RESEARCH ARTICLE

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The Apoptotic Potential Activity of *Terminalia Brownii* Extracts against Various Cancer Cell Lines

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Abstract

The present study aimed to investigate the potential inhibitory activities of various *Terminalia brownie* extracts against the proliferation of different cancer cell lines. Fractionation was carried out through the process of liquid-liquid extraction produced four fraction types using polarity-enhancing solvents such as, n-hexane, chloroform, ethyl acetate, and water. All extracts have been tested for their cytotoxicity against cancer cell lines using SRB assay and their inhibitory activity on cell cycle phases using flow cytometry. The chloroform extract exhibited a cytotoxic effect against all three types of cancer cell lines and especially the HepG2 cells at 100-µg concentration. Moreover, the most prominent plant extracts of chloroform, acetyl acetate, and hexane showed significant induction of cell death during the S phase cell cycle. The findings of this study suggest that compounds in the *Terminalia brownie* plant used in traditional Saudi medicine may have the potential to suppress the growth of cancer cell lines.

Keywords: *Terminalia brownie*- anticancer- apoptosis- phenolic compounds

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Introduction

Cancer is a disease with a high mortality risk that constitutes a major health burden in developed and developing countries alike. The transformation of normal cells into cancerous cells exhibits a wide range of biological alterations. At global level, cancer remains the second cause of human mortality, despite the availability of a multitude of treatments (Abdel-Magid, 2014). The progression of cancerous cells to different pathological and metabolic conditions is greatly aided by mutations (Kuppusamy et al., 2016). Normal cells are transformed to be cancerous when mutant genes are generated in greater amount due to the dysfunction of anti-apoptotic mechanisms and pathways by various transcriptional pathway disorders (Bradner et al., 2017). Furthermore, the regular functionality of neighboring cells may be disrupted due to growing of cancerous cells, irregular proliferation and differentiation. The expression of apoptotic genes inhibits chemical response, making cells unresponsive to signals or chemicals promoting programmed cell death (Newman et al., 2000). Among mechanisms that modulate the differentiation and proliferation of cancerous cells is the equilibrium of gene expression pathways respectively promoting and suppressing cancerous cells, as well as inhibition of anti-cancer genes and promotion of pro-cancer genes (Newman et al., 2000). A wide range of terrestrial and marine flora and fauna have supplied natural product medicines (Machumi et al., 2013). Indeed, ample literature has addressed the value of natural products in modern medicine (Newman et al., 2000), with a number of studies focusing particularly on cancer (Cragg et al., 2009). Furthermore, natural products are of great help in the search for and production of new drugs. In addition, secondary metabolites of natural origin exhibit potential for drug development because, compared to synthetic compounds, they have demonstrated effects more similar to drugs and better biological compatibility when introduced in living systems (Drahl et al., 2005).

Terminalia brownii (TB) is a member of the family *Combrataceae,* which is found in tropical and sub-tropical areas of East and Central Africa. Various compounds of pharmaceutical importance have been extracted from these plants, including tannins, flavonoids, pentacyclic triterpenoids, and compounds with effects against malaria, fungi, bacteria, oxidants, diabetes, as well as promoting immunoregulation (Machumi et al., 2013). Various interventions are available for treating cancer, including chemotherapy and drugs, but these are associated with numerous side-effects. In the present study, the crude extract of TB is investigated for its anticancer activity, its safety and its potential as a substitute for existing treatments accompanied by side-effects.

Materials and Methods

Plant preparation and extraction

The TB leaves were gathered from the Faifa

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Mountains of Saudi Arabia during the year 2017 where the identification process was conducted at the Herbarium of Biological Sciences, Biology Department, King Khalid University. Fresh plant branches were cut and the leaves were plucked and air-dried at ambient temperature (Ediriweera et al., 2016). After drying, the leaves were ground to a fine powder. A technique involving 80% aqueous methanol was employed to perform the extraction process (Ediriweera et al., 2016). Fractionation was carried out through the process of liquid-liquid extraction, which involved the production of four fractions through the use of four polarity-enhancing solvents, namely, n-hexane, chloroform, ethylacetate and water. The excess solvents were removed after every fraction through rotary evaporation.

Cell culture

Human breast cancer cells (MCF-7), human liver cancer cells (HEPG-2) and human colon cancer cells (HCT 116) were the cancer cell types that were employed for the purposes of this work. These cells were subjected to culturing with 10% foetal bovine serum and antibiotic-containing fresh growth medium (RBMI) in an incubator with 5% carbon dioxide at a temperature of 37°C for 24 hours. Following the period allowed for culturing, an inverted microscope was used to verify that the flask in which the culturing took place did not contain any fungi or bacteria, as well as to assess cell confluence. The trypsinization procedure was subsequently implemented to collect cells by employing 1 ml of trypsin/EDTA solution for a duration of 2 - 5 minutes in the incubator. To ensure comprehensive cell coverage with trypsin, the tissue culture flask was rotated, while an inverted microscope facilitated the observation of cellular dislocation and rounding. The requisite quantity of cells was then transferred to distinctly labelled 96-well plates acquired from Sigma Aldrich, with each well accommodating 1,000 - 2,000 cells. In anticipation of the sulforhodamine B (SRB) assay, the plates underwent a 24-hour incubation period (Keepers et al., 1991). The crude extract, along with its respective fractions, was employed at varying concentrations (0.01, 0.1, 1, 10, 100, and 1,000 µg/µl), with dimethyl sulfoxide (DMSO) serving as the positive control.

Analysis of cell survival via SRB assay

The SRB protein stain assay was conducted alongside another calorimetric assay. The *in vitro* SRB assay exhibited preferable linearity when the number of cells was assessed, while it exhibited heightened sensitivity during staining (Alshehri, 2016). All three types of cancer cells were used to determine the cytotoxic effect of the TB crude extract and associated fractions. Live and dead cells could be distinguished from one another through the SRB assay (Van Tonder et al., 2015).

The three types of cancer cells were subjected to culturing in labeled microtiter plates with 96 wells, with each well containing 1,000-2,000 cells, followed by 24-hour incubation (Gaidhani et al., 2009; Van Tonder et al., 2015). The next step was applying the TB crude extract and its fractions from a stock solution in different concentrations to the wells, followed by 72-hour incubation (Gaidhani et al., 2009; Van Tonder et al., 2015). 10% trichloroacetic acid (TCA) was used for fixation of the incubation medium for 60 minutes at 4°C. The medium was subsequently washed with tap water 3-5 times and air-dried for 60 minutes.0.4% SRB stain was afterwards used for staining the wells, followed by dissolution in 1% acetic acid for 10-30 minutes at 20-25°C. 1% acetic acid was then used to wash the stain and eliminate the unbound dye until the acetic acid became transparent. This was followed by 24-hour air-drying without UV exposure. The next step was solubilization of the dye with 10 Mm tris base with 7.4 pH (150µl/well) for 5 minutes (Liu et al., 2015). An ELISA microplate reader was employed for measurement of the optical density of the wells and the rate of cell survival at a wavelength of 454-540 nm (Van Tonder et al., 2015).

