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Evaluation of chemical composition, cytostatic and anti-proliferative effects of ethanolic roots extract of *Securidaca longepedunculata* (Fresen)

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Abstract
The crave for evidence on the safety and efficacy of traditional medicines has led to this study on evaluation of the chemical composition, anti-proliferative and cytostatic effects of *S. longepedunculata* roots extract. The phytochemical investigation on the ethanol extract revealed the presence of tannins, saponins, saponin glycosides, alkaloids, flavonoids, cardiac glycosides, terpenes and steroids. The HPLC chromatogram showed seventeen peaks and the compounds with retention time of 4.891, 7.247, 8.745, 18.522 and 25.889 minutes corresponded to caffeic acid, rutin, ferulic acid, apigenin and quercetin respectively. Growth inhibition test of the roots extract on *Sorghum bicolor* seeds showed a significant ($P<0.0001$) result throughout the experiment against the control seeds (5% DMSO and Methotrexate). At 96h, percentage growth inhibitions for the seeds treated with 1mg/ml, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml were 42.97%, 72.69%, 83.94%, 86.14%, 91.26% and 91.57% respectively. The results suggest the probable use of the plant in traditional medicine for the treatment of tumour related ailments and other diseases. However, further works using appropriate human cell lines are needed to find a novel drug.

Keywords: Anti-proliferative, Cytostatic effects, Phytochemistry, *Securidaca longepedunculata* root.

Introduction
Man since creation has depended on plants to enhance his health and other things. Herbs are generally valued for their virtues as food as well as medicine [1]. The use of traditional medicine, complementary and alternative medicine (TM/CAM) has been on the increase. There is therefore a great need for evidence on the safety, efficacy and quality of TM/CAM products and practices. In general, however, increased use of TM/CAM has not been accompanied by an increase in the quantity, quality and accessibility of clinical evidence to support TM/CAM claims [2].

Cancer has been defined as a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells [1]. Breast cancer was by far the most common in women (25% of all new cases diagnosed in women). Cancer also accounted for about more than 8.2 million deaths world-wide in 2012 [3].

Securidaca longepedunculata (Fresen) belongs to the family Polygalaceae. It is a shrub or small tree to 8–9 m height with conspicuous violet (or white) flowers, common in savannah woodland from Senegal to North and South Nigeria, and generally widespread in tropical Africa [4]. It is commonly known as Rhodesian violet, violet tree and also as "*Ezeogwu, Ipeta* and *Uwar Magunguna*" (king of drugs) in Igbo, Yoruba and Hausa languages in Nigeria [5]. The plant parts are used to treat purgative, diuretic, diaphoretic, emetics, conjunctivitis, malaria, venereal diseases, infertility problems, urethral discharges, stomach problems, dysentery, fever, rheumatism, leprosy, fibrositis, toothache, headache, sleeping sickness, cough, chest complaints, snakebite, and wound dressing, and as aphrodisiac, vermifuge and expectorant and as an arrow poison antidote [4, 6, 7]. The concoction of the root is used in treatment of breast cancer and other diseases. This work evaluated the chemical composition, anti-proliferative and cytostatic effects of ethanolic roots extract of *Securidaca longepedunculata* on the rapidly growing cells using *Sorghum bicolor* (guinea corn).The obtained result was compared with that of the standard drug (Methotrexate) used in breast cancer treatment.

Materials and Methods

Reagents used were of JHD grade and were procured from Zayo-Sigma Abuja.

Materials

Petri dishes, filter papers, muslin cloth, sample bottles, DMSO, ethanol, distilled water, cotton wool, Methotrexate injection.

Plant Material Collection and Certification

The fresh roots of *Securidaca longepedunculata* were harvested in the month of May, 2015 from Uhunowerre in Igbo–eze South Local Government Area, Enugu state, Nigeria. It was identified and authenticated by Mr Felix Nwafor at the Herbarium, International Centre for Ethno-medicine and Drug Development (InterCEDD), No. 110, Aku Road, Nsukka, Enugu state with voucher number, InterCEDD/1600.

