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Screening of *Entada pursaetha* (DC) seed kernels and *Wagatea spicata* (Dalzell) roots on cancer cell lines for cytotoxic activity.

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Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Though chemotherapy is a successful therapy for various types of cancers, there are many severe side effects of the same. To overcome this problem, the discovery of anticancer agents from natural sources is the need of the day. The aim of present study was to screen the methanol, ethyl acetate and acetone extracts of *Entada pursaetha* seed kernel and *Wagatea spicata* root for anticancer activity on the Colo 205, MCF-7, B16F10 cell lines by MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide)] assay. The extracts have also been tested on normal mouse fibroblast cells L929. Final results were analysed using GraphPad prism 10.1.2. *Entada pursaetha* acetone extract (EPA) showed the most promising 85.47%, 86.03% and 89.64% inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/ml respectively with 40.46µg/mL IC_{50} against colo 205 cell line while, *Wagatea spicata* acetone extract (WSA) showed 32.76µg/mL IC_{50} and cell inhibition was 82.05%, 83.28% and 85.37% with concentrations of 10µg/mL, 30µg/mL and 100µg/mL, respectively. Against MCF-7 cell line methanol extract of *E. pursaetha* (EPM) and *W. spicata* (WSM) showed maximum cell inhibition 68.17% (10µg/mL), 68.68% (30µg/mL), 70.96% (100µg/mL) and 70.73% (10µg/mL), 71.04% (30µg/mL), 73.81% (100µg/mL) respectively with IC_{50} >100. Against the B16F10 cell line, all extracts of both plants showed moderate percentage cell inhibition. All extracts showed minimal toxicity against normal mouse fibroblast cells L929.

Keywords: African dream herb, Vakeri mul, MTT assay

Worldwide cancer represents one of the prime health challenges and continues to be the second leading cause of death after cardiovascular disease among non-communicable ailment[1]. As per the estimates, the overall number of new cancer cases will rise by 25% every decade, reaching 24 million per year in 2050; the total number of deaths will increase from 6 million in 2000 to 10 million in 2020 to over 16 million in 2050; and there will be 17 million new cases[2]. Although chemotherapy is still widely used as therapy for cancer, its cytotoxic effect leads to damage of rapidly dividing healthy cells in tissues like the gastrointestinal tract, bone marrow, and hair follicles resulting in side effects like nausea, vomiting, alopecia, and myelosuppression. Furthermore, the risk of developing secondary cancers has been linked to certain antitumor drugs. These drawbacks highlight the urgent need for safer and more effective alternatives. Several antioxidants and phytochemicals have been proposed as adjuvant therapies in cancer treatment because they possess pro-apoptotic and antiproliferative properties making them promising candidates for anticancer research. Hence, continued investigation of plant-based compounds is essential, as their bioactive constituents hold the potential to create effective therapies with fewer toxic effects than standard chemotherapy.

The present study aimed to evaluate the preliminary cytotoxic potential of seed kernels of *Entada pursaetha* and root of *Wagatea spicata* on the colo 205 colon cancer cell line, MCF-7 human breast cancer cell line and B16F10 human skin cancer cell line.

Entada pursaetha DC (*E. scanden* auct. non Benth, *E. monostachya* DC) commonly called as African dream nut, Elephant creeper has occurred in the tropical area of South Africa, Sri Lanka, India, China, Malaysia and Australia. The bark and seeds of this plant are used for ulcers, stems for skin diseases. Seeds are used for stomach aches, anti-rheumatic,

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anti-inflammatory, dietary supplements and for hallucinatory effects[3]. The preliminary phytochemical screening showed the presence of flavonoids, glycosides, triterpenoids, saponins, and tannins in various plant parts[4]. Ethanolic extract of stem of *Entada pursaetha* suppresses monosodium iodoacetate induced osteoarthritis pain and disease progression by histopathological changes in joints in a rat model[5]. Ethanolic extract of seed of *Entada pursaetha* shows anti-inflammatory activity against carrageenan-induced paw oedema[6].

