentrations of *Eurycoma longifolia* at various time points [57]. They further explored the anti-proliferative and cytotoxicity mechanisms of *Eurycoma longifolia.* By evaluating the biochemical parameters of early apoptosis (Annexin-V positive) and late apoptosis/necrosis (Annexin-V/PI positive), they suggested that treatment of K-562 cells with various fractions of *Eurycoma longifolia* caused induction of apoptosis in a dose- and time-dependent manner. Nuclear changes such as chromatin condensation and DNA fragmentation which are the hallmark features of apoptosis were also identified in K-562 cells treated with *Eurycoma longifolia* (Hoechst 33342 staining). A promising anti-leukemic potential of *Eurycoma longifolia* was also confirmed by an *in vivo* animal study that showed a remarkable decrease in the tumor volume, number of viable tumor cells (VC), increased numbers of apoptotic cells (AC) and the necrotic cells (NC) in the *Eurycoma longifolia* (TAF273)-treated mice compared to the control groups (Fig. **3**). Further analysis of histological micrographs using Image J software revealed a significantly higher percentage of necrosis in *Eurycoma longifolia* (TAF273)-treated group compared to the control group. The mean count of apoptotic cells were 2566 and 103611 in the control and TAF273 groups, respectively (Fig. **3**). These findings suggested that *Eurycoma longifolia* exhibits a strong anti-leukemic potential [57]. The anti-proliferative and anticancer efficacy of alcoholic and aqueous extracts of *Eurycoma longifolia* has also been investigated by other researchers [58-60]. *Eurycoma longifolia* has also been used in combination with *Dipterocarpus obtusifolius* and *Tamilnadia uliginosa* for the treatment of various types of human cancers [61].

The standardized quassinoids extract of *Eurycoma longifolia* (SQ40) was also investigated for the treatment of human prostate cancer [62]. SQ40 is an extract that contains 40% (w/w) of quassinoids which was tested for anti-proliferative and anticancer activities against the human prostate cancer using a series of *in vitro* and *in vivo* experiments. The cell viability analysis revealed that SQ40 showed a strong dose-dependent cytotoxicity against LNCaP cells (human prostate cancer cell line); however, showed no cytotoxicity against human normal prostate (RWPE-1) and liver (WRL-68) cells. The concentrations of SQ40 that cause maximal half inhibitory effects (IC_{50}) against RWPE-1, WRL-68 and LNCaP cell lines were 59.26 μg/mL, 27.69 μg/mL, and 5.97 μg/mL, which indicated that SQ40 exhibits potent cytotoxicity against human prostate cancer. The analysis of growth kinetics of LNCaP, RWPE-1 and WRL-68 cells using impedance-based cell sensing measurement system further validated that QS40 showed cytostatic effects at lower concentrations (2.5-10 μg/mL) and cytotoxic response at higher concentrations (20-80 μg/mL). A significant dose-dependent down-regulation of cell cycle regulatory proteins such as CDK4, CDK2, Cyclin D1 and Cyclin D3 and subsequent up-regulation of cell cycle inhibitory protein ($p21^{Waf1/Kip1}$) in SQ40-treated LNCaP cells further validated the anti-proliferative and cytotoxic mechanisms of this quassinoids extract against human prostate cancer [62]. The anticancer activity of SQ40 was also evidenced using the *in vivo* LNCaP tumor xenograft growth in nude mice. An intraperitoneal administration of SQ40 for a period of six weeks in prostate cancer induced nude mice showed a significant dose-dependent decrease in the tumor volume compared to the control groups (Fig. **4**). These findings evidenced that *Eurycoma longifolia* exhibit strong anticancer potential against human prostate cancer [62]. The potency of quassinoids extract of *Eurycoma longifolia* has also been demonstrated by other researchers [63, 64]. They suggested that the quassinoids from the leave extract of *Eurycoma longifolia* exhibits promising potential against A-549 (human lung cancer cells) and other human cancers.