Evaluation of apoptosis

The AO/EB staining technique was utilized to assess apoptosis, focusing on quantifying live cells, early and late apoptotic cells, and necrotic cells, and examining aberrantly organized cells (Amna, 2019). AO penetration and staining of viable cells with a green color by employing a suitable filter is the basis of this method. On the other hand, EB can penetrate solely non-viable cells with compromised membrane, so the dead cells were noticeable by the orange color from EB staining.

The MCF-7, HEPG-2 and HCT 116 cancer cells were subjected to culturing in tissue culture plates with 96 wells, each well containing 1,000-2,000 cells, followed by 24-hour incubation. IC_{50} of crude extract and its fractions was then applied to the cells before another round of incubation for 48 hours. The next step was transferring 2 µl of AO/EB mixture (100 µg/ml of AO and 100 µg/ml of EB in phosphate buffer saline) to the 96-well tissue culture plates. A fluorescence microscope (Nikon Eclipse E400) was then used to observe the dead cells, the necrotic cells and the live cells. AO-stained viable cells exhibited a bright green color and their structure was uncompromised, while AO-stained cells in early apoptosis exhibited a bright green region in the nucleus. By contrast, cells in late apoptosis subjected to both AO and EB staining were small in size and displayed a red-orange color, with dense orange regions indicating chromatin condensation (Elbehairi et al., 2020).

Flow cytometric analysis of cell cycle with propidium iodide DNA staining

Cell cycle analysis was conducted according to the manufacturer's protocol as follows: cells were harvested appropriately and washed in PBS; fixed in cold 70% ethanol, which was added dropwise to the pellet while vortexing to ensure fixation of all cells and minimize clumping; fixed for 30 minutes at 4°C; washed twice in PBS; centrifuged at 850 g, ensuring care was taken to avoid cell loss when discarding the supernatant, particularly after spinning out of ethanol; treated with ribonuclease by adding 50 µl of a 100 µg/ml stock of RNase, ensuring only DNA, not RNA, was stained; and finally, 200 µl of PI (from a 50 µg/ml stock solution) was added.

Statistical analysis

All data were presented as the mean ± SE. Statistical analysis was performed using SPSS statistics software (Version 16.0) one-way ANOVA for all assays. However, statistical study of obesity induction follow-up parameters before starting the treatment was analyzed using SPSS statistics software (Version 16.0) Paired sample T-test. For all analyses, P-value < 0.05 was considered to be statistically significant.

Results

Ion mass spectrum (EPI-MS)

Several compounds were detected in the methanolic extract of TB leaves (see Table 1). To name a few, the deprotonated molecular ion peak [M – H]+ for a component eluted at a retention time (Rt) 27.6 sec and observed at m/z 92.8 (Figure 1A) can be assigned as phenolate anion. As shown in the mass spectrum of the compound eluted at Rt = 29.9 sec, the main peak was observed at m/z 110.8 could be ascribed to deprotonation {[M – H]+} of another phenolic compound,catechol. Besides, two additional peaks were observed at m/z 92.8 and 82.8 attributable for the loss of water [M – H_2O]+. and carbonyl [M – CO]+. groups, respectively. The participation of carboxylic acids in the chemical composition of this extract was revealed from notice of peak at m/z 113.8 for the compound eluted at Rt = 46.1 (Figure 1C) corresponding to [M – H] of the

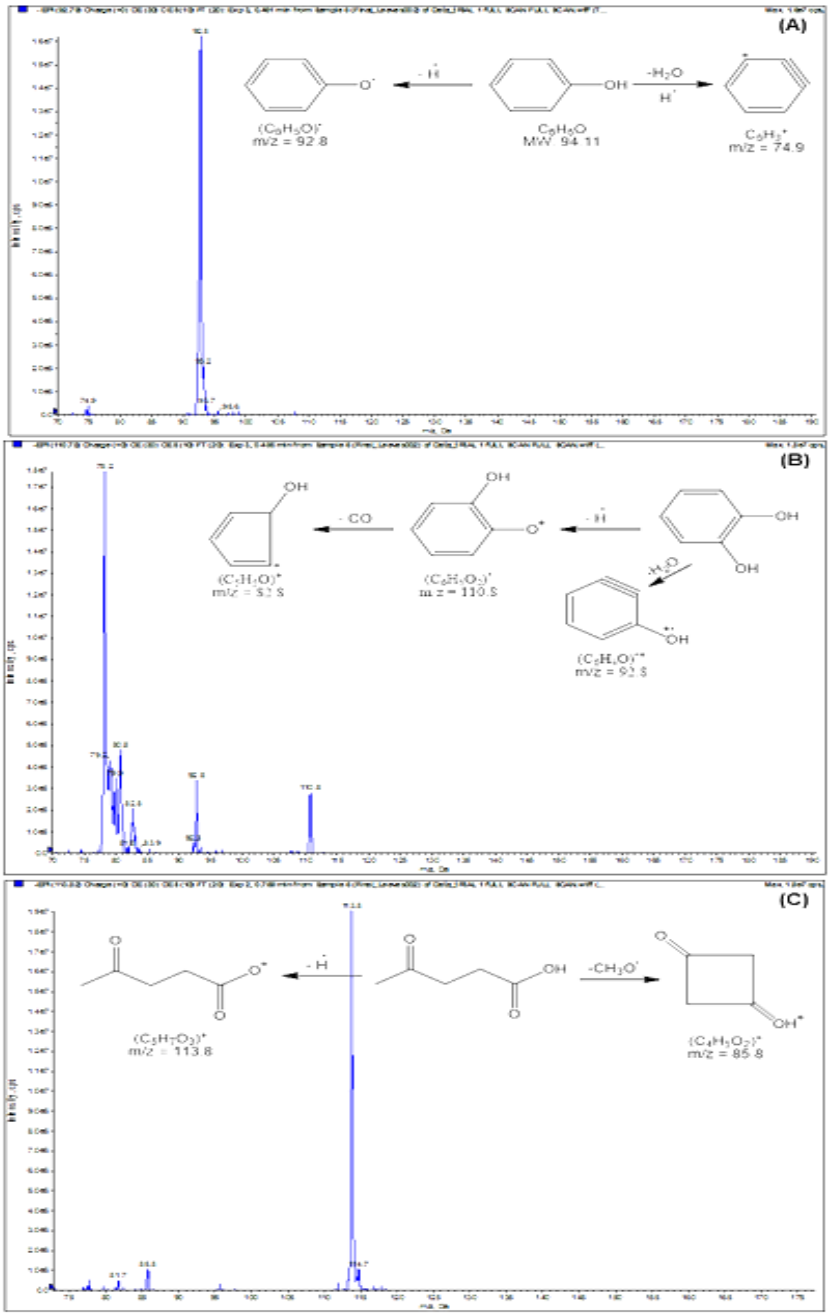

Figure 1. A. Enhanced product ion mass spectrum (EPI-MS) of the eluted compound at Rt = 27.6 sec. The data collected under negative mode (i.e. Molecular weight – 1). B. Enhanced product ion mass spectrum (EPI-MS) of the eluted compound at Rt = 29.9 sec. The data collected under negative mode (i.e. Molecular weight – 1); C. Enhanced product ion mass spectrum (EPI-MS) of the eluted compound at Rt = 46.1 sec. The data collected under negative mode (i.e. Molecular weight – 1).

