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A detailed study of phytochemical and in-vitro anticancer study on euphorbia heterophylla .L

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Abstract

Health is the main issue of today's life. Most diseases are due to improper life-style, Taking improper functional foods, and depending on fast, junk foods. Due to these foods, oxidation takes place and oxidation causes lysis of cells and it causes cancer Plants such as Euphorbia heterophylla are commonly found in our surroundings. They are also used for the management of different human diseases. The anticancer activities of leaves from these plants were investigated using aqueous extracts. The extracts are subjected to phytochemical analysis. The in-vitro methods were used for significant cytotoxicity on Human ovarian cancer cell lines, viz., Pa-1 cells with the IC50 concentrations (The Concentration of the Compound has the capacity to kill 50% of Viable Cells) at 57.08ug/ml respectively after the incubation period of 24hrs. The MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on the reduction of yellow-colored red water-soluble tetrazolium dye MTT to formazan crystals. The obtained results suggesting us that the EHE may have effective anticancer potential against the Human ovarian cancer (Pa-1) cells due to its low IC50 value.

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Introduction

Euphorbia heterophylla Linn. (EH) is a plant belongs to family Euphorbiaceae. It is a hardy, ruderal species, growing between 30 and 70 cm in height, nears 4-5 lobed leaves and stem with milky exudation. The fruits are small, segmented capsules. It is distributed worldwide and has been used as a folk medicine. Euphorbia heterophylla leaf is used in traditional medical practices as laxative, antigonorrheal, migraine and wart cures. The plant lattices have been used as fish poison, insecticide and ordeal poisons [1, 2]. Its leaves are commonly used for antioxidant, an anti- inflammatory and a laxative agent [3, 4]. In some parts of Kogi State, Nigeria, the leaves are used as anticonvulsant and cough remedy.

The leaves of E. heterohylla have been reported to contain quercetin [4]. Diterpenoids have been reported in the root of E. heterophylla. The skin irritant, tumor promoting anti-tumor/anti-cancer and recently anti-HIV activities of Euphorbia species have also been reported in E. heterophylla leaf [5].

The leaves extract also shown antibacterial, antioxidant, nutritive anti- diabetic potential [2, 6, and 7]. Ethanol and aqueous extract E. heterophylla leaf has shown cytotoxic

potential and wound healing ability in experimental animals [8]. A recent study concluded that the aqueous extract of E. heterophylla leaf can be used as an anticoagulant for long storage of whole human blood [9]. However, available literature revealed that no pharmacognostic study has been carried out on the leaves; hence the present investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of leaves; ash values, extractive values, microscopical characteristics of powdered fruit and preliminary phytochemical analysis of Euphorbia heterophylla Linn leaves.

Phytochemical Extract

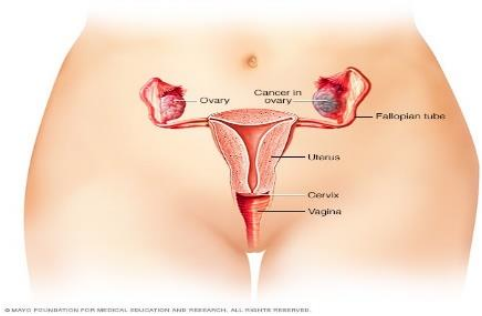
Plants such as Euphorbia heterophylla are commonly found in our surroundings. They are also used for the management of different human diseases. The anticancer activities of leaves from these plants were investigated using aqueous extracts.

The extracts are subjected to phytochemical analysis. Euphorbia heterophylla was found to contain alkaloids, flavanoids, carbohydrates, saponins, glycosides, sitosterols, fats and fixed oils, phenols, proteins, amino acids, tannis terpenoids.

About Ovarian Cancer

Types of Cancer on the Human Body

Bladder Cancer
Breast Cancer
Colon and Rectal Cancer
Endometrial Cancer
Kidney Cancer
Leukemia
Liver Cancer
Lung Cancer
Melanoma
Non-Hodgkin Lymphoma
Pancreatic Cancer
Prostate Cancer
Thyroid Cancer



In Cancer, brief ovarian cancer is a growth of cells that forms in the ovaries. The cells multiply quickly and can invade and destroy healthy body tissue. The female reproductive system contains two ovaries, one on each side of the uterus. The ovaries — each about the size of an almond — produce eggs (ova) as well as the hormones estrogen and progesterone. Ovarian cancer treatment usually involves surgery and chemotherapy.