Several other medicinal compounds of *Eurycoma longifolia* including eurycomanol, 13-β, 21-dihydroxyeurycomanone, 14-hydroxyglaucarubol, eurycomalactone, eurycomadilactone, 5-iso-eurycomadilactone, 13-epi-eurycomadilactone, longilactone, 6-dehyroxylongilactone, canthin-6-one, 9-methoxycanthin-6-one, canthin-6-one 9-*O*-beta-glucopyranoside, 14,15 beta-dihydroxyklaineanone, pasakbumin B, and pasakbumin C have also shown strong anti-proliferative and anticancer activities against a wide variety of human cancers *in vitro* and *in vivo* [1, 2, 20]. A summary of

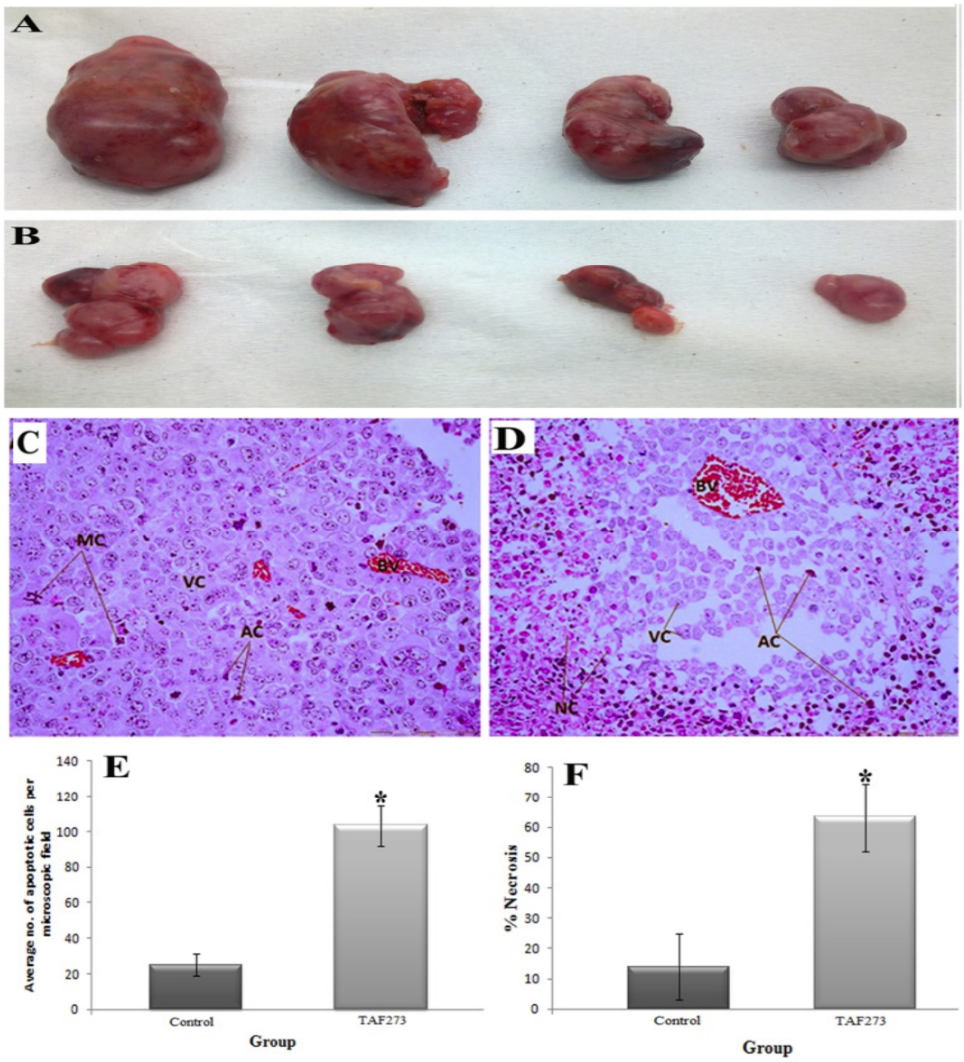

Fig. (3). Effect of TAF273 on the size and histological appearance of subcutaneous tumor induced by injecting the K-562 cells in nude mice. (**A**) Gross appearance of tumors in the control mice. (**B**) Gross appearance of tumors in TAF273-treated mice. (**C**) An H&E-stained tumor section (original magnification of 40×) of the control group is composed of compact sheet of aggressively proliferating viable tumor cells (VC), abundance of blood vessels (BV) and the presence of mitotic figures (MC). (**D**) The tumor section (original magnification of 40×) of TAF273 (50 mg/kg) treated cells revealed notable changes in tumor histology, as significant loss of compact arrangement of viable tumor cells (VC), with less number of blood vessels (BV), abundance of apoptotic cells (AC) surrounded by necrotic regions (NC) and absence of mitotic figures. (**E**) Graphical comparison of the mean apoptotic cells/microscopic field (control *vs* TAF273). (**F**) Graphical comparison of the mean necrotic areas (control *vs* TAF273) as calculated by using image J software. Values are presented as mean ± SD, (n=4) [57]. Reprinted with permission from Al-Salahi et al. [57] (Copyright © 2014).

anti-proliferative and anticancer activities of *Eurycoma longifolia* and its medicinal compounds is presented in (Table **1**).