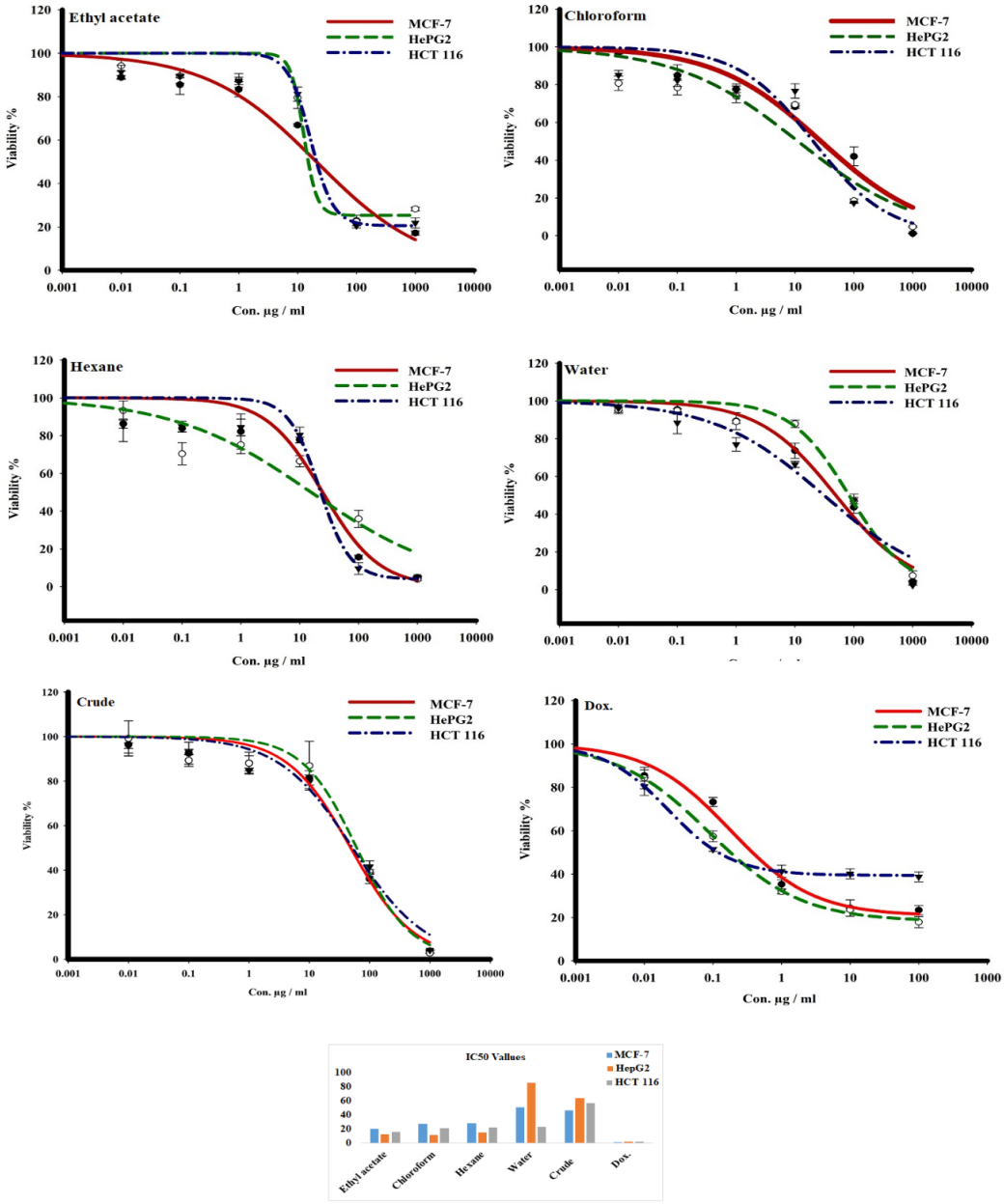

Figure 2. Dose Response Curve of Different Extracts of *T. brownii* and the IC_{50}s (µg/ml). All are against three-tumor cell line. Cells were treated for 72 hr. and cell viability was determined using SRB assay. Display broad efficacy in reducing cell viability.

deprotonated Levulinic acid form. Moreover, its peak at m/z 85.8 is assigned to [M–CH3OH] (Figure 1C).

The activity of different extraction methods on cell viability

The extraction process was conducted to determine the plant extract efficiency and their activity on cell viability and potency of the extraction products against cancer cell development and proliferation. The chloroform and ethyl acetate-based extraction of TB leaves displayed the highest level of toxicity against HepG2 cells, with IC50 11.6 ± 0.7 µg/ml and 12.3 ± 0.86 µg/ml, respectively. The action of the water extract and crude whole extract showed moderate action of activity against the three types of cancer cells was moderate Furthermore; the greatest and lowest sensitivity to the various extracts was exhibited for HepG2 cells and MCF-7 cells, respectively (Figure 2A). Additionally, TB ethyl acetate extract for MCF-7, HepG-2, and HCT 116 cancer cells at 100 µg showed a significant suppressing rate accompanied with reducing the viability, and their action was increased beyond this action as shown in Figure 2B.

The growth analysis was conducted 48 hours after treatment with the different extracts. As the extract concentration was elevated up to 100, growth was suppressed at a growing rate, reducing the viability; however, beyond the 100-µg concentration, there was a rise in viability, which might be indicative of drug interactions. The TB chloroform extract displayed great toxicity towards all three types of cells, with maximum toxicity at 1000 µg/ml, as shown in (Figure 2C). Similarly,