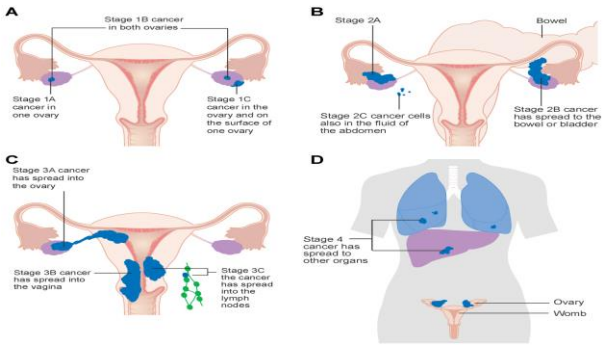


Fig: 01 Stages of ovarian cancer and its spreading nature in body organs

Symptoms

When ovarian cancer first develops, it might not cause any noticeable symptoms. When ovarian cancer symptoms happen, they're usually attributed to other, more common conditions.

Signs and symptoms of ovarian cancer may include:

- Abdominal bloating or swelling
- Quickly feeling full when eating
- Weight loss
- Discomfort in the pelvic area
- Fatigue
- Back pain
- Changes in bowel habits, such as constipation
- A frequent need to urinate

Causes

It's not clear what causes ovarian cancer, though doctors have identified things that can increase the risk of the disease. Doctors know that ovarian cancer begins when cells in or near the ovaries develop changes (mutations) in their DNA. A cell's DNA contains the instructions that tell the cell what to do. The changes tell the cells to grow and multiply quickly, creating a mass (tumor) of cancer cells. The cancer cells continue living when healthy cells would die. They can invade nearby tissues and break off from an initial tumor to spread (metastasize) to other parts of the body

Types of ovarian cancer

The type of cell where cancer begins determines the type of ovarian cancer you have and helps your doctor determine which treatments are best for you. Ovarian cancer types include:

Epithelial ovarian cancer. This type is the most common. It includes several subtypes, including serous carcinoma and mucinous carcinoma.

Stromal tumors. These rare tumors are usually diagnosed at an earlier stage than other ovarian cancers.

Germ cell tumors. These rare ovarian cancers tend to occur at a younger age

Risk factors

Factors that can increase your risk of ovarian cancer include:

Older age. The risk of ovarian cancer increases as you age. It's most often diagnosed in older adults.

Inherited gene changes. A small percentage of ovarian cancers are caused by gene changes you inherit from your parents. The genes that increase the risk of ovarian cancer include *BRCA1* and *BRCA2*. These genes also increase the risk of breast cancer. Several other gene changes are known to increase the risk of ovarian cancer, including gene changes associated with Lynch syndrome and the genes *BRIP1*, *RAD51C* and *RAD51D*.

Family history of ovarian cancer. If you have blood relatives who have been diagnosed with ovarian cancer, you may have an increased risk of the disease.

Being overweight or obese. Being overweight or obese increases the risk of ovarian cancer.

Postmenopausal hormone replacement therapy. Taking hormone replacement therapy to control menopause signs and symptoms may increase the risk of ovarian cancer.

Endometriosis. Endometriosis is an often painful disorder in which tissue similar to the tissue that lines the inside of your uterus grows outside your uterus.

Age when menstruation started and ended. Beginning menstruation at an early age or starting menopause at a later age, or both, may increase the risk of ovarian cancer.

Never having been pregnant. If you've never been pregnant, you may have an increased risk of ovarian cancer.

Prevention

Symptoms and causes

There's no sure way to prevent ovarian cancer. But there may be ways to reduce your risk:

Consider taking birth control pills. Ask your doctor whether birth control pills (oral contraceptives) may be right for you. Taking birth control pills reduces the risk of ovarian cancer. But these medications do have risks, so discuss whether the benefits outweigh those risks based on your situation.

Discuss your risk factors with your doctor. If you have a family history of breast and ovarian cancers, bring this up with your doctor. Your doctor can determine what this may mean for your own risk of cancer. You may be referred to a genetic counselor who can help you decide whether genetic testing may be right for you. If you're found to have a gene change that increases your risk of ovarian cancer, you may consider surgery to remove your ovaries to prevent cancer.

Pathology of Ovarian Tumors

Most tumors of the ovary can be placed into one of three major categories—*surface epithelial-stromal* tumors, *sex cord-stromal* tumors, and *germ cell* tumors (Fig. 1)—according to the anatomic structures from which the tumors presumably originate. Each category includes a number of subtypes. Combinations of different subtypes, either intimately intermixed or side-by-side within a single tumor, are found with some frequency. Tumors that combine two or more subtypes are designated as *mixed*, with the contributing subtypes specified in the designation. By convention, for classification purposes, tumor subtypes making up < 10% of the total tumor mass are ignored.

