

Anti-Cancer Activity of Three *Terminalia* Species and Preliminary Phytochemical Screening

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ABSTRACT

This study evaluated anticancer potential of three *Terminalia* species, *Terminalia muelleri*, *Terminalia bellerica*, and *Terminalia laxiflora* and also their phytochemical content were determined. Anticancer potential of the plant extracts was measured according to MTT assay. The results showed that *T. muelleri* methanolic extract was active against breast cancer cell line with IC₅₀ 40 µg/mL. *T. bellerica* methanolic extract exerted cytotoxic effects only against colon cancer and liver cancer cell lines with IC₅₀ of 50 and 15µg/mL, respectively. While *T. laxiflora* methanolic extract did not inhibit the proliferation of all cancer cell lines tested. Phytochemical investigation of the three plant species proved the presence of carbohydrates, flavonoids, tannins, and triterpenes. The methanolic extracts of *T. muelleri* and *T. bellerica* had a significant anticancer activity and so further phytochemical study to isolate and identify the bioactive molecules responsible for the observed anticancer activity is necessary.

Keywords: *Terminalia* species, bark, anticancer, phyto-constituents.

1. INTRODUCTION

Cancer is a disease that causes the death all over the world and represents a major public health burden⁽¹⁾. Treatments of cancers vary depending on the type and stages of cancer. The modalities of treatment include surgery, chemotherapy, hormonal therapy, immune therapy and supportive therapy. Cancer treatments kill cancer cells; however, these treatments can also damage the healthy cells, a matter that causes various side effects⁽²⁾. Well known side effects such as nausea, vomiting, hair loss, fatigue, constipation are very common. Many researches tried to find effective treatments for cancer and also the search for compounds

having significant anticancer activity from plants which may help to find effective anticancer therapeutics with less side effects. Plants represent the important sources of bio- active phytoconstituents. It has been estimated that about 50% of the prescription products in the world are originating from natural products or their derivatives⁽³⁾.

Terminalia is a tree from Combretaceae family. *Terminalia* genus contained cyclic triterpene and its derivatives, flavonoid, tannin and other aromatic compounds. In traditional medicine, *T. bellerica* was used for high cholesterol and digestive disorders, including both diarrhea and constipation, and indigestion. It was used to protect the liver and to treat respiratory conditions, including respiratory tract infections, and at⁽⁴⁾. The ethanolic extract of *T. muelleri* leaves was used as antioxidants⁽⁵⁾ and to inhibit the growth of *E. coli*, *S. aureus* bacteria and *C. albicans* fungi⁽⁶⁾. *T. laxiflora* bark

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is applied to wounds, yaws and haemorrhoids because of its haemostatic and healing effects. The bark has been used in the treatment of malaria. Root decoctions are taken to treat diarrhoea, dysentery and jaundice, and applied to itchy eyes. Leaf extracts are also used against diarrhoea, whereas extracts of various plant parts are administered to treat tuberculosis and cough⁽⁷⁾. In the present study, we performed anticancer assay of three methanolic plant extracts of *terminalia* species against four human cancer cell lines and also phytochemical composition of the extracts was detected.

Materials and Methods

Plants Materials

Terminalia muelleri L. and *Terminalia bellerica* L. barks were collected from Zoo garden, while *Terminalia laxiflora* W. bark was collected from Al-Zohiriya garden, Giza, Egypt in May 2012. The plants were identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereza Labib at the Ministry of Agriculture and director of Orman Botanical Garden, Giza, Egypt.

Chemical and Reagents

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA and penicillin-streptomycin were purchased from PAA (Austria). Trypan blue, MTT ([4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide, dimethyl sulfoxide (DMSO) and commercial phytic acid were obtained from Sigma (USA). 96-well plate (Eppendorf, USA), 96-well plate sealing film (Eppendorf, USA), were purchased from Sigma (USA). Hematoxylin and Eosin were bought from Surgipath (USA). All other chemicals and reagents used were of the highest purity grade available.

Cell Lines

The colon cancer (HT-29), liver cancer (HepG2), breast cancer (MCF-7, MDA-MB 231) and BALB/c 3T3 (mouse fibroblast) cell lines were obtained from the American Type Culture Collection (USA).

Preparation of the Extracts

Air dried bark of *Terminalia muelleri* (320 g), *Terminalia bellerica* (450 g) and *Terminalia laxiflora* (280 g) were extracted with methanol:water (80:20, v/v) at room temperature several times (five times) until exhaustion by maceration method for three days. The extract was concentrated under reduced pressure to give 18.5 g, 26 g and 12.8 g of crude extracts, respectively. Each extract was subjected to phytochemical analysis to detect the phyto-constituents according to methods described by Yadav and Agarwala⁽⁸⁾.