The wound-healing activity of isolated phytoconstituents of seed kernels of *Entada pursaetha* was proved[6]. In molecular docking studies of ethanolic extract of *Entada pursaetha*, the plant compounds were analysed as potential inhibitors of breast cancer[7]. Methanolic leaf extract of *Entada pursaetha* was screened for anticancer activity using brine shrimp lethality bioassay[8].

Wagatea spicata Dalzell, family Leguminosae (Caesalpiniaceae), commonly known as Vagati or Vakeri, is a monotypic genus of a robust woody, climbing shrub, with long trailing prickly branches growing abundantly in Konkan jungles[9]. Near about 25 species are available of this genus. In phytochemical screening, it shows the presence of alkaloids, proteins, tannins, starch, and saponins.

Traditionally, the bark of *Wagatea spicata* has been used for treating skin disorders, and its roots are employed in the management of respiratory conditions such as pulmonary tuberculosis and pneumonia. It is reported that the whole plant is used in respiratory and cardiovascular diseases[10]. The main active chemical constituents reported are vakerin (bergenin), epifriedelin, friedelin, lupeol, and taraxerol, β-sitosterol, lignoceryl alcohol and melissic acid, β-sitosterol P-D-glucoside, quercetin[11]. Cytotoxicity and antioxidant activity of methanolic extract of leaf of the plant were reported[11,12]. Leaves, flowers and bark of *Wagatea spicata* proved as antibacterial against methicillin-resistant *Staphylococcus aureus* bacteria[13]. Due to its rich phenolic and flavonoid content flower, leaf and bark extracts showed significant scavenging action against superoxide radicals and hydroxyl radicals[14]. Limited information is available regarding the effects of these plants on the selected cancer cell lines. In the present study, cell lines were selected based on their translational relevance and the experimental conditions. Colo 205 (human colon carcinoma) and MCF-7 (human breast adenocarcinoma) were included as they represent two of the most prevalent and well-characterised human cancers. The murine melanoma cell line B16F10 serves as a well-established preclinical model for studying tumor growth and metastasis. The normal murine fibroblast cell line L929 was employed to assess cytotoxic selectivity, helping distinguish cancer-specific effects from general toxicity. The combined use of human and murine cell lines reinforces the conclusion that the observed anticancer effects are broadly relevant across different models, thereby strengthening the preliminary evidence for further isolation and validation of phytoconstituents.

Materials and Methods

Plant collection and identification

Seed kernels of *Entada pursaetha* were collected from Tiranelveli District, Tamil Nadu (Voucher No: 087) and authenticated by scientist Dr. S. Mutheeswaran, Xavier Research Foundation, St. Xavier's College, Palayamkottai, Tamil Nadu. Roots of *Wagatea spicata* were collected from Raigad District, Maharashtra and authenticated by scientist Dr. R. K. Choudhary Agharkar Research Institute Pune, Maharashtra.

Preparation of extract

1000 g of dried and powdered plant material of *Entada pursaetha* seed and *Wagatea spicata* root were used for successive extraction with 700 mL of solvents ranging from non-polar to polar such as petroleum ether, chloroform, ethyl acetate, acetone, methanol and water. The extracts were concentrated by evaporating the solvent using a water bath maintained at 60-80^{0}C. The evaporated extracts were stored in an airtight container at 4^{0}C .

Preliminary phytochemical screening

All the extracts of *Entada pursaetha* and *Wagatea spicata* were screened for the presence of secondary metabolites like carbohydrates, proteins, phenols, tannins, saponins, alkaloids, flavonoids and triterpenoids[15].

Preparation of the TLC plates

The precoated TLC plates (Merk, Germany) made up of silica gel G as an adsorbent were activated in an oven for 30 minutes at 110°C. All extracts of *Entada pursaetha* and *Wagatea spicata* prepared by successive extraction were subjected to thin layer

chromatographic studies for preliminary identification of compounds.