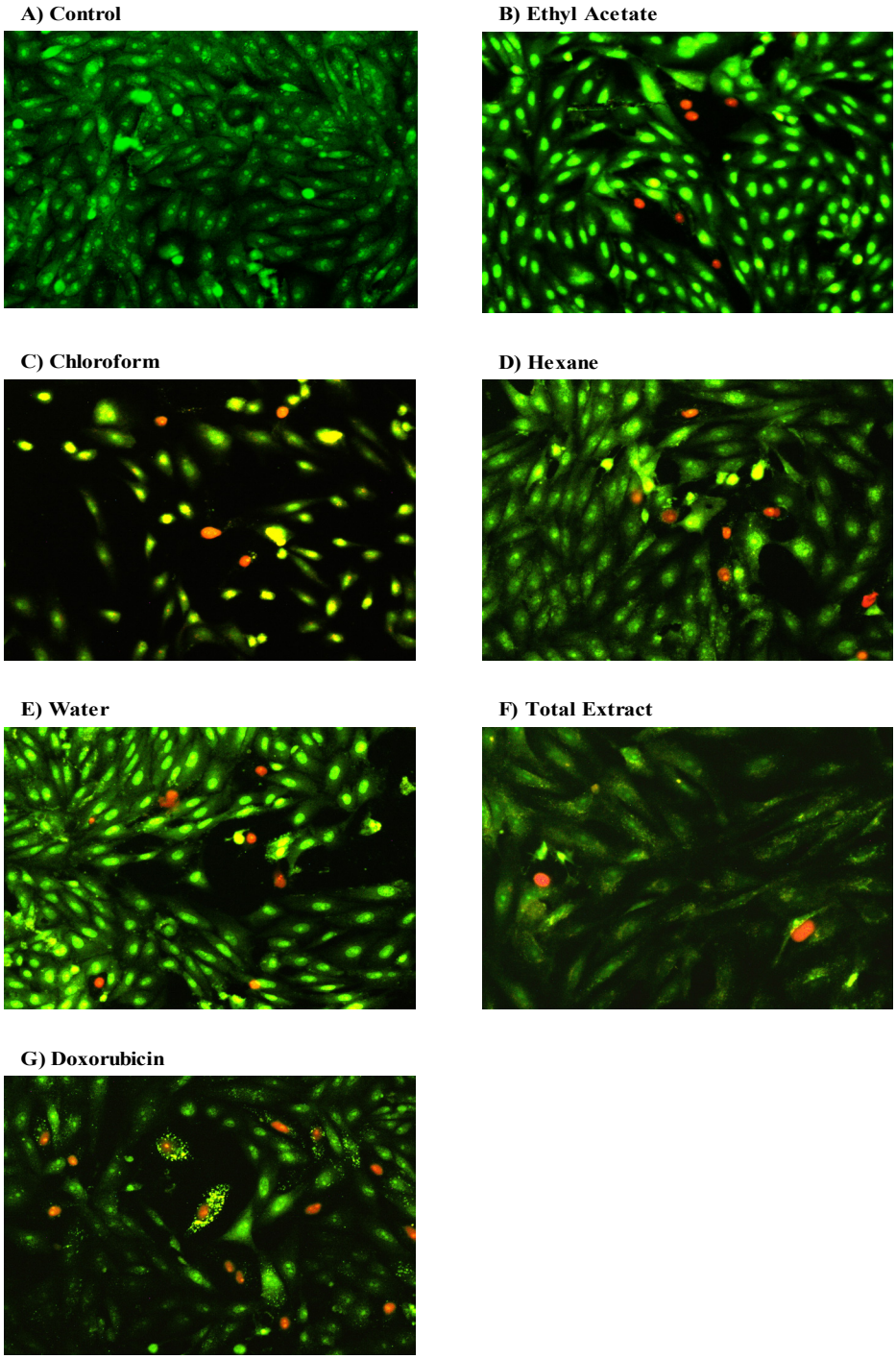

Figure 3. Morphological Changes in MCF-7 Cells after 48 hrs. Treatment with extract of TB and its fractions. The cells were stained with Acridine orange and ethidium bromide. (Magnification at 20X).

the TB hexane extract was most toxic at 1000 µg/ml concentration, while at a concentration of 100 µg/ml it displayed cytotoxicity against the MCF-7 and HCT 116 cells and more moderately against HepG2 cells (Figure 2D). The TB water extract had a toxic effect solely at 1,000 µg/ml, whereas at lower concentrations it lacked toxicity as shown in figure 2E. The crude extract also had a toxic effect only at 1,000 µg/ml, whereas at lower concentrations it did not display any toxic effect (Figure 2F).

On the morphological basis, Figure 3 (A-G) illustrated the modifications in the morphology of MCF-7 cells exposed to different TB extracts and their various fractions at IC_{50} concentration for 48 hours. Figure 3A shows the untreated cells exhibiting rounding and relative deterioration of the nuclear membrane. Figure 3B reveals that the majority of cells have chromatin regions of a greenish color and high density, while some other cells are in various stages of apoptosis with necrotic aspects, as reflected by their rounding and red color. Figure 3C shows that the majority of cells are green or yellowish, while other cells are red and had fragmented nucleases, suggesting they are in final apoptosis. Figure 3D illustrates that cells have rounded and normal nuclei, with a couple of distributed cells displaying a yellow color and high

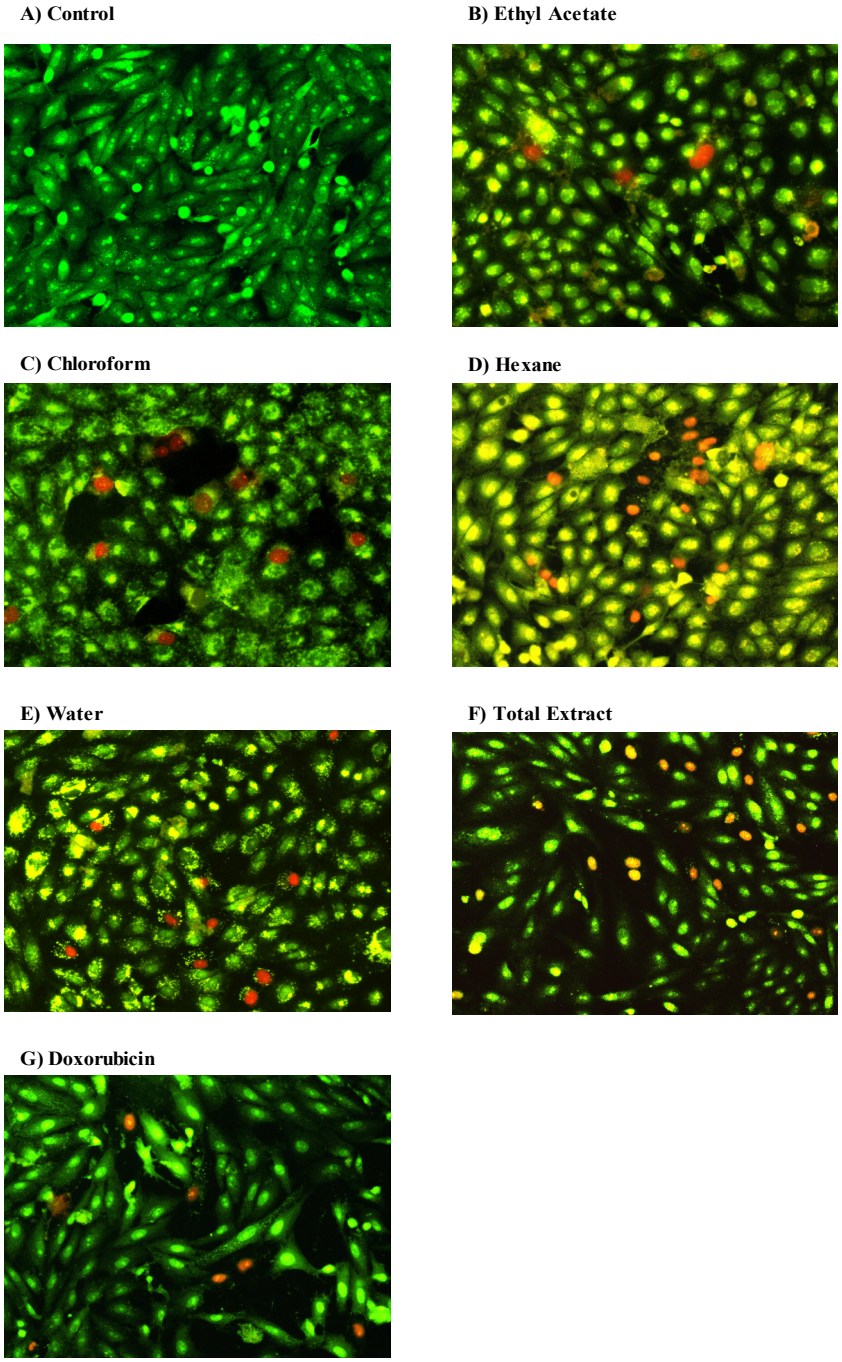

Figure 4. Morphological Changes in HePG2 Cells after 48 hrs. The treatment with extract of TB fractions. The cells were stained with Acridine orange and ethidium bromide. (Magnification at 20X).