The prime mode of cytotoxicity of *Eurycoma longifolia* and its medicinally active compounds in cancerous cells is the induction of apoptosis (programmed cell death) *via* the up-regulation of the expression of p53 (a tumor suppressor protein) and pro-apoptotic protein (Bax), and the down-regulation of the expression of anti-apoptotic protein (Bcl-2) [17, 55]. However, other studies suggested that activation of Caspases (apoptotic signaling cascades) [65] and/or inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) have also been identified as the important molecular targets of *Eurycoma longifolia* and its medicinal compounds to provoke anti-proliferative and anticancer activities [32, 66].

Apoptosis is a physiological cascade of programmed cell death to eradicate abnormal/cancerous cells while preserving the overall structure of surrounding tissue. It is a prime barricade to oncogenesis, hence, an important mechanism to maintain homeostasis within an organism. Among several regulators of apoptosis and suppressor of carcinogenesis, p53 play crucial role in regulating apoptosis of cancerous cells and maintaining internal environment constant [67, 68]. Generally, apoptosis is regulated by the two main pathways; an extrinsic "death receptor signaling" pathway and an intrinsic "mitochondria" pathway. Various regulatory proteins including Bcl-2, Bax and Caspases family members also impart prominent roles in the regulation of apoptosis. Because of its potent tumor suppressive effects, p53 is a fundamental molecular/pharmacological target for anticancer therapeutics [69-71]. It can be activated in response to

Table 1. Summary of *in vitro* and *in vivo* studies for the anti-proliferative and anticancer efficacies of *Eurycoma longifolia* and its medicinal compounds.