The Experimental Plant

Sorghum bicolor (Guinea corn) seed was used as the experimental plant. It was purchased from Gwa-Gwa market in Abuja Municipal Area Council (AMAC), F.C.T, Abuja. The viability of the seeds were determined by placing them inside a beaker containing water. The seeds that floated were discarded while the totally submerged ones were cleansed with methylated spirit and dried for usage.

Plant Preparation and Extraction

The fresh roots of *Securidaca longepedunculata* were sun dried for four(4) days and then air dried at room temperature of about 28-30 ºC for six(6) weeks. The dried plant sample was pulverized into powder with hammer ball mill. Then 220g of the powdered material was weighed and macerated using ethanol in a stoppered vessel for 48 h; 1:5w/v of the sample and ethanol at the ambient temperature of 28-30ºC. The resultant mixture was vacuum filtered using whatman No.1 filter paper. The filtrate was dried below 40ºC over a water bath to yield *S. longepedunculata* root ethanol extract (SLREE).

Phytochemical Screening

Phytochemical analyses were conducted on ethanol extract of *S. longepedunculata* root (SLREE) for the presence of tannins, alkaloids, Anthraquinone derivatives, saponins, Saponin glycosides, cardiac glycosides, terpenes, steroids and flavonoids using standard methods [8-12].

High Performance Liquid Chromatography Analysis

The bioactive constituents of the ethanolic roots extract of *S. longepedunculata* was analysed by high performance liquid chromatography. The HPLC consisted of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, 5µm VP-ODS C_{18} and dimensions (4.6 x 150 mm). The chromatographic conditions included mobile phase: 0.2% v/v formic acid and acetonitrile (20:80); mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 100 mg/ml solution of extract in methanol; detection UV 254 nm. The HPLC operating conditions were programmed to give solvent B: 20%. Column oven temperature was 40 ºC. The total run time was 30 minutes. Flavonoids and phenolic acid standards such as apigenin, rutin, quercetin, caffeic acid, ferulic acid were employed for the identification of the phytoconstituents of ethanol extract by comparing the retention time under similar experimental conditions [13].

Anti-Proliferative and Cytostatic Test on Guinea Corn

The modification of methods obtained from the Literature [14, 15] were used in this study. The Petri dishes were layered with two fitter papers (whatman No 1) each with cotton wool in between them. Then 200mg of *S. longepedunculata* roots ethanol extract was dissolved in10ml of 5% dimethyl sulfoxide (DMSO) in distilled water to obtained 20mg/ml stock solution of the extract. Then various concentrations (1mg/ml, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml) of *S. longepedunculata* roots ethanol extract (SLREE) were prepared. The Methotrexate (Standard) was made to a concentration of 166.66µg/ml. Twenty (20) seeds of *Sorghum bicolor* (guinea corn) each were placed in sample bottles and treated with different concentrations of the extract. The control seeds were treated with 10ml of 5% dimethyl sulfoxide distilled water. The treated seeds were incubated in a dark room for 24 h. The seeds were then poured into the various 9 cm wide Petri dishes and observed for growth after 24 h. The length of the radicles of the seeds were measured in mm after 24, 48, 72 and 96 h. The percentage inhibition was calculated as [(mean radicle length control - mean radicle length treated)/mean radicle length control] x 100. Percentage growth was calculated as 100 - % inhibition and growth rate as mean radicle length/time.

Statistical analysis

The data obtained were expressed as mean ± standard error mean and analyzed using Graph pad prism (version 6.07). Two way analysis of variance test was used to test for significance. $P<0.0001$ was considered to be significant.

Results

Extractive Value and Phytochemical analyses

The extraction yielded 11.6% w/w of the plant sample (Table 1)

Table 1: Extractive value of the *S. longepedunculata* roots.