Table 1. Chemical Compounds and Their Daughter Ions Identified in Extract by Using the Liquid Chromatography Mass Spectrometry (LC-MS)

No.	Rt (min)	[M – 1]+	m/z	Compound	Relative abundance
1	0.461	92.8	92.8, 74.9	Phenol	$1.6x10^6$
2	0.498	110.8	110.8, 92.8, 82.8, 78.2	Catechol	$3.0x10^6$
3	0.769	114.7	114.7, 113.8, 85.8	Levulinic acid	$1.0x10^6$
4	0.788	127.8	127.8, 94.8, 76.7	Pyrogallol	$1.7x10^7$
5	0.761	145.9	145.9, 143.6, 125.9, 107.8	Cinnamic acid	$2.0x10^6$
6	0.739	178.8	178.8, 160.8, 132.7, 86.8	Caffeic acid	$3.5x10^6$
7	0.72	182.8	182.8, 167.8, 139.8, 123.6, 90.6	Methyl gallate	$1.7x10^7$
8	0.547	190.7	190.7, 172.9, 126.8, 110.8, 84.6	Quinic acid	$1.8x10^7$
9	0.517	300.4	300.4, 257.3, 229.2	Quercetin	$1.5x10^7$

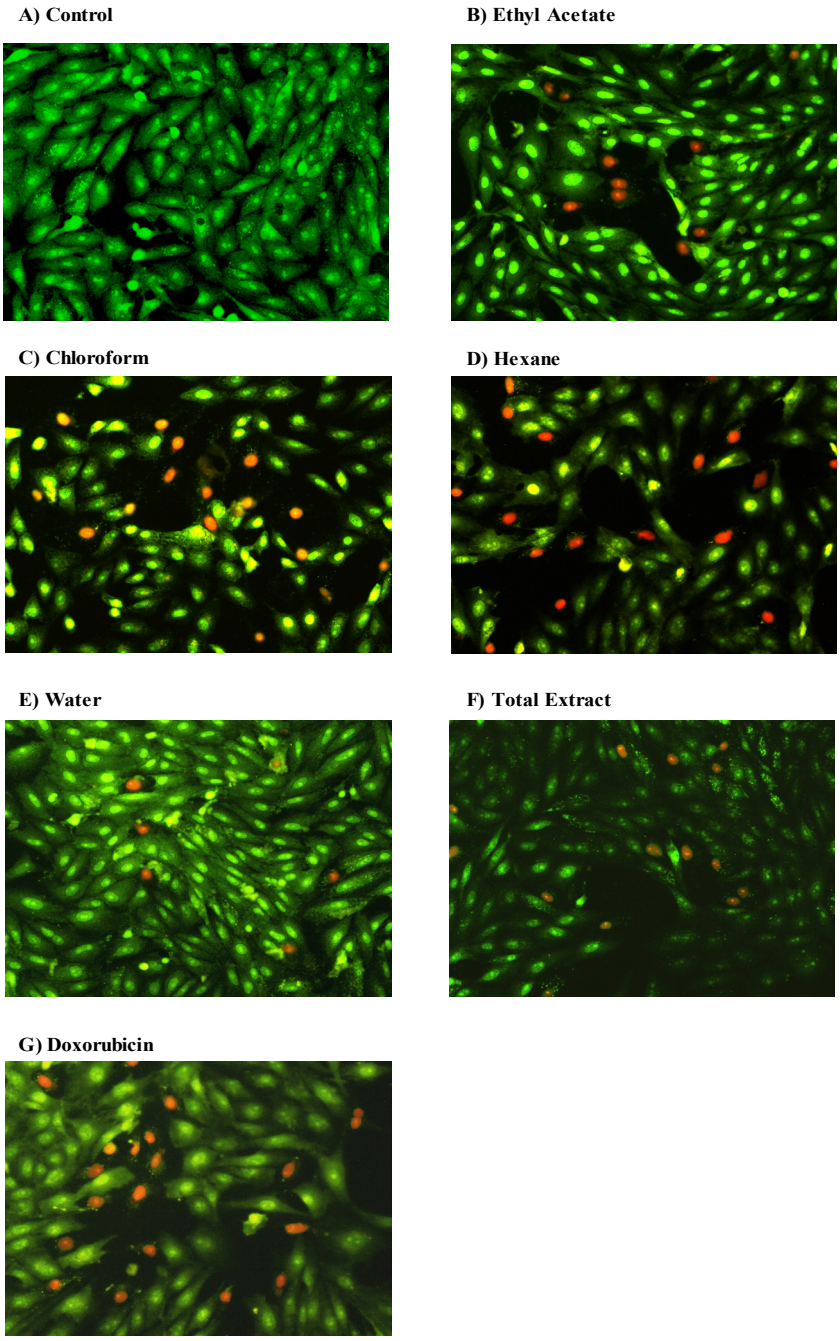

Figure 5. Morphological Changes in HCT 116 Cells after 48 hrs. Treatment with extract of TB and its fractions. The cells were stained with Acridine orange and ethidium bromide. (Magnification at 20X).

chromatin density and small fragmented cells of red color. Figure 3E shows cells in early apoptosis and a couple of distributed cells in late apoptosis. Figure 3F reveals massively deteriorated cells of green color. Figure 3G shows the positive control, with early apoptotic green cells and late apoptotic red cells. Figure 4 (A-G) illustrates the modifications in morphology suffered by the HepG2 cells exposed to TB extract and its various fractions at IC50 concentration for 48 hours. Figure 4A shows that every cell is viable and normal, displaying a green color and rounded nucleus and nuclear membrane. Figure 4B shows that most cells have structural deterioration and fragmentation. Figure 4C reveals distributed fragmented cells of green and red color. Figure 4D illustrates that the majority of cells are viable and of green or yellowish color, with a couple of distributed fragmented cells of red color. Figure 4E shows that most cells have structural fragmentation and are of green and red color. Figure 4F shows distributed and viable cells of green and yellowish color. Figure 4G illustrates the positive control with viable green- and red-colored cells in early and late apoptosis, respectively. Figure 5 (A-G) illustrates the modifications in morphology suffered by the HCT 116 cells exposed to TB extract and its various fractions at IC_{50} concentration for 48 hours. Figure 5A shows fully viable green cells with rounded and normal nuclei. Figure 5B shows viable cells of green color and high chromatin density. Figure 5C shows viable cells of green color and a couple of fragmented and late apoptotic cells of red color. Figure 5D illustrates a couple of distributed cells of greenish color