Development of mobile phase

Several solvent systems were tried for the identification of phytoconstituents present in various extracts of *Entada pursaetha* and *Wagatea spicata.* After development, the plates were air-dried, and spots were visualised under UV at 254nm and 366nm by spraying with Vanillin Sulphuric acid Reagent (VSR) and Anisaldehyde Sulphuric acid Reagent (ASR) to identify different compounds present in the extract. Numbers of spots were noted, and Rf values were calculated.

Cell lines and culture conditions

Three cell lines Colo 205 (Colon cancer cell lines), B16F10 (Skin cancer cell lines), MCF-7 (Human breast cancer cell lines) and (L929) normal mouse fibroblast cells were procured from the National Centre for Cell Science, Pune. The cell lines were maintained in RPMI-1646 medium. The medium composition is 10% fetal bovine serum, glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100g/mL). The cultures were maintained in a humidified 5% CO_2 incubator at 37^0C and the cells were sub-cultured every 3-4 days to maintain logarithmic growth and were allowed to grow for 24 hours before use.

Chemicals and reagents

MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Fetal bovine serum (Gibco, Invitrogen) Cat No -10270106), Trypsin, Penicillin, DMEM with high glucose (Cat No-11965-092), Antibiotic – Antimycotic 100× solution (Thermo Fisher Scientific) (Cat No-15240062).

Cytotoxicity assay

MTT assay

Cells were incubated at a concentration of 1×10^4 cells/mL in a culture medium for 24 hours at 37°C with 5% CO_2. Cells were seeded 1 x 10^4 cells/well in 100µL culture medium and 100µL samples of extracts *E. pursaetha* methanol extract (EPM), *E. pursaetha* acetone extract (EPA), *E. pursaetha* ethyl acetate extract (EPEA), *W. spicata* methanol extract (WSM), *W. spicata* acetone extract (WSA), *W. spicata* ethyl acetate extract (WSEA) at concentrations of 10µg/mL, 30µg/mL and 100µg/mL into microplates respectively (tissue culture grade and 96 wells). The control wells were incubated with DMSO (0.2% in PBS) and cell lines (all samples were incubated in triplicate). The controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 hours. at 37°C and 5% CO_2 in a CO_2 incubator (Thermo Scientific BB150). After incubation, the medium was removed, and 20 µL of MTT reagent (5mg/min PBS) was added. After the addition of MTT, cells were incubated for 4 hrs. at 37°C in the CO_2 incubator. The wells were observed for formazan crystal formation under the microscope. The yellowish 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was reduced to dark-coloured formazan by viable cells only. After completely removing the medium, 200 µL of DMSO was added, incubated for 10 min at 370C (wrapped in aluminium foil). Triplicate samples were analysed by measuring the absorbance of each sample by a microplate reader (Benesphera E21) at a wavelength of 550 nm.

Statistical analysis

Statistical analysis was done by using GraphPad Prism 10.1.2 Software. Data were assessed using one way analysis of variance (ANOVA). Results were presented as mean ± standard error of mean (SEM). *P* values less than 0.05 were considered significant.

Results

Percentage yield of extracts

Methanol was observed the solvent with maximum yield 10.7% w/w from *Entada purseatha* seeds and 5.60% w/w from *Wagatea spicata* seeds. The extraction yield of *Entada purseatha* in other solvents is 3.12% w/w in petroleum ether, 2.34% w/w in chloroform, 1.83% w/w in ethyl acetate, 0.93% w/w in acetone, and 3.77% w/w in water. *Wagatea spicata* root extracts, on the other hand, demonstrated percentage yields of 0.95% w/w in petroleum ether, 3.10% w/w in chloroform, 4.22% w/w in ethyl acetate, and 0.56% w/w in acetone and 2.77% w/w in water.

Preliminary phytochemical screening

Preliminary phytochemical analysis of six extracts of *Entada pursaetha* and *Wagatea spicata* is shown in Table 1 & 2 respectively. Carbohydrates, protein, phenol and alkaloids are not present in all the extracts of *Entada pursaetha.* Flavonoids and terpenoids are present in almost all the extracts except water.