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
1999	Eurycomanone from the root extract	*In vitro* cell culture	Breast cancer (MCF-7 cells) Colon cancer Fibrosarcoma (HT-1080 cells) Lung cancer (A-549 cells) Melanoma KB, and KB-V1 (a multi-drug resistant cell line derived from KB)	-	Extraction, isolation and structure elucidation of bioactive compounds from root extract of *Eurycoma longifolia,* cell proliferation and cell cytotoxicity	1. Strong anti-proliferative and cytotoxic effects were observed against all human cancer cell lines; however, the anti-proliferative effects were moderate against murine cell lines.	[53]
2002	Quassinoids from leave extract	*In vitro* cell culture	Lung cancer (A-549 cells)	-	Extraction, isolation and structure elucidation of 6 bioactive compounds, MTT assay, cell cytotoxicity	1. Significant anti-proliferative and cell toxicity were observed against human lung cancer cell line (A-549).	[60]
2002	Methanol, methanol-water (1 : 1) and water extracts	*In vitro* cell culture	Fibrosarcoma (HT-1080 cells) Cervical adenocarcinoma (HeLa cells) Lung cancer (A-549 cells) Murine colon carcinoma (26-L5 cells) Murine Lewis lung carcinoma (LLC cells) Murine melanoma (B16-BL6 cells)	Morphological change in cell features, DNA fragmentation and induction of apoptosis	MTT assay, *In vitro* growth inhibition test, morphological changes using phase contrast microscopy, DNA fragmentation	1. Significant anti-proliferative activity was observed against most of the human and murine leukemic cell lines. 2. Obvious morphological changes were observed under phase contrast microscopy in all the human and murine leukemic cell lines after treatment with *Eurycoma longifolia* extract. 3. DNA fragmentation was also observed in most of the leukemic cell lines.	[55]
2004	Sixty-five bioactive compounds extracted and isolated from roots	*In vitro* cell culture	Lung cancer (A-549 cells) Breast cancer (MCF-7 cells)	Cell cytotoxicity	Bioactive compounds extraction, isolation and purification and cell cytotoxicity	1. Significant anti-proliferative effects against A-549 and MCF-7 cell lines.	[17]
2005	Methanol, n-butanol, chloroform and water extracts of roots	*In vitro* cell culture	Leukemia (KB cells) Prostate cancer (DU-145 cells) Rhabdomyosarcoma (RD cells) Breast cancer (MCF-7 cells) Ovarian carcinoma (CaOV-3 cells) Madin-Darby bovine normal kidney epithelial cells (MDBK cells)	Cell cytotoxicity	Bioactive compounds extraction, cell anti-proliferation assay and cell cytotoxicity	1. Significant anti-proliferative and cytotoxicity effects were observed against KB, DU-145, MCF-7 and CaOV-3 cells. 2. No cytotoxicity was observed against normal kidney epithelial cells (MDBK).	[56]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2005	Chromatographic fraction from root extracts	*In vitro* cell culture	Breast cancer (MCF-7 cells)	Bcl-2-mediated apoptosis and DNA damage	Cell proliferation, cell cytotoxicity, Tdt-mediated dUTP nick end labelling assay, nuclear staining and Western blotting	1. Significant anti-proliferation efficacy was observed against MCF-7 cell line; however, no cytotoxicity was observed against normal human breast cells (MCF-10A). 2. Significantly increased apoptosis in MCF-7 cells, as evaluated by the Tdt-mediated dUTP nick end labelling assay and nuclear morphology. 3. Significant down-regulation of the anti-apoptotic Bcl-2 protein expression; however, no effect on the relative expression of pro-apoptotic protein, Bax.	[18]
2007	F16, a plant-derived pharmacologically active fraction	*In vitro* cell culture	Breast cancer (MCF-7 cells)	Apoptosis *via* Caspase-9 independent pathway Bcl-2-mediated apoptosis and DNA damage	Cell proliferation, cell viability, protein extraction, Western blotting	1. Strong anti-proliferative activity of F16 was observed against human breast cancer (MCF-7) caused by induction of apoptosis. 2. F16 induce apoptosis in MCF-7 cell by the cleavage of caspase-7 and PARP-1, independent of caspase-9 and p53.	[62]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2008	Eurycomanone extracted from the roots	*In vitro* cell culture	Liver carcinoma (HepG2 cells) Malignant melanoma (HM3KO cells) Cervical cancer (Hela cells) Breast cancer (MCF-7 cells) Ovarian carcinoma (CaOV-3 cells) MDBK and Vero cells	Apoptosis induction mediated *via* the up-regulation of p53 and Bax proteins and down-regulation of Bcl-2 protein.	Methylene blue staining assay, Hoechst 33258 nuclear staining, TUNEL assay, flow cytometry with Annexin-V/propidium iodide double staining, Western blotting, and immunostaining assay	1. Remarkable cytotoxicity was observed against all the human cancerous cell lines (HeLa, CaOv-3, HM3KO, HepG2, MCF-7) and no cytotoxicity against normal human cell lines (MDBK and Vero cells). 2. The characteristic features including DNA fragmentation, chromatin condensation, and apoptotic bodies evidenced significant apoptosis in all the cancerous cell line. 3. The mechanism of apoptosis was further evidenced from the up-regulation of p53 followed by a significant increase in pro-apoptotic protein, Bax, and down-regulation of anti-apoptotic protein, Bcl-2.	[52]