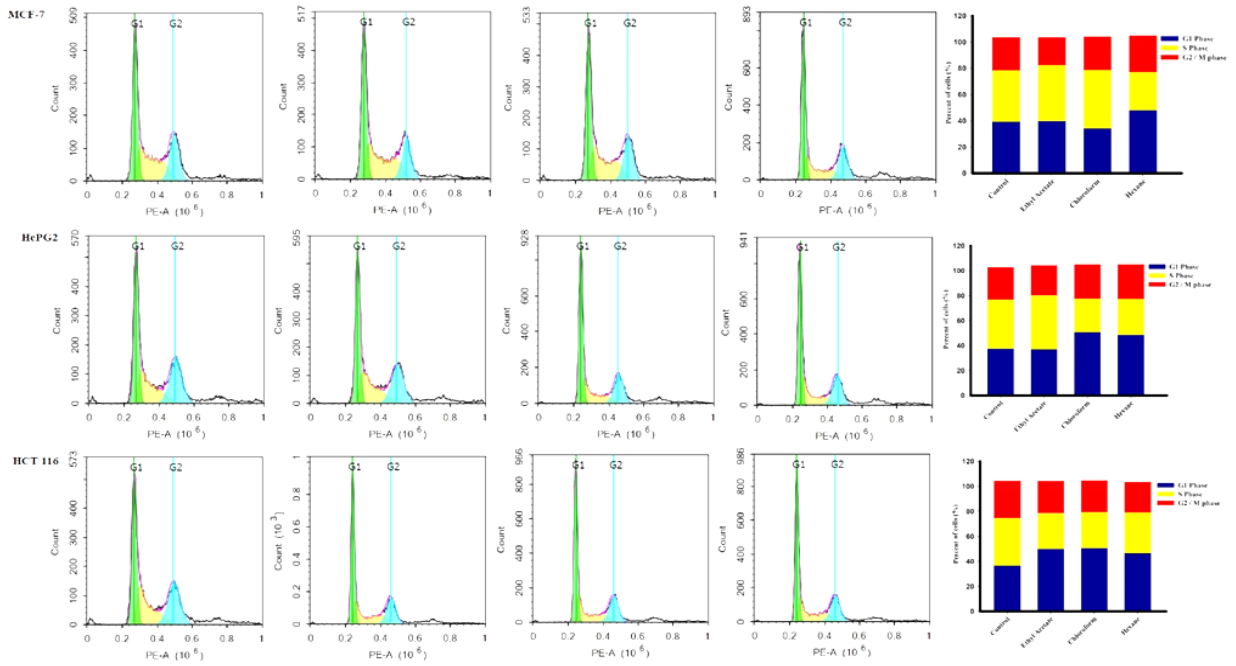

Figure 6. Effect of Different Extracts on Cell Cycle Phase and DNA Damage on MCF-7, HePG2, and HCT 116.

Table 2. Cell Cycle Distributions of Cancer Cell Lines after Treatment with Plant Extract for 48hr.

Cell line	Extracts	Cell Phases		
		G1	S	G2 / M
MCF-7	Control	38.23 ± 1.2	38.22 ± 0.9	23.55 ± 1.5
	Ethyl Acetate	39.09 ± 0.88	41.17 ± 1.4	19.74 ± 1.3
	Chloroform	32.66 ± 1.7	43.52 ± 0.98	23.82 ± 0.78
	Hexane	46.58 ± 1.7	27.41 ± 1.5	26.01 ± 1.6
HePG2	Control	36.79 ± 0.98	38.23 ± 0.9	24.98 ± 0.89
	Ethyl Acetate	36.33 ± 0.89	41.23 ± 1.9	22.44 ± 1.4
	Chloroform	50.04 ± 0.9	24.92 ± 1.8	25.04 ± 2.1
	Hexane	47.43 ± 1.4	26.98 ± 1.6	25.59 ± 1.9
HCT 116	Control	35.03 ± 1.7	36.64 ± 1.3	28.33 ± 1.4
	Ethyl Acetate	48.98 ± 1.2	27.08 ± 1.4	23.94 ± 1.7
	Chloroform	49.25 ± 1.5	27.24 ± 1.6	23.51 ± 1.4

data are presented as the mean ± SE

alongside rounded necrotic cells of red color. Figure 5E shows green viable cells with high chromatin density and a couple of interstitial necrotic cells of red color. Figure 5F illustrates partially viable cells of green color alongside a couple of necrotic cells of red color. Figure 5G shows the positive control, with green viable cells and red cells in early and late apoptosis, respectively.

Flow cytometry and cell cycle

The cell cycle results showed that the S phase was the most prominent in the phases through the chloroform extracts for the TB among treated MCF-7 cells, followed by ethyl acetate extracts for HepG2, and finally, Hexane extracts for HCT11 as shown in Table 2, Figure 6.

Discussion

Cancer continues to be associated with a high mortality rate throughout the world, despite the various available treatments and preventive measures, including surgical intervention, which alleviates the tumor burden, chemotherapy, which eradicates residual and micro-metastatic tumors systematically and in a surgical setting, and radiation, which eradicates residual tumor cells within a surgical setting. However, radiation and chemotherapy lack tumor specificity, so they can affect healthy cells with fast proliferation just like tumors, such as the cells of the bone marrow, hair follicles and gastrointestinal tract lining. Another limitation of these interventions is that they are severely invasive and occasionally their effect is only a palliative one. Therefore, new interventions of greater efficiency are urgently needed (Elbehairi et al., 2020).

Although, radiotherapy and chemotherapy lack tumor specificity, they affect healthy cells with fast proliferation just like tumors, such as the cells of the bone marrow, hair follicles, and gastrointestinal tract lining. Additionally, another limitation of these interventions is that they are severely invasive and occasionally have a palliative action. Therefore, new interventions of greater efficiency are urgently needed (Haupt et al., 2002). On this background, increasing attention is being paid to immunological strategies for cancer treatment as more and more insight is gained into the immune system and its regulatory mechanisms (Haupt et al., 2002). The immune system displays exceptional specificity, so cancer cells could be targeted whilst healthy cells are left unaffected.

Tumor development is highly dependent on excessive expression of oncogenes or mutated tumor inhibiting genes, which could therefore be targeted in treatment strategies. Cancer is typically associated with mutations in the tumor inhibiting genes p53 (Nigro et al., 1989; Chiba et al., 1990; Berzofsky et al., 2004) and HER-2/neu, which represents an extra oncogene exhibiting excessive expression rather than mutation in several carcinomas (Yanuck et al., 1993). Exposure to chemotherapy or radiation causes the death of cancer cells primarily through the mechanism of apoptosis. By evolving mechanisms that confer resistance to apoptosis, cancer cells become less susceptible to clinical interventions (Slamon et al., 1989).

The phytochemistry of Terminalia laxiflora and *Terminalia brownie* has been the focus of several studies. It has been discovered that the roots of both species and the stem bark of TB contain medium-polar-to-polar phenolic compounds, including gallic acid, ellagic acid, and its derivatives, gallotannins and the ellagitannins punicalagin, terchebulin, methyl-(S)-flavogallonate, and its isomer (Hanahan and Weinberg, 2011; Schrader et al., 2016; Yamauchi et al., 2016; Salih et al., 2017). Furthermore, the stem wood of T. laxiflora has been found to contain terchebulin, flavogallonic acid dilactone, and ellagic acid and its derivatives (Muddathir and Mitsunaga, 2013). The stem bark of both species and the root bark of T. laxiflora have also been reported to contain sterols (β-sitosterol and stigmasterol), triterpenoids (betulinic acid, arjungenin, terminolic acid and monogynol A), a diterpenoid (laxiflorin), and a chromone (terminalianone) (Rashed et al., 2016).