Table 1 — Phytochemical analysis of *Entada pursaetha* seed kernel extract

Solvent	Carbohydrate	Protein	Phenol	Tannin	Saponin	Alkaloid	Flavonoid	Terpenoid
Petroleum ether	-	-	-	-	-	-	+	+
Chloroform	-	-	-	-	-	-	+	+
Ethyl acetate	-	-	-	-	-	-	+	+
Acetone	-	-	-	-	+	-	+	+
Methanol	-	-	-	+	+	-	+	+
Water	-	-	-	+	+	-	-	-

['+' – positive and '-' - Negative]

Table 2 — Phytochemical analysis of *Wagatea spicata* root extract

Solvent	Carbohydrate	Protein	Phenol	Tannin	Saponin	Alkaloid	Flavonoid	Terpenoid	Glycosides
Petroleum ether	+	-	+	-	-	-	-	+	+
Chloroform	+	-	+	+	+	-	+	+	+
Ethyl acetate	+	-	+	-	-	-	-	+	+
Acetone	+	-	+	-	-	-	+	+	-
Methanol	+	-	+	+	+	-	+	+	+
Water	+	-	+	+	+	-	+	-	-

['+' – positive and '-' - Negative]

Saponins are present in acetone, methanol, and water extracts. Tannins are present only in methanol and water extract. Carbohydrates, phenols and terpenoids are present in all the extracts of *Wagatea spicata*. Proteins and alkaloids are absent in all the extracts, tannins and saponins are present in chloroform, methanol and water extract. Glycosides are absent in acetone extract while flavonoids are absent in ethyl acetate extract.

Thin Layer Chromatography

Thin-layer chromatography was used to identify phytoconstituents in both plant extracts using various mobile phases. EPEA, EPA, EPM comparatively showed a greater number of separation of compounds with mobile phase ethyl acetate: methanol: water (8:1:1). EPEA showed separation of 7 spots with Rf values 0.88, 0.86, 0.68, 0.50, 0.39, 0.17 and 0.07 under UV at 366 nm wavelength. EPA showed the separation of 6 spots with Rf values 0.86, 0.82, 0.59, 0.34, and 0.17 under UV at 366 nm wavelength. EPM showed one spot with Rf value 0.55 under UV at 366 nm wavelength.

WSEA, WSA and WSM are comparatively showed good separation of compounds with mobile phase toluene: ethyl acetate: formic acid (5:4:1). WSEA shows separation of 4 spots with Rf values 0.51, 0.46, 0.41and 0.26 under UV at 366 nm wavelength. WSA shows separation of 6 spots with Rf values 0.82, 0.74, 0.66, 0.60, 0.54 and 0.35 under UV at 366 nm wavelength. WSM shows the separation of 4 spots with Rf values 0.64, 0.58, 0.54 and 0.20 under UV at 366 nm wavelength.

Colo 205 (Colon cancer cell line)

The highest inhibitory effect on the Colo 205 cell line was shown by EPA compared with EPEA and EPM with the minimum cell inhibition of normal mouse fibroblast cells L929. EPA extract showed 85.47%, 86.03% and 89.64% inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively and IC_{50} of this extract was 40.46µg/mL. The percent inhibition for this cell line was not detected by EPM extract (Table 3).

WSA showed maximum cell inhibition 82.05%, 83.28% and 85.37% with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively with 32.76µg/mL IC_{50} as compared with WSM and WSEA while the less sensitivity shown for normal mouse fibroblast cells L929 as compared to standard compound 5 Fluorouracil (5FU) (Table 4). The graphical presentation of cell inhibition of all six extracts and standard (5FU) against normal mouse fibroblast cells L929, Colo 205 cell lines, MCF-7 cell lines and B16F10 cel lines are shown in Fig. 1. The cytotoxicity of extracts against Colo 205 cell line is presented in Fig. 2.

MCF-7 (Breast cancer cell line)

EPM, EPEA showed better percent inhibition on MCF-7 cell lines than EPA with $IC_{50} \geq 100$ µg/mL. EPM showed 68.17%, 68.68%, 70.96%, cell inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively. EPEA showed 63.55%, 64.37%, 68.88%, cell inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively (Table 3).