Sample	Sample (wt)g	Wt of empty dish (g) W_2	Wt of dish + extract W_3	Wt of dried extract (W_3 -W_2) W4	% yield
S. longepedunculata root (Ethanol extract)	220	37.5	63.1	25.6	11.6

The phytochemical analyses of *S. longepedunculata* ethanol root extract were found to show the presence of tannins, alkaloids, anthraquinone derivatives, saponins, saponin glycosides, cardiac glycosides, Terpenes, steroids and absence of anthraquinone (Table 2)

Table 2: Phytochemical results of *S. longepedunculata* ethanol root extract

Test	Inference
Tannins	+
Alkaloids	+
Saponins	+
Saponin glycosides	+
Cardiac glycosides	+
Flavonoids	+
Anthraquinone derivatives	-
Terpenes and Steroids	+

Key: + presence of secondary metabolites - absence of secondary metabolites

HPLC Chromatogram.

The HPLC chromatogram of the ethanol extract of *S. longepedunculata* roots as in Figure 1, showed that seventeen peaks were detected as the constituents with retention times in minutes of 4.891, 6.183, 6.722, 7.247, 8.745, 10.048, 12.869, 15.949, 17.639, 18.522, 22.406, 24.376, 25.889, 30.397, 32.064, 34.568 and 36.030. Compounds with retention time in minute of 4.891, 7.247, 8.745, 18.522 and 25.889 minute corresponded to caffeic acid, rutin, ferulic acid, apigenin and quercetin respectively.

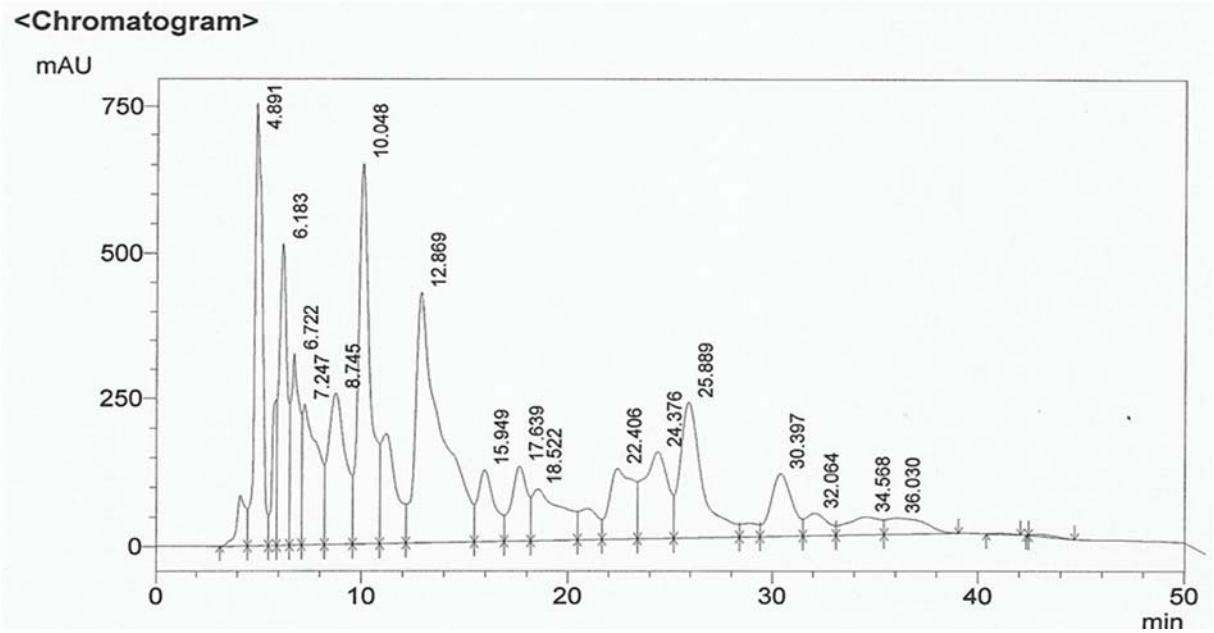

Fig 1: HPLC chromatogram of ethanol roots extract of *S. longepedunculata*

Anti-Proliferative and Cytostatic Effect on Guinea Corn

The result showed a significant (P<0.0001) anti-proliferation and cytostaticity as there was an appreciable reduction on the length of radicles of the seeds treated with the various concentration of the extract when compared with the control and the standard drug (Methotrexate) throughout the experiment. A rapid and progressive growths were observed in the control seeds radicle lengths. The length of radicles of the seeds increased with the incubation period 24 – 96 h. The SLREE was observed to inhibit the radicle lengths of *Sorghum bicolor* seeds treated with the various concentrations. The inhibition was concentration-dependent, the higher the concentration, the higher the inhibitory effect on the radicles.