2009	Eurycomanone from the root extract	*In vitro* cell culture and *in vivo* animal study using nude mice	Liver carcinoma (HepG2 cells) Malignant Melanoma (HM3KO cells) Cervical cancer (Hela cells) Ovarian carcinoma (CaOV-3 cells)	Apoptosis induced by up-regulation of p53 and Bax and down-regulation of Bcl-2 proteins	MTT assay, cell cycle analysis, cytotoxicity study, flow cytometry, detection of proteins involve in apoptosis (Bcl-2; Bax; p53; Cytochrome C), relative tumor growth ratio and relative tumor volume	1. Significant antiproliferative effects against human cervical cancer cell (Hela), human malignant melanoma cell (HM3KO), human ovarian carcinoma cell (CaOV3), and human liver cancer cell (HepG2) and with IC_{50} values of 60 ± 0.25 µg/mL, 60 ± 0.25 µg/mL, 79 ± 0.16 µg/mL, and 45 ± 0.15 µg/mL, respectively. 2. No cytotoxicity against human normal cells such as skin cell (CCD11114sk), liver cells (Chang's liver), and WLR-68. 3. Significant decrease in tumor volume in nude mice after an intraperitoneal dose of 17 mg/kg.	[14]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2009	Eurycomanone from the root extract	*In vitro* cell culture	Liver carcinoma (HepG2 cells) Malignant melanoma (HM3KO cells) Cervical cancer (Hela cells) Breast cancer (MCF-7 cells) Ovarian carcinoma (CaOV-3 cells) MDBK and Vero cells	Apoptosis, DNA damage and nuclear condensation	Extraction, cell proliferation, cell cytotoxicity, Giemsa staining, Hoechst 33258 nuclear staining, TUNEL assay, flow cytometry, and annexin-V/PI double staining	1. Dose-dependent anti-proliferative effects and decreased viability of cancerous cells (CaOv-3, HeLa, HepG2, HM3KO and MCF-7) with IC_{50} value of values of <20 µg/mL. 2. No cytotoxicity was observed against non-cancerous cells (MDBK and Vero). 3. Obvious morphological changes observed in cellular features including the loss of adhesion, rounding, and sporadical distribution in cancerous cells. 4. Obvious apoptosis observed in cancerous cells compared to the normal cells with normal cellular features.	[15]
2010	Twenty four quassinoids isolated from the roots extract	*In vitro* cell culture	Lung adenocarcinoma (A-549 cells) Murine colon carcinoma (26-L5 cells) Murine melanoma (B16-BL6) Murine lung carcinoma (LLC cells)	-	Extraction, isolation and structure elucidation of bioactive compounds, cell proliferation, and cell cytotoxicity	1. Among the tested compounds, eurycomalactone displayed the most potent activity against all the tested cell lines; murine colon cancer cell line (IC_{50} = 0.70 µM), murine melanoma (IC_{50} = 0.59 µM), murine lung carcinoma cell line (IC_{50} = 0.78 µM), as well as human lung adrenocarcinoma (IC_{50} = 0.73 µM). 2. Anti-cancer efficacy was comparable to doxorubicin (control).	[61]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2012	Eurycomanone from the root extract	*In vitro* cell culture	Lung carcinoma (A-549 cells)	p53-induced apoptosis by enhanced expression of Bax and decreased expression of Bcl-2 proteins	Extraction and isolation, cell viability, cell proliferation, soft agar colony formation assay, immunoblotting analysis, reverse transcription real-time quantitative PCR (RT-qPCR)	1. Strong dose-dependent anti-proliferative effects of eurycomanone were observed against human lung carcinoma cells (A549) at concentrations ranging from 5-20 µg/mL. 2. Significant (>25%) suppression of anchorage-independent growth of A-549 cells. 3. Promising down-regulation in the expression of human lung cancer markers, heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1, p53 tumor suppressor protein and other cancer-associated genes including prohibitin (PHB), annexin 1 (ANX1) and endoplasmic reticulum protein 28 (ERp28) but not the house keeping genes.	[36]
2013	Methanolic root extract	*In vitro* cell culture	Promyelocytic leukemia (HL-60 cells)	Apoptosis, DNA damage, nuclear condensation and down-regulated CD14	Bioactive compound extraction, cell proliferation assay, cell morphology, measurement of apoptosis using flow cytometry, cell differentiation (expression of CD14)	1. Significant inhibition of cell growth of HL-60 cells in time- and dose dependent manner with mean IC_{50} values of 15.2 µg/mL, compared to vincristine (IC_{50} = 0.3 µg/mL). 2. Obvious nuclear condensation, DNA damage and membrane blebbing were observed in HL-60 cells in dose- and time-dependent manner at 100 µg/mL. 3. Induction of apoptosis in HL-60 cells was observed in time- and dose-dependent manner.	[16]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2013	Ethanolic extracts of root, leaves and stem	*In vitro* cell culture	Colorectal cancer (HT-29 cells) Breast cancer (MCF-7 cells) Ovarian carcinoma (A-2780 cells) Normal liver cells (WRL-68)	Apoptosis and DNA damage	Cell viability, and cell cytotoxicity	1. Significant anti-proliferative activity against all the cancerous cell lines (HT-29, MCF-7 and A2780 cells). 2. Comparative analysis revealed a higher cytotoxicity efficacy of root and stem extracts against human colorectal cancer cell line (HT-29) and of the leaf extract against human ovarian carcinoma cell line (A2780).	[57]
2014	Quassinoids includeing 14-hydroxyglaucarubol, 5-iso-eurycomadilactone, eurycomadilactone, 13-epi-eurycomadilactone, eurycomanone, eurycomanol, and 13β, 21-dihydroxy-eurycomanone isolated from the roots	*In vitro* cell culture	Colorectal cancer (HT-29 cells) Breast cancer (MCF-7 cells) Intestinal cancer (LOVO cells) Gastric cancer (BGC-823 & MGC-803 cells) Liver carcinoma (HepG2 cells), Cervical carcinoma (HeLa cells) Lung adenocarcinoma (A-549 cells)	Cytotoxicity induced *via* activation of p53	Extraction, isolation, and structure elucidation, cytotoxicity against HT-29, MCF-7, LOVO, BGC-823, MGC-803, HepG2, HeLa, and A549 cancer cell lines	1. Anti-proliferative efficacy of quassinoids was more obvious and potent against the human breast cancer cell line (MCF-7) with IC_{50} values of 24.9 μM, 11.8 μM, 44.1 μM, and 14.1 μM of various bioactive compounds. 2. Moderate anti-proliferative effects against other cancer cell lines (HT-29, LOVO, BGC-823, MGC-803, HepG2, HeLa, and A549) compared to the fluorouracil.	[2]
2014	Quassinoids includeing Eurylactone E, Eurylactone F, Eurylactone G, eurycomalide D, Eurycomalide E isolated from the root extracts	*In vitro* cell culture	Lung cancer (A-549 cells) Cervical cancer (HeLa cells)	-	Extraction and isolation of quassinoids, structure elucidation, cytotoxicity against A549 and HeLa cell lines	1. Significant cytotoxicity of all the tested quassionoids on human lung carcinoma (A-549 cells). 2. Remarkable anti-proliferative effects of all the tested quassionoids against the human cervical carcinoma (HeLa cells).	[1]