Table 3 — Cell inhibition and IC_{50} value of EPM, EPA, EPEA, WSM, WSA, and WSEA extracts on L929, Colo205, MCF-7 and B16F10 cell lines

Extract	Concentration µg/mL	L929 cell line		Colo 205 cell line		MCF7 Cell line		B16F10 Cell line	
		% Inhibition	IC_{50} µg/mL	% Inhibition	IC_{50} µg/mL	% Inhibition	IC_{50} µg/mL	% Inhibition	IC_{50} µg/mL
	10	47.5 ± 0.12		80.91 ± 0.12		79.76 ± 0.12		48.35 ± 0.12	
5FU	30	71.82 ± 0.45	2.23	88.31 ± 0.05	18.56	84.27 ± 0.03	46.79	58.31 ± 0.08	8.9
	100	83.21 ± 0.12		89.07 ± 0.15		86.63 ± 0.02		68.53 ± 0.14	
	10	24.82 ± 0.12		-		68.17 ± 0.12		38.7 ± 0.12	
EPM	30	31.72 ± 0.12	47.26*	-	-	68.68 ± 0.01	>100**	35.11 ± 0.10	115.63**
	100	58.57 ± 0.12		-		70.96 ± 0.14		48.76 ± 0.32	
	10	28.83 ± 0.12		85.47 ± 0.12		38.72 ± 0.12		32.19 ± 0.12	
EPA	30	34.88 ± 0.12	47.90*	86.03 ± 0.01	40.46*	40.47 ± 0.15	>100**	44.2 ± 0.07	84.89*
	100	46.79 ± 0.12		89.64 ± 0.13		48.37 ± 0.08		51.9 ± 0.01	
	10	24.65 ± 0.12		26.78 ± 0.12		63.55 ± 0.12		37.57 ± 0.12	
EPEA	30	32.76 ± 0.12	49.21*	36.46 ± 0.16	32.55	64.37 ± 0.19	>100**	43.42 ± 0.67	136.3*
	100	50.43 ± 0.12		52.32 ± 0.14		68.88 ± 0.02		46.43 ± 0.29	

Values are expressed as Mean ± S.E.M. (n=3). Data were assessed using one way analysis of variance (ANOVA).[***$P < 0.001$, **$P < 0.01$, *$P < 0.05$]

Table 4 — Cell inhibition and IC_{50} value of WSM, WSA, and WSEA extracts on L929, Colo205, MCF-7 and B16F10 cell lines

Extract	Concentration µg/mL	L929 cell line		Colo 205 cell line		MCF7 Cell line		B16F10 Cell line	
		% Inhibition	IC_{50} µg/mL	% Inhibition	IC_{50} µg/mL	% Inhibition	IC_{50} µg/mL	% Inhibition	IC_{50} µg/mL
	10	47.5 ± 0.12		80.91 ± 0.12		79.76 ± 0.12		48.35 ± 0.12	
5FU	30	71.82 ± 0.45	2.23	88.31 ± 0.05	18.56	84.27 ± 0.03	46.79	58.31 ± 0.08	8.9
	100	83.21 ± 0.12		89.07 ± 0.15		86.63 ± 0.02		68.53 ± 0.14	
	10	23.39 ± 0.12		26.49 ± 0.12		70.73 ± 0.12		31.46 ± 0.12	
WSM	30	32.02 ± 0.12	48.96*	32.28 ± 0.04	28.39***	71.04 ± 0.21	>100*	34.74 ± 0.13	101.05*
	100	45.54 ± 0.12		36.46 ± 0.04		73.81 ± 0.12		49.95 ± 0.25	
	10	26.42 ± 0.12		82.05 ± 0.12		38.03 ± 0.12		39.9 ± 0.12	
WSA	30	35.83 ± 0.12	48.78*	83.28 ± 0.07	32.76*	39.9 ± 0.01	>100**	43.24 ± 0.04	124.39*
	100	49.57 ± 0.12		85.37 ± 0.25		39.95 ± 0.02		47.89 ± 0.10	
	10	27.18 ± 0.12		41.88 ± 0.12		63.17 ± 0.12		29.58 ± 0.12	
WSEA	30	34.76 ± 0.01	48.76*	44.06 ± 0.31	36.51**	63.34 ± 0.06	>100**	35.11 ± 0.14	117.15**
	100	44.54 ± 0.09		51.28 ± 0.46		65.86 ± 0.10		46.66 ± 0.05	