Growth Inhibition Assay

The colorectal cancer (HT-29) and breast cancer (MCF-7, MDA-MB 231) cell lines was cultured in DMEM while the liver cancer (HepG2), and the normal fibroblast (3T3) cell lines were cultured in RPMI medium supplemented with 10% fetal bovine serum and 1% of penicillin-streptomycin. All cells were incubated in a humidified atmosphere, at 37°C under 5% CO₂. The tetrazolium salt assay was used to evaluate the proliferation of the cells following the modified methods of Shamsuddin *et al.*⁽⁹⁾ and Vucenic *et al.*⁽¹⁰⁾. After the cells reached 70-80% confluence, they were detached by trypsinization. The cells marked with trypan blue were counted and seeded at a density of 1×10^5 cells/well in 96-well microtiter plates overnight in an incubator. The medium was removed the next day, and 0 to 250 µg/mL of each samples in DMSO was added to each well. After 72 h, 20 µL of MTT solution was added and left for 4 h in the incubator. The formazan product was solubilized in 100 µL of dimethyl sulfoxide, and the absorbance was measured at 570 nm using an Elisa plate reader.

Results and Discussion

Anticancer assay

The 3- (4, 5- dimethylthiazol- 2- yl) -2, 5- diphenyltetrazolium bromide (MTT) assay is a simple and reliable assay that measures the viability of cells, and it can be used to screen antiproliferative agents⁽¹¹⁾. Different doses of the sample solution ranging from 0-

250 µg/mL, were applied to colon cancer (HT-29), breast cancer (MCF-7, MDA-MB 231), liver cancer (HepG2) cell lines. The cytotoxicity of the methanolic plant extracts is expressed as IC₅₀, and the results are shown in table 1. The results showed that *T. muelleri* had cytotoxicity activity against all the studied cancer cell except for the breast cancer. However, the breast cancer (MCF-7) cell lines was the most sensitive to *T. muelleri* with IC₅₀ 40 µg/mL. On the other hand, *T. bellerica* exerted cytotoxic effects only against the HT-29 and

HepG-2 with IC₅₀ values of 50 and 15 µg/mL, respectively after 72 h of exposure (Table 1). As indicated in the National Cancer Institute Guidelines (USA), extracts with IC₅₀<100 µg/mL are considered antiproliferative agents⁽¹²⁾. MDA-MB-231 however, was not sensitive for all sample studied (*T. muelleri*, *T. bellerica*, and *T. laxiflora*). *T. laxiflora* did not inhibit the proliferation of all cancer cell lines tested even for HepG-2 showing an IC₅₀ value more than 100 µg/mL.

Table 1. Results of anticancer activity of the three methanolic plant extracts

Plant Extract	IC ₅₀ (ug/mL)-72 hrs incubation				
	<i>HT-29</i>	<i>HepG-2</i>	<i>MCF-7</i>	<i>MDA</i>	<i>3T3 (normal cell line)</i>
<i>T. muelleri</i>	50	40	35	n.d	130
<i>T. bellerica</i>	50	15	200	n.d	125
<i>T. laxiflora</i>	n.d	140	n.d	n.d	n.d

Identification of lowest cytotoxic dose of the plant extract for non-tumorigenic cells was performed using a normal cell lines (mouse fibroblast, BalB/c 3T3) as comparison. BalB/c 3T3 cell line is recommended by US National Institute of Environmental Health Sciences, Interagency Coordinating Committee in the Validation of Alternative Methods for determining basal cytotoxicity⁽¹³⁾. No toxic effect on 3T3 cell was observed for all the samples IC₅₀ was more than 100 µg/mL (Table 1). The presented results showed that all the methanolic plant extracts tested are selective anticancer agents because they caused cell death in the HT-29, HepG2, MCF 7 cell lines but not on normal cell (BalB/c 3T3 cell line).

Phytochemicals Analysis

Phytochemical screening of the three *Terminalia* species extracts proved the presence of carbohydrates, tannins, flavonoids and triterpenes (Table 2). The

presence of these active compounds in the studied extracts would attribute the anticancer potential of these extracts.

Polyphenolic compounds (flavonoids and tannins) displayed a wide range of biological activities, including anti-inflammatory, antioxidant, antimutagenic, anticarcinogenic, and modulation of enzymatic activities^(14,15,16). They can be considered as chemopreventive or therapeutic agents against cancer⁽¹⁷⁾. The flavonol quercetin inhibits the growth of pancreatic cancer tumor cell line by inducing apoptosis⁽¹⁸⁾.