(Table 1) contd….

Study Year	***Eurycoma longifolia*/ Bioactive Compound(s)**	**Study Design**	**Types of Human Cancer (Cell Line)**	**Mechanism(s) of Cell Death**	**Study Parameters**	**Major Findings**	**Ref.**
2014	Quassinoids: eurycomanone and eurycomanol from root extracts	*In vitro* cell culture	Acute T-lymphocyte leukemia (Jurkat) Chronic myelogenous leukemia (K562 cells)	Inhibition of NF-κB signalling through the inhibition of IκBα phosphorylation and upstream mitogen activated protein kinase (MAPK) signaling and apoptosis	Cell viability, cell proliferation, IncuCyteTM video microscopy-based approach, Hoechst staining, flow cytometry	1. Significant anti-proliferative effects of eurycomanone were observed against Jurkat and K562 cell lines in dose- and time- dependent manner; however, moderate effects were shown by eurycomanol. 2. Significant dose- and time-dependent apoptosis was observed in both leukemic cell lines treated with eurycomanone; however, moderate anti-proliferative effects were observed in cells treated with eurycomanol. 3. No cytotoxicity was observed against peripheral blood mononuclear cells (PBMCs) from healthy donors by both the quassinoids. 4. Significant inhibition of NF-κB and MAPK by TNFα without strongly affecting the viability of healthy cells.	[63]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2014	Methanolic extract of root	*In vitro* cell culture and *in vivo* animal study using nude mice	Chronic myelocytic leukemic (K-562 cells)	Bax- and p53 induced apoptosis, Cell cycle arrest, inhibition of angiogenesis and DNA damage	Cell viability assay, clonogenic assay, annexin V-FITC/PI assay, Hoechst 33342 staining, cell cycle analysis, RT^2 profiler^{TM} PCR array, *in vivo* experiments using nude mice, histological examination	1. Potent-to-moderate anti-proliferative effects were observed against K-562 cells with IC_{50} values varied from 6 ± 1 µg/mL to 62 ± 7 µg/mL. 2. Significant dose- and time-dependent cytotoxicity was observed. 3. Significant dose- and time-dependent apoptosis was observed in K-562 cells. 4. Significant changes in the chromatin structure including fragmentation, uniform condensation and forming clusters against the nuclear periphery were observed in K-562 cells. 5. Significant growth inhibition of subcutaneous tumor in nude mice after the intra-peritoneal administration.	[54]
2015	Standardized quassinoid extract from roots	*In vitro* cell culture and *in vivo* LNCaP xenograft study	Prostate cancer (LNCaP cells)	Cell cycle arrest, down-regulation of expression of prostate cancer markers and reduction in volume of tumor	Cell viability assay, soft agar colony formation assay, Real-time cell proliferation analysis, trypan blue exclusion test, cell cycle analysis, immunoblotting analysis, prostate specific antigen (PSA) ELISA and i*n vivo* LNCaP xenograft study	1. Significant anti-proliferative effects and cytotoxicity against human prostate cancer (LNCaP). 2. Significant down-regulation of relative expression of G1-to-S phase transition regulatory proteins and up-regulation of inhibitory protein which subsequently led to cell cycle arrest in G0/G1 phase. 3. Promising anticancer activity against mouse xenograft model.	[59]

(Table 1) contd….