Values are expressed as Mean ± S.E.M. (n=3). Data were assessed using one way analysis of variance (ANOVA).[***$P < 0.001$, **$P < 0.01$, *$P < 0.05$]

WSM and WSEA showed good percent inhibition on MCF-7 cell lines than WSA. WSM showed 70.73%, 71.08% and 73.81%, cell inhibition with concentrations of 10µg/mL, 30µg/mL and 100 µg/mL respectively. WSEA showed 63.17%, 63.34%, 65.86%, cell inhibition with concentrations of 10 µg/mL, 30µg/mL and 100µg/mL respectively. Both the extracts showed $IC_{50} \geq 100$ µg/mL (Table 4). Fig. 3 representing cytotoxicity of extracts against MCF-7 cell line.

EPM, EPEA, WSM and WSEA all four extracts proved a promising percent cell inhibition on MCF-7 breast cancer cell lines with minimum cell inhibition in normal mouse fibroblast cells L929 as compared to standard compound 5 Fluorouracil (5FU).

B16F10 (Skin cancer cell line)

EPA showed better cell inhibition as 32.19%, 44.20%, 51.90%, with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively and IC_{50} value 84.89 µg/mL as compared to EPM and EPEA. EPM showed 38.70%, 35.11%, and 48.76% cell inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively and IC_{50} value 115.63µg/mL. EPEA showed 37.57%, 43.42%, 46.43% cell inhibition with concentrations of 10µg/mL, 30µg/mL

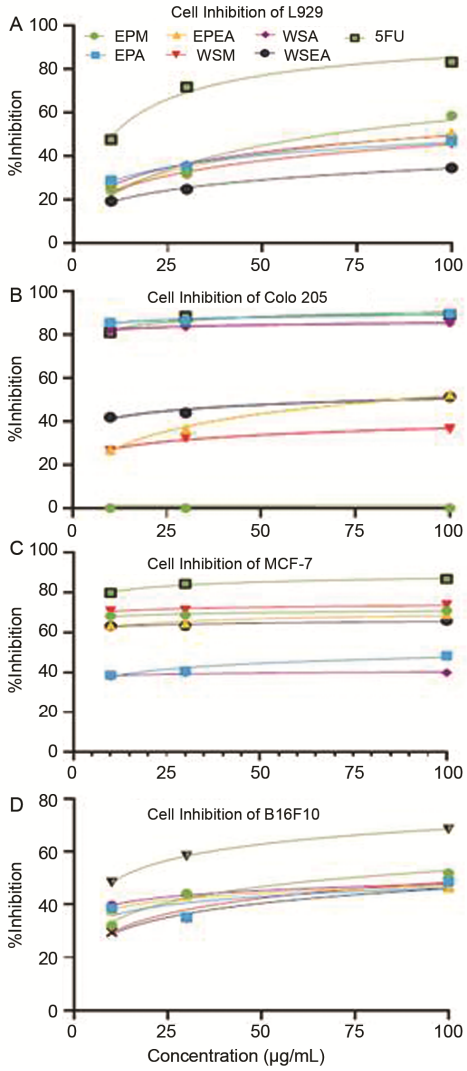

Fig. 1 — Cell inhibition of all six extracts and standard (5FU) against normal mouse fibroblast cells L929 (A). Cell inhibition of all six extracts and standard (5FU) against Colo 205 cell line (B). Cell inhibition of all six extracts and standard (5FU) against MCF-7 cell lines (C). Cell inhibition of all six extracts and standard (5FU) against B16F10 cell lines (D).

and 100µg/mL respectively IC_{50} value 136.30µg/mL (Table 3).