The mean radical length of the control seeds at 24 h was 8.30±0.82mm while the mean radical lengths of the seeds treated with the standard (166.66µg/ml Methotrexate), 1mg/ml, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml of *S. longepedunculata* roots ethanol extract (SLREE) were 1.60±0.25mm, 1.65±0.40mm, 0.85±0.32mm, 0.80±0.31mm, 0.25±0.17mm and 0.00±0.00 respectively, indicating inhibition of 80.72%, 80.12%, 89.76%, 90.36%, 96.99% and 100% (Table 3a and Figure 1). At 48 h, the mean radical length of the control seeds was16.05±1.44mm whereas it was reduced to 2.70±0.46mm, 4.60±0.78mm, 2.30±0.59mm, 2.10±0.51mm, 1.30±0.40mm and1.00±0.31mm in the seeds treated with (166.66µg/ml Methotrexate), 1mg/ml, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml of concentration of the standard and the extract, indicating inhibition of 83.18%, 71.34%, 85.67%, 86.92%, 91.90% and 93.77% respectively.

Table 3a: Anti – proliferative and cytostatic effects of *S. longepedunculata* root ethanol extract on *S. bicolor* seeds.

Treatment	Mean radicle length in (mm)				Percentage(%) inhibition			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control (5%DMSO)	8.30±0.82	16.05±1.44	22.30±2.09	24.90±2.25	0.00	0.00	0.00	0.00
MTX (166.66µg/ml)	1.60±0.25	2.70±0.46	7.30±1.30	14.20±2.15	80.72	83.18	67.26	42.97
S.L (1mg/ml)	1.65±0.40	4.60±0.78	6.70±1.39	6.80±1.39	80.12	71.34	69.96	72.69
S.L (2 mg/ml)	0.85±0.32	2.30±0.59	3.60±1.90	4.00±0.14	89.76	85.67	83.86	83.94
S.L (4mg/ml)	0.80±0.31	2.10±0.51	3.30±0.74	3.45±0.79	90.36	86.92	85.20	86.14
S.L (6 mg/ml)	0.25±0.17	1.30±0.40	1.95±0.60	2.20±0.60	96.99	91.90	91.26	91.16
S.L (8 mg/ml)	0.00±0.00	1.00±0.31	1.80±0.52	2.10±0.65	100.0	93.77	91.93	91.57

Table 3b: Percentage growth and growth rate of guinea corn (*Sorghum bicolor)* seeds radicle

Treatment	Percentage (%) Growth				Growth Rate in (mm/h)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control (5%DMSO)	100	100	100	100	0.35	0.33	0.31	0.26
MTX(166.66µg/ml)	19.28	16.82	32.74	57.03	0.07	0.06	0.10	0.15
S.L (1mg/ml)	19.88	28.66	30.04	27.31	0.07	0.10	0.09	0.07
S.L (2 mg/ml)	10.24	14.33	16.14	16.06	0.04	0.05	0.05	0.04
S.L (4mg/ml)	9.64	13.08	14.80	13.86	0.03	0.04	0.05	0.04
S.L (6 mg/ml)	3.01	8.10	8.74	8.84	0.01	0.03	0.03	0.02
S.L (8 mg/ml)	0.00	6.23	8.07	8.43	0.00	0.02	0.03	0.02

At 72 h, the mean radicle lengths were7.30±1.30mm, 6.70±1.39mm, 3.60±1.90mm, 3.60±1.90mm, 3.30±0.74mm and 1.95±0.60mm respectively compared to the control seeds with 22.30±2.09mm. After 96 h, the mean radicle length of the control was 24.90±2.25mm, while the mean radical length of the seeds treated with 166.66µg/ml (Methotrexate), 1mg/ml, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml concentration were 14.20±2.15mm, 6.80±1.39mm, 4.00±0.14mm, 3.45±0.79mm, 2.20±0.60mm and 2.10±0.65mm, indicating 42.97%, 72.69%, 83.94%, 86.14%, 91.26% and 91.57% respectively reductions in the radicle lengths of the seeds respectively.