Study Year	*Eurycoma longifolia*/ Bioactive Compound(s)	Study Design	Types of Human Cancer (Cell Line)	Mechanism(s) of Cell Death	Study Parameters	Major Findings	Ref.
2017	Thai herbal recipe containing roots extract of three medicinal plants such as *Eurycoma longifolia, Dipterocarpus obtusifolius* and *Tamilnadia uliginosa* in a ratio of approximately 7:4:3.	*In vitro* cell culture	Breast cancer (MCF-7, SKBR3 and MDA-MB435 cells) Lung cancer (A-549 cells) Colon cancer (HCT116 cells) Cervical cancer (HeLa cells) Leukemia (NB4 & K562 cells) Liver cancer (Hep3B & C3A cells)	Cell cycle arrest and apoptosis	Extraction and formulation of Thai herbal recipe, MTT assay, cell cytotoxicity, and cell cycle analysis	1. A potent anti-proliferative effect of Thai herbal recipe against HCT116, HeLa, SKBR3, K562 and NB4 with ED_{50} values of 70, 80, 85, 75, and 70 µg/mL, respectively. 2. Moderate anti proliferative effect was observed against A549, MCF-7 and MDA-MB435 cancer cell lines (<250 µg/mL); however, weaker anti-proliferative efficacy was observed against Hep3B and C3A cancer cell lines. 3. Higher percentage of apoptosis was observed in all cancer cell lines except HCT116 which showed significant S phase arrest.	[58]

oncogene activation, DNA damage, cellular abnormality or hypoxia, and trigger biological output such as programmed death of abnormal cells, cell-cycle arrest and modulation of autophagy [72-74]. p53 mainly functions as a transcriptional factor, and can trigger or stimulate variety of anti-proliferative cascades by activating or suppressing key effectors genes [75, 76].

Caspase family members have also been established as essential mediators of apoptosis [77-79]. These cell regulatory proteins are created within the cell as inactive zymogens that lack significant protease activity. They activate in response to molecular or chemical signals of cells death [80]. After receiving specific cell death signals, they started the cleavage and dismantling of cellular machinery (apoptosis) [81, 82]. Similarly, mitochondrial dysfunction, loss of survival signals, or DNA damage might cause activation of caspases may predispose to apoptosis. The inactivation or functional defect of caspases may lead to oncogenesis. On the other hand, over-expression of caspases may stimulate cellular suicide, and this may the basis for degenerative diseases such as Alzheimer's disease and Huntington's disease.

CONCLUSION

The critical analysis of the literature revealed that aqueous, methanolic, ethanolic, or butanolic extracts of various parts (roots, stem, bark, or leaves) of *Eurycoma longifolia* and its medicinally active compounds have shown potent anti-proliferative and anticancer efficacy against various types of human cancers including hepatocellular carcinoma, malignant melanoma, cervical cancer, ovarian carcinoma, breast cancer, colorectal cancer and lung cancer. A plethora of *in vitro* and *in vivo* studies have demonstrated their safety, efficacy, tolerability, pharmacological efficacy and therapeutic feasibility against various types of human cancer cell lines. The anti-proliferative and anticancer activity of *Eurycoma longifolia* is attributed to its ability to provoke induction of apoptosis (programmed cell death) *via* the up-regulation of the expression of p53 (tumor suppressor protein) and Bax (pro-apoptotic protein) and down-regulation of Bcl-2 (anti-apoptotic protein). Activation of caspases (apoptotic signaling cascades) and inhibition of NF-κB have also been recognized as the important molecular targets for *Eurycoma longifolia* and its medicinally active compounds. *Eurycoma longifolia*-mediated treatment of cancer cell lines have shown remarkable efficacy in down-regulating the cell growth and causing nuclear condensation,

DNA fragmentation, membrane blebbing and change in the cell morphology. Treatment of cancer induced animals with *Eurycoma longifolia* showed significant decrease in the cancerous lump size, tumor volume and cancer-related mediators locally and in the plasma.