WSA showed maximum cell inhibition 39.90%, 43.24%, 47.89% with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively and IC_{50} value 124.39 µg/mL as compared to WSEA and WSM. WSEA showed 29.58%, 35.11%, 46.66%, cell inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively and IC_{50} value 117.15 µg/mL. WSM showed 31.46%, 34.74%, 49.95%, cell inhibition with concentrations of 10µg/mL, 30µg/mL and 100µg/mL respectively and IC_{50} value 101.05 µg/mL. All extracts percentage inhibition was compared to standard compound 5 Fluorouracil (5FU) (Table 4). Cytotoxicity against B16F10 is represented in Fig. 4. The effect of all extracts on normal mouse fibroblast cells L929 is shown in Fig. 5.

Discussion

Despite enormous progress in drug discovery, cancer is still one of the world's leading causes of death, and researchers are constantly looking for safer and more efficient treatment alternatives[16]. Conventional treatments such as chemotherapy, radiation, and surgery are often associated with severe side effects, highlighting the need for complementary or alternative approaches, such as herbal remedies[17]. Natural products, rich in diverse phytochemicals, have historically served as important sources of anticancer agents. Several classes of compounds, including saponins, terpenoids, flavonoids, tannins, and phenols, have been reported to exhibit cytotoxic or apoptosis-inducing effects in various cancer model.

In the current investigation cytotoxic potential of *E. pursaetha* seed kernel and *W. spicata* root extracts were evaluated using MTT assay against Colo 205, B16F10 and MCF-7 cell lines. Phytochemical

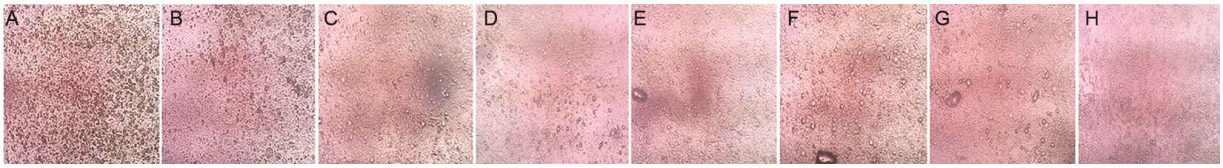

Fig. 2 — Cytotoxicity of (A) Control, (B) Standard (5FU), (C) EPM, (D) EPA, (E) EPEA, (F) WSM, (G) WSA, (H) WSEA extracts on Colo 205 Cell line.

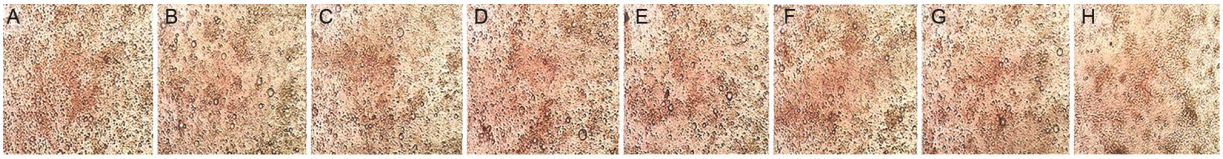

Fig. 3 — Cytotoxicity of of (A) Control, (B) Standard (5FU), (C) EPM, (D) EPA, (E) EPEA, (F) WSM, (G) WSA, (H) WSEA extracts on MCF-7 Cell line.

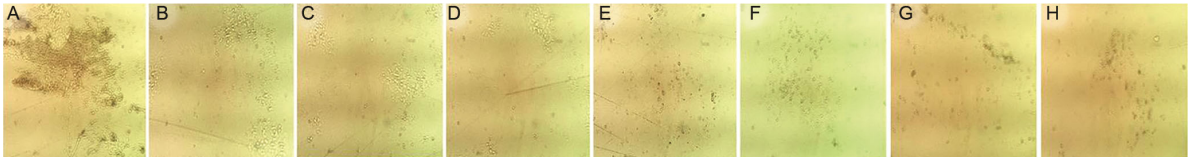

Fig. 4 — Cytotoxicity of of (A) Control, (B) Standard (5FU), (C) EPM, (D) EPA, (E) EPEA, (F) WSM, (G) WSA, (H) WSEA extracts on B16F10 Cell line.