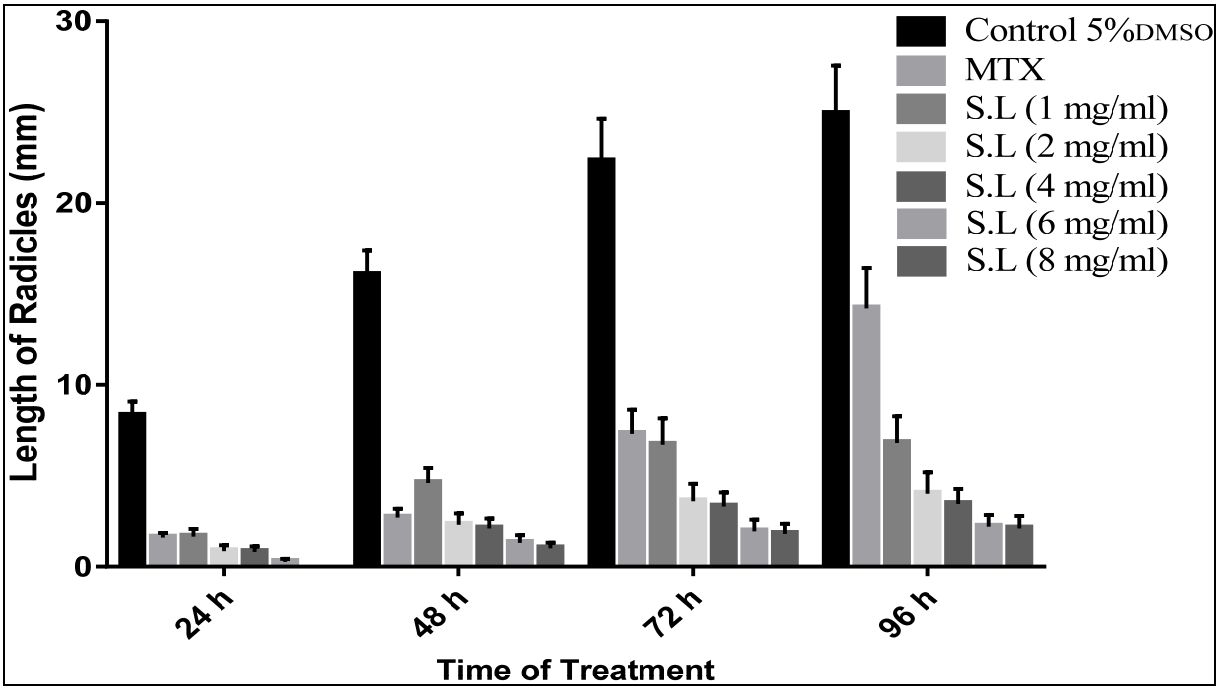

Fig 2: Antiproliferative and cytostatic effects of *S. longepedunculata* roots ethanol extract on the growth of guinea corn radicles

Discussion

The phytochemical screening of *S. longepedunculata* root extract (Table 2) showed the presence of tannins, alkaloids, saponins, saponin glycosides, cardiac glycosides, flavonoids, terpenes, steroids and no trace of anthraquinone.