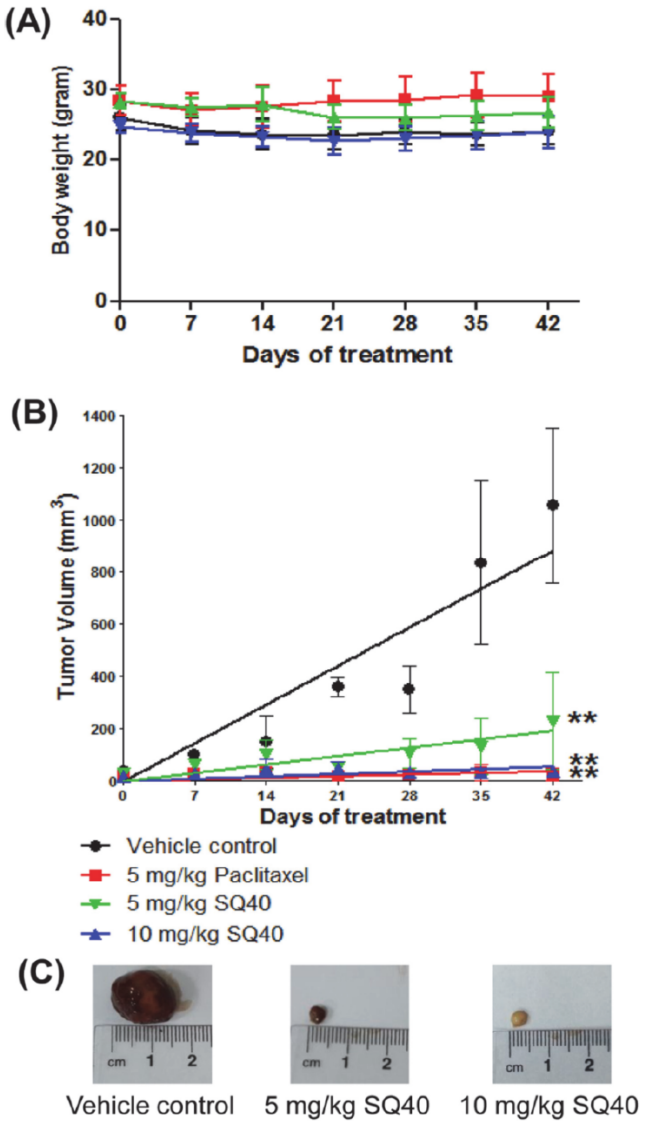

Fig. (4). Anti-tumor activity of SQ40 against subcutaneous LNCaP cell tumors. LNCaP cells at 2 × 10^6 were injected subcutaneously into right flank of NC nude mice. SQ40 treatment was initiated when the tumor was palpable. Vehicle control (saline) and SQ40 were given intraperitoneally thrice a week for 6 weeks with a total of 18 doses. (**A**) Mean body weight and (**B**) tumor volume for each treatment *versus* the number of days after initial injection of LNCaP cells. (**C**) Representative images of tumors isolated from vehicle control, 5 mg/kg and 10 mg/kg of SQ40-treated animals. Each point represents the mean ± SEM of data (n = 6). ** indicates $p<0.01$ *versus* vehicle control [62]. Reprinted with permission from Tong *et al*. [62] (Copyright © 2015).

FUTURE PROSPECTS

Even though numerous studies have explored the pharmacological significance and therapeutic viability of *Eurycoma longifolia* and its bioactive compounds for the treatment of various types of human cancers, much has yet to be executed and learned. To gain further insight into the anticancer trends of *Eurycoma longifolia*, we have noticed substantial gaps in research which include but not limited to; 1) lack of integration of medicinal chemistry, biology, pharmacology and toxicology which could be a promising way to further explore the anticancer specificity of *Eurycoma longifolia* and its compounds against each specific type of human cancer, 2) lack of sufficient attention on pharmacologically active constituents and their determination, identification, standardization and structural manipulation for future developments of new structural and functional analogs, 3) lack of research on individual translational anticancer mechanism of most active medicinal compounds against various types of human cancers, 4) lack of comparative anticancer analysis of most active constituents of *Eurycoma longifolia,* 5) lack of sufficient *in vivo* and human clinical studies to further explore demographic specificity and variations, and 6) lack of sufficient safety profile and toxicity data to conduct human clinical trials.

LIST OF ABBREVIATIONS

A-549	=	Human lung adenocarcinoma cells
hnRNP	=	Heterogeneous nuclear ribonucleoprotein
PHB	=	Prohibitin
ANX1	=	Annexin-1
ERp28	=	Endoplasmic reticulum protein-28
ACS	=	American Cancer Society
CAM	=	Complementary and alternative medicines
WHO	=	World Health Organization
MCF-7	=	Human breast carcinoma cells
HeLa	=	Human cervical carcinoma cells
HepG2	=	Hepatocellular carcinoma cells
HM3KO	=	Human malignant melanoma cells
CaOV-3	=	Human ovarian carcinoma cells
HT-29	=	Human colorectal cancer cells
LNCaP	=	Human prostate cancer cells
RWPE-1	=	Human normal prostate cells
and WRL-68	=	Human normal liver cells
K562	=	Human chronic myelogenous leukemia cells
MAPK	=	Mitogen activated protein kinase
LC-MS	=	Liquid chromatography-mass spectrometry

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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