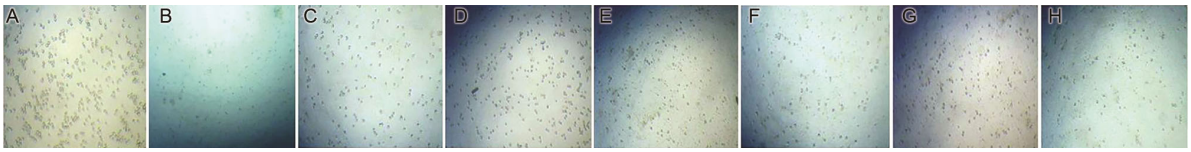

Fig. 5 — Cytotoxicity of of (A) Control, (B) Standard (5FU), (C) EPM, (D) EPA, (E) EPEA, (F) WSM, (G) WSA, (H) WSEA extracts on L929 Cell line.

screening revealed the presence of phenolic chemicals, flavonoids, terpenoids, tannins, and saponins all of which have been previously reported to contribute to anticancer activity[18,19,20-]. However, it is important to note that this investigation is preliminary, and no specific bioactive compounds were isolated or identified[21,23].

Several medicinal plants can exert anticancer effects by modulating signaling pathways, inhibiting key enzymes, and promoting DNA repair[9,21,22]. According to NCI and Geran's cytotoxicity criteria, all extracts demonstrated activity within the moderate cytotoxic range (IC50 values 21–200 µg/mL)[17].

The requirement of higher concentrations for achieving 50% inhibition may be attributed to the intrinsic resistance of the tested cancer cell lines toward crude plant extracts. Among the tested extracts, (EPA) and (WSA) extracts produced comparatively greater inhibition against the Colo 205 colon cancer cell line and while EPM, EPEA, WSA, and WSEA showed better activity against MCF-7 cells. The cytotoxicity observed might correlate with the phytoconstituents present in the extracts, suggesting their possible role in the activity. For example, phenolic compounds and triterpenoid saponins are known to induce apoptosis through extrinsic and intrinsic pathways[17,23], while flavonoids such as quercetin and apigenin promote apoptosis and autophagy under stress conditions[23]. Additionally, triterpenoid saponins like glycyrrhizin and polyphenols such as ellagic acid have been reported to exert antiproliferative effects by modulating signaling pathways (e.g., MAPK, PI3K/Akt) and inhibiting cyclin-dependent kinases[19,24,25]. Activity against B16F10 cells, however, was relatively modest, possibly reflecting higher levels of oncogenic mutations and resistance mechanisms in this line. While these parallels provide a possible rationale for the observed effects, the current study did not directly assess mechanistic pathways or isolate specific compounds, and thus conclusions remain speculative.

Additionally, at the tested concentrations, none of the extracts demonstrated appreciable cytotoxicity against normal mouse fibroblast cells (L929), indicating a good safety margin. This supports the plants' potential importance in cancer management, though further validation is essential. In general, the current study provides preliminary evidence that E. pursaetha and W. spicata extracts show moderate cytotoxic activity against breast and colon cancer cell lines. These results lend credibility to the plant's ethnomedical significance and raise the possibility that it contains bioactive components deserving of further research. However, before any definitive conclusions about the plant's therapeutic potential can be made, thorough mechanistic research, chemical isolation, and in vivo validation are needed.

Conclusion

The present research study confirmed that the acetone extract of the seed kernel of *Entada pursaetha* and root of *Wagatea spicata* has a strong inhibitory effect on Colo 205 cell lines. The methanol and ethyl acetate extract of the seed kernel of *Entada pursaetha* and root of *Wagatea spicata* shows notable cytotoxic effect against MCF-7 cell lines, while all other extracts showed moderate cytotoxicity against B16F10 cell lines. These findings demonstrate that both plants have strong potential as anticancer agents. Hence, these extracts can be further explored for isolating the phytoconstituents responsible for anticancer activity.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

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