C. hieronymi Griseb Shoots proved a strong activity against lung carcinoma cells, mouse lymphoma, and human colon carcinoma and this observed activity is due to the presence of flavonoids⁽¹⁹⁾. The CH₂Cl₂ extract of *C. macrobothrys* Baill. leaves and *C. zambesicus* Müll. Arg. proved cytotoxic effect against human lung, leukemia and cervix carcinoma cells⁽²⁰⁾, the observed activity is due to the presence of flavonoids⁽²¹⁾.

Table 2. Phytochemical analysis of the three methanolic plant extracts

Constituents	<i>T. muelleri</i>	<i>T. bellerica</i>	<i>T. laxiflora</i>
Triterpenes and /or Sterols	+	+	+
Carbohydrates and/or glycosides	+	+	+
Flavonoids	+	+	+
Coumarins	-	-	-
Alkaloids and/or nitrogenous compounds	-	-	-
Tannins	+	+	+
Saponins	-	-	-
(+), presence of constituents, (-) absence of constituents			

Betulinic acid, Triterpenic compound, may be considered a potential anti-cancer agent. In many experiments betulinic acid was selective against neuroblastoma cells and lacked side-effects^(22,23). The cytotoxic activity of betulinic acid was tested on more human melanoma cell lines, and a significant inhibition of cell growth was observed and betulinic acid was shown to be responsible for a marked reduction of tumor mass and did not induce any side effects such as weight loss⁽²⁴⁾. Pedunculagin which is a tannin derivative molecule proved a strong cytotoxic effect against human hepatocellular carcinoma, human colon cancer cells, mild cytotoxic effect on human breast cancer cells. Tannins, Pedunculagin, castalagin, and grandinin had a selective cytotoxic effect against PRMI-7951 melanoma cells but a weak cytotoxic effect on lung carcinoma, ileocecal adenocarcinoma, epidermoid carcinoma of nasopharynx

(KB) and medulloblastoma (TE-671) tumour cells⁽²⁵⁾.

CONCLUSION

Based on the results obtained in the presented study, all the methanolic plant extracts did not inhibit the proliferation of breast cancer (MDA-MB-231) cell lines. *T. muelleri* extract was most effective for MCF-7, followed by HepG-2 and HT-29, *T. bellerica* extract is most effective for HepG2 followed by HT-29 but with no effect on MCF-7, on the other hand, *T. laxiflora* extract did not show any inhibition of the proliferation of all cancer cell lines. Also all the extracts did not inhibit the growth of normal cell lines (3T3) a matter that proves their selective towards cancer cells. In conclusion: *T. muelleri* and *T. laxiflora* extracts can be considered as a source of potential anticancer agents.

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الفاعلية المضادة للسرطان من ثلاثة أنواع من الترميناليا والتحليل الفيتوكيميائي المبدئي

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ملخص

هذه الدراسة قيمت الفاعلية المضادة للسرطان لثلاثة أنواع من الترميناليا وهي ترميناليا موليري وترميناليا بليريك وترميناليا لاسيفلورا وكذلك محتواها الكيميائي النباتي. وقد تم قياس إمكانات المضادة للسرطان من المستخلصات النباتية وفقاً لفحص MTT. وأظهرت النتائج أن المستخلص الميثانولي من ترميناليا موليري فعال ضد خط خلايا سرطان الثدي مع تركيز تثبيط =40 ميكروجرام/مل. أما المستخلص الميثانولي من ترميناليا بليريك فقد كان فاعل فقط على خلايا سرطان القولون وسرطان الكبد مع تركيز تثبيط بين 50 و15 ميكروجرام/مل على التوالي. في حين أن المستخلص الميثانولي من ترميناليا لاسيفلورا لم يمنع انتشار نمو كافة خطوط الخلايا السرطانية التي تم اختبارها. في حين أثبت التحقيق الكيميائي النباتي من الأنواع النباتية الثلاثة تواجد الكريوهيدرات، الفلافونويد والعفصيات، والتربينات الثلاثية. وحيث إن المستخلصات الميثانولية من ترميناليا موليري وترميناليا بليريك ذات نشاط كبير مضاد للسرطان ولذا فالدراسة الكيميائية النباتية الأبعد الهادفة إلى عزل المركبات والتعرف إلى الجزئيات الحيوية النشطة المسؤولة عن النشاط المضاد للسرطان يكون ضرورياً.

الكلمات الدالة: أنواع الترميناليا، الساق، المضاد للسرطان، المكونات الفيتوكيميائية.

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