The premise postulated by the present study is that the ratios of the different polyphenolic compounds, particularly those of ellagitannins, may be the reason that explains the activity of extracts from various parts of T. laxiflora and TB differ in their effectiveness as anti-cancer drug. The compounds contained in the roots might have the ideal ratio since extracts from those parts were observed to suppress growth most efficiently. Indeed, by comparison to other plant parts, the roots have been reported to have a greater proportion of compounds with antimicrobial effect (Balmer and Mauch-Mani, 2013). This might be explained by the occurrence of synergistic interaction between certain compounds such as ellagitannins and other phenolic compounds in the roots of T. laxiflora and TB (Balmer and Mauch-Mani, 2013). A correlation between the antioxidant action and the antimicrobial effect of phenolic compounds has been proposed, meaning that the pathogenic activity of microorganisms is diminished by phenols through reduction of the generation of stress-associated defense compounds in those microorganisms (de Freitas Araújo et al., 2012). Thus, inflammatory events caused by microbial infections could be managed with plant extracts containing antioxidant compounds (Courtney et al., 2015).

The findings of this study are consistent with those of Courtney et al., (2015) which discovered that ellagitannin-containing Terminalia ferdinandiana extracts displayed satisfactory antimicrobial and antioxidant actions, despite the lack of correlation between the two properties. A particularly potent antioxidant effect has been demonstrated by corilagin, with 53 dg/ml IC_{50} (Anokwuru et al., 2015). Different phytochemicals are present in the methanolic bark extracts of TB and some of them may underpin its effect against inflammation. Flavonoids also have an anti-inflammatory effect because they suppress the action of synthetase, a prostaglandin enzyme (Tapas et al., 2008; Chatterjee et al., 2015). Furthermore, inflammatory events are alleviated by steroids through suppression of phospholipase A2, which causes prostanoids and leukotrienes to form by hydrolyzing arachidonic acid from the membrane phospholipids (Mencarelli et al., 2009). Additional compounds with anti-inflammatory effects are triterpenoids, which block not only prostaglandin secretion but also macrophage and neutrophil function (Salminen et al., 2008). Hence, the anti-inflammatory effect observed in the present study might have been generated by the individual or synergistic action of the flavonoids, steroids, and terpenoids in the TB extract.

The current study primarily sought to assess the suppressing effect of diluted TB leaf extracts in different concentrations on three types of cancer cells. Employing a range of extraction techniques, the cytotoxicity of the extracts and their disruption of cancer cell viability were demonstrated. The findings were validated via SRB cell survival assay and dual staining techniques for examination of cell morphology. Similarly, Wani et al., (2016) stated that the homeopathic preparations of Terminalia chebula were shown to hinder the proliferation of the breast cancer cell line MCF-7, whilst leaving normal cells unaffected. Likewise, at 10 µg/ml LC_{50} T. chebula affected leukemia cell line K562, which is equivalent to prostate cancer cell line.

On one side, the anti-cancer potential activity of T. chebula was attributed to the highly effective bioactive secondary metabolites gallic acid, ellagic acid, chebulic acid, and tannin (Bupesh et al., 2016). On the other side, it was shown that T. bellerica extracts were more efficient at suppressing the viability of MCF-7 cells in high dosage (Diab et al., 2015). Furthermore, n-hexane extract from other types of plants was reported to make HepG2 cell line significantly less viable, while the common fruit extract malvidin has been proven to suppress the migration of HCT 116 cells. Additionally, the chloroform, ethyl acetate, and Hexane extract revealed significant changes in the replication of the cancer cell for MCF-7, HepG2, and HCT116, respectively through the S phase cell cycle

(Al Groshi et al., 2018). That may trigger the activity of the TB treatment with their different extractions that enhance DNA damage that occurs in the S phase due to replication forks, nucleotide excision/repair process, or as intermediates of DSB resolution, the intra S phase checkpoint is activated to prevent further replication (Errico et al., 2012). Additionally, Peasland et al., (2011) stated drugs targeting the S and G2/M checkpoint are considered potent promising pathways that may be developed against breast and ovarian carcinoma cell lines.

The findings of this study suggest that compounds in the TB plant used in traditional Saudi medicine may have the potential to suppress the growth of cancer cells. The extracts obtained with ethyl acetate and chloroform displayed particularly high cytotoxicity against the different types of cancer cells that were examined. More specifically, the chloroform extract exhibited a cytotoxic effect against all three types of cancer cells and especially the HepG2 cells at 100 μM concentration. By contrast, the crude extract demonstrated cytotoxicity solely against the HepG2 cancer cells. Given that the IC50 value of the TB plant is less than 20, further research is required to determine how extracts of this plant affect healthy cells. At the same time, it is also worth conducting in vivo studies to find out how its suppressing effects are underpinned by molecular and cellular mechanisms. Moreover, the chloroform, ethyl acetate, and Hexan extracts of the TB plant are considered good inhibitors that can sensitize cancer cells to DNA damaging drugs forcing the cells with DNA damage to bypass the S and G2/M arrest and enter mitosis, leading to cell death by mitotic inhibition.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical approval Not provided

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

References

Abdel–Magid AF (2014). Inhibitors of Mutant IDH for the Treatment of Cancer. *ACS Med Chem Lett*, **11**, 1184–5.

Al Groshi A, Evans AR, Ismail FM, Nahar L, Sarker SD (2018). Cytotoxicity of Libyan Juniperus phoenicea against Human Cancer Cell Lines A549, EJ138, Hepg2 and MCF7. *Pharm Sci*, **24**, 3–7.

Alshehri MA (2016). Anticancer activity of methanolic extarct of Momordica charantia against human colon, liver and breast cancer cell lines– In vitro. *Int J Agric Biol*, **6**, 106–11.

Amna U, Halimatussakdiah, Wahyuningsih P, Saidi N, Nasution R (2019). Evaluation of cytotoxic activity from Temurui (Murraya koenigii [Linn.] Spreng) leaf extracts against HeLa cell line using MTT assay. *J Adv Pharm Technol Res*, **10**, 51–5.

Anokwuru CP, Sinisi A, Samie A, Taglialatela–Scafati O (2015). Antibacterial and antioxidant constituents of Acalypha wilkesiana. *Nat Prod Res*, **29**, 1180–3.

Balmer D, Mauch–Mani B (2013). More beneath the surface? Root versus shoot antifungal plant defenses. *Front Plant Sci*, **4**, 256.

Berzofsky JA, Terabe M, Oh S, et al (2004). Progress on new vaccine strategies for the immunotherapy and prevention of cancer. *J Clin Investig*, **113**, 1515–25.

Bradner JE, Hnisz D, Young RA (2017).Transcriptional addiction in cancer. *Cell*, **168**, 629–43.