Production of tumour cells is characterised by an uncontrolled multiplication of cells. This can be linked to the rapid growth and multiplication exhibited by the meristematic cells of a germinating seed or a growing radicle. Bench top assay methods have been variously used to study the ability of plant extracts to impact cytotoxic and cytostatic on certain organisms like tadpoles, mosquito larvae and plant seeds. By this, the degree plant extracts can inhibit growth of tumour-producing cells, induce dormancy in seeds (cytostatic) or produce allelopathic effects are determined [14]. Numerous ant-cancer agents have been developed and are currently in use

today but these have not been able to effectively curtail the ailment although various levels of successes have been achieved. Moreover, the fact that most of these therapies have the tendency of causing dangerous adverse effects has led to the search for a more effective anti-cancer agent with less side effects. It has been stated that measures aimed at preventing the disease (i.e. those that cause cytostatic effect to the cancerous cells) are better than treatment procedures as damage cannot be reverted. Therefore, a search for chemo-preventive agents is also a priority [15].

The curative value of medicinal herbs largely depends on their secondary metabolites, especially alkaloids, terpenoids, flavonoids and phenolic compounds. Results given in (Table 2) show that the occurrence of different secondary metabolites suggests a wide range of biological application of the plant. Several alkaloids like vinblastine, vincristine, camptothecin, taxol etc. are successfully employed in cancer treatment [16]. Alkaloids have many medicinal uses as drugs for malaria, colds, cough, hypertension, diabetes, cancer, and other diseases [17]. Kunle and Egharevba suggested to consider the presence of flavonoids in a plant as indication of its antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties. Tannins were reported to exhibit antiviral, antibacterial and anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic [18]. However, glycoside derivatives showed very promising activity in vitro and in vivo and two of them, ethylidene derivative etoposide and ethylidene derivative teniposide were developed as anticancer drugs. Terpenoids and steroids are capable of preventing cancer, because of their anti-carcinogenic effects. Some researchers have also reported that some saponins have anticancer and immunomodulatory properties [16]. Saponin is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc. [18]. Digitoxin is a cardiac glycoside. Digitoxin and related cardenolides display potent anticancer activity against a range of human cancer cell lines in vitro but the clinical use of digitoxin to treat cancer has been restricted by its narrow therapeutic index. It is also used to treat heart failure, and also used to treat a certain type of irregular heartbeat [19]. The therapeutic value of medicinal plants depend largely on their secondary metabolites that they contain [14, 16-20].

However in this study, the results of the growth inhibitory effects of SLREE on *S. biolor* (guinea corn) seeds showed a significant ($P<0.0001$) inhibition when compared with the controls. The length of the radicles of the seeds treated with different concentrations of the extract showed continually remarkable reduction when compare with the control throughout the experiments. This may be attributed to the interference of the constituents of the extracts with the certain biochemical process in the seeds. At 96 h, the mean radicle length of the control was 24.90±2.25mm, while the mean radical length of the seeds treated with 166.66µg/ml (Methotrexate), 1mg/ml, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml concentration were 14.20±2.15mm, 6.80±1.39mm, 4.00±0.14mm, 3.45±0.79mm, 2.20±0.60mm and 2.10±0.65mm, indicating 42.97%, 72.69%, 83.94%, 86.14%, 91.26% and 91.57% reductions in the radicle lengths of the seeds respectively (Table 3a and Figure 2). Therefore, the reduction in the length of radicles is said to be concentration – dependent (i.e. the higher the concentration of the extract, the higher the inhibitory effect). Some authors have suggested that inhibition of cell proliferation by the plant medicine is a measure of the biological activities of the chemical constituents. These bioactive constituents have been reported as possible therapeutic agents in the treatment and prevention of various diseases such as cancer, cardiovascular, asthma, inflammations and others [16, 21]. The presence of the major chemical constituents could be responsible for these activities in the aqueous extract of the plant. This plants is known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases.

The aqueous extract of *S. longepedunculata* root exhibited growth inhibitory effects on proliferating cells (*S. bicolor* seeds) hence can be attributed to tumour cells. Therefore, this work has supported the ethno-medicinal uses of this plant (*S. longepedunculata*) root in the treatment of breast cancer, wound, venereal disease and other diseases. However, further works on this same plant using appropriate human cell lines including other biological activities are recommended.

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