Bupesh G, Manikandan E, Thanigaiarul K, et al (2016). Enhanced antibacterial, anticancer activity from Terminalia chebula medicinal plant rapid extract by phytosynthesis of silver nanoparticles core–shell structures. *J Nanomed Nanotechnol*, **7**, 1–6.

Chatterjee A, Sen B, Das S, Chatterjee TK (2015). Anti–inflammatory and analgesic activity of methanolic xtract of medicinal plant Rhodiola rosea I. Rhizomes. *Int J Pharm Res Rev*, **4**, 1–8.

Chiba I, Takahashi T, Nau MM, et al (1990). Mutations in the p53 gene are frequent in primary, resected non–small cell lung cancer. Lung Cancer Study Group. *Oncogene*, **5**, 1603–10.

Courtney R, Sirdaarta J, Matthews B, Cock I (2015). Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. *Phcog*, **7**, 18–31.

Cragg GM, Grothaus PG, Newman DJ (2009). Impact of natural products on developing new anti–cancer agents. *Chem Rev*, **109**, 3012–43.

De Freitas Araújo MG, Hilário F, Vilegas W, et al (2012). Correlation among antioxidant, antimicrobial, hemolytic, and antiproliferative properties of Leiothrix spiralis leaves extract. *Int J Mol Sci*, **13**, 9260–77.

Diab KA, Guru SK, Bhushan S, Saxena AK (2015). *In Vitro* Anticancer Activities of Anogeissus latifolia, Terminalia bellerica, Acacia catechu and Moringa oleiferna Indian Plants. *Asian Pac J Cancer Prev*, **16**, 6423–8.

Drahl C, Cravatt BF, Sorensen EJ (2005). Protein-reactive natural products. *Angewandte Chemie*, **44**, 5788–5809.

Ediriweera MK, Tennekoon KH, Samarakoon SR, et al (2016). A study of the potential anticancer activity of Mangifera zeylanica bark: Evaluation of cytotoxic and apoptotic effects of the hexane extract and bioassay–guided fractionation to identify phytochemical constituents. *Oncol Lett*, **11**, 1335–44.

Elbehairi SEI, Ismail LA, Alfaifi MY, Elshaarawy RFM, Hafez HS (2020). Chitosan nano-vehicles as biocompatible delivering tools for a new Ag (I) curcuminoid-Gboxin analog complex in cancer and inflammation therapy. *Int J Biol Macromol*, **165**, 2750–64.

Errico A, Costanzo V (2012). Mechanisms of replication fork protection: a safeguard for genome stability. *Crit Rev Biochem Mol Biol*, **47**, 222–35.

Gaidhani S, Lavekar G, Juvekar A, et al (2009). In–vitro anticancer activity of standard extracts used in ayurveda. *Pharmacogn Mag*, **5**, 425–9.

Hanahan D, Weinberg RA. (2011). Hallmarks of cancer: the next

generation. *Cell*, **144**, 646–74.
Haupt K, Roggendorf M, Mann K (2002). The potential of DNA vaccination against tumor–associated antigens for antitumor therapy. *Exp Biol Med*, **227**, 227–37.
Keepers YP, Pizao PE, Peters GJ, et al (1991). Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for *in vitro* chemosensitivity testing. *Eur J Cancer*, **27**, 897–900.
Kuppusamy P, Ichwan SJA, Al–Zikri PNH, et al (2016). *In Vitro* Anticancer Activity of Au, Ag Nanoparticles Synthesized Using Commelina nudiflora L. Aqueous Extract Against HCT–116 Colon Cancer Cells. *Biol Trace Elem Res*, **173**, 297–305.
Liu K, Liu P–C, Liu R, Wu X (2015). Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. *Med Sci Monit Basic Res*, **21**, 15–20.
Machumi F, Midiwo JO, Jacob MR, et al (2013) Phytochemical, antimicrobial and antiplasmodial investigations of Terminalia brownii. *Nat Prod Commun*, **8**, 761–4.
Mencarelli A, Renga B, Palladino G, Distrutti E, Fiorucci S (2009). The plant sterol guggulsterone attenuates inflammation and immune dysfunction in murine models of inflammatory bowel disease. *Biochem Pharmacol*, **78**, 1214–23.
Muddathir AM, Mitsunaga T (2013). Evaluation of anti–acne activity of selected Sudanese medicinal plants. *J Wood Sci*, **59**, 73–9.
Newman DJ, Cragg GM, Snader KM (2003). Natural products as sources of new drugs over the period 1981− 2002. *J Nat Prod*, **66**, 1022–37.
Newman DJ, Cragg GM, Snader KM (2000). The influence of natural products upon drug discovery. *Nat Prod Rep*, **17**, 215–34.
Nigro JM, Baker SJ, Preisinger AC, et al (1989). Mutations in the p53 gene occur in diverse human tumour types. *Nature*, **342**, 705–8.
Peasland A, Wang LZ, Rowling E, et al (2011). Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. *Br J Cancer*, **105**, 372-81.
Rashed K, Mohd Esa N, Ismail S (2016). Anti–Cancer Activity of Three Terminalia Species and Preliminary Phytochemical Screening. *Jordan J Pharm Sci*, **9**, 1–9.
Salih E, Kanninen M, Sipi M, et al (2017). Tannins, flavonoids and stilbenes in extracts of African savanna woodland trees Terminalia brownii, Terminalia laxiflora and Anogeissus leiocarpus showing promising antibacterial potential. *S Afr J Bot*, **108**, 370–86.
Salminen A, Lehtonen M, Suuronen T, Kaarniranta K, Huuskonen J (2008). Terpenoids: natural inhibitors of NF–κB signaling with anti–inflammatory and anticancer potential. *Cell Mol Life Sci*, **65**, 2979–99.
Schrader KK, Cantrell CL, Midiwo JO, Muhammad I (2016). Compounds from Terminalli brownii Extracts with Toxicity against the Fish Pathogenic Bacterium Flavobacterium columnare. *Nat Prod Commun*, **11**, 1679–82.
Slamon DJ, Godolphin W, Jones LA, et al (1989). Studies of the HER–2/neu proto–oncogene in human breast and ovarian cancer. *Science*, **244**, 707–12.
Tapas AR, Sakarkar D, Kakde R (2008). Flavonoids as nutraceuticals: a review. Trop J Pharm Res, 7, 1089–1099.
Van Tonder A, Joubert AM, Cromarty AD (2015). Limitations of the 3–(4, 5–dimethylthiazol–2–yl)–2, 5–diphenyl–2H–tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. *BMC Res Notes*, **8**, 47.
Wani K, Shah N, Prabhune A, et al (2016). Evaluating the anticancer activity and nanoparticulate nature of homeopathic preparations of Terminalia chebula. *Homeopathy*, **105**, 318–26.
Yamauchi K, Mitsunaga T, Muddathir AM (2016). Screening for melanogenesis–controlled agents using Sudanese medicinal plants and identification of active compounds in the methanol extract of Terminalia brownii bark. *J wood Sci*, **62**, 285–93.
Yanuck M, Carbone DP, Pendleton CD, et al (1993). A mutant p53 tumor suppressor protein is a target for peptide–induced CD8+ cytotoxic T–cells. *Cancer Res*, **53**, 3257–61.