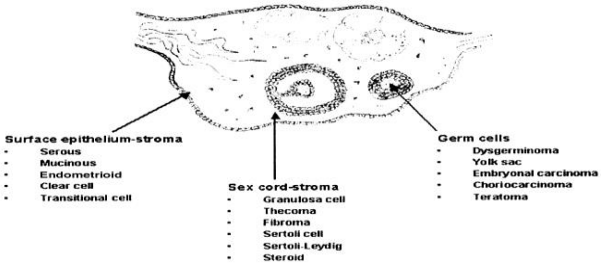


Fig: 02 Origins of the three main types of ovarian tumors

The ovarian surface epithelium is histological similar to the mesothelium, which is the epithelium that lines the interior of the pelvic and abdominal cavities. This similarity, as well as the close morphologic resemblance of ovarian epithelial-stromal tumors to some epithelial tumors arising elsewhere within the pelvis and abdomen, may be explained by the shared origin (i.e., the primitive coelomic epithelium) of the ovarian surface epithelium and the mesothelium.

The sex cord-stromal group includes tumors of mesenchymal and mesonephric origin. Some of these tumors, namely fibromas and thecomas, have a fibrous appearance, and some appear to be derived from the granulosa cells or their testicular sex cord counterparts, the Leydig and Sertoli cells.

The ovarian germ cells are the origin of a number of tumors that are identical to testicular germ cell tumors. Germ cells that are stranded or have gone astray during their migration between the yolk sac and the developing gonads may develop into germ cell tumors outside the

gonads. The three main types of ovarian cancer and their subtypes are discussed briefly with special consideration of key aspects related to tumor registration and epidemiology. A more comprehensive and detailed discussion of the pathology of ovarian tumors can be found in specialized publications [23-26].

Surface Epithelial-Stromal Tumors

Surface epithelial-stromal tumors are believed to originate from the surface epithelium of the ovary. They are classified as *benign* if they lack exuberant cellular proliferation and invasive behavior; as *borderline* (also known as *atypically proliferating* or *of low malignant potential*) if there is exuberant cellular proliferation but no invasive behavior; and as *malignant* if there is invasive behavior. Surface epithelial-stromal tumors account for approximately 60% of all ovarian tumors and approximately 90% of malignant ovarian tumors. Most borderline tumors behave clinically as benign tumors and have good prognosis, but some may recur after surgical removal and some may seed extensive *implants* within the abdominal cavity. Surface epithelial-stromal tumors occur primarily in women who are middle-aged or older and are rare in young adults, particularly before puberty.

Five major subtypes are included within the surface epithelial-stromal group. They are designated as follows: serous, mucinous, endometrioid, clear cell, and transitional cell (or Brenner type). Highly malignant epithelial-stromal tumors lacking any specific differentiation are classified as undifferentiated.

Epithelial-stromal tumors that are not designated as having a specific subtype commonly are recorded as adenocarcinomas not otherwise specified (NOS).

Serous or mucinous tumors identical to those occurring in the ovary may arise in multiple locations within the pelvic and abdominal cavities. They sometimes coincide with ovarian tumors of identical type. When they do so, it may be difficult to establish whether the extraovarian sites represent seedlings or implants originating from the ovarian tumor or de novo malignancies. By convention, when the ovaries appear to be incidentally involved and do not appear to be the primary origin of the tumor, the tumor is recorded as an extraovarian peritoneal carcinoma.

Serous tumors

Serous tumors are epithelial-stromal tumors formed by cells that resemble those of the internal lining of the fallopian tube. Benign serous tumors are thin-walled cysts formed by a single chamber filled with a watery, straw-colored fluid. The internal lining of the cyst is usually flat but may display a few coarse papillary projections.

Mucinous tumors

Mucinous tumors are epithelial ovarian tumors formed by cells that resemble either those of the endocervical epithelium (*endocervical* or *müllerian type*) or, more frequently, those of the intestinal epithelium (*intestinal type*).

Endometrioid tumors

Endometriod tumors are epithelial ovarian tumors formed by cells that resemble those of the internal lining of the uterus (the *endometrium*). They may be associated with the aberrant presence of endometrium outside the uterus (*endometriosis*) and with overgrowth (*hyperplasia*) or cancer of the endometrium.

Clear cell tumors

Clear cell tumors are epithelial ovarian tumors that are formed by clear, peglike or hobnail-like cells. Benign and borderline clear cell tumors are quite rare. Most clear cell ovarian tumors are malignant. They can be predominantly solid or cystic with one or more polypoid masses protruding into the lumen. Clear cell tumors represent 4–5% of all malignant ovarian epithelial tumors. On average, diagnosis occurs in the fifth decade of life. [23, 25, 26]

Transitional cell (Brenner) tumors

Transitional cell tumors are epithelial ovarian tumors formed by cells that resemble those of the internal lining of the urinary bladder (the transitional epithelium or urothelium). These tumors presumably are derived from surface ovarian epithelium that undergoes urotheliumlike transformation (e.g., urothelial metaplasia, Walthard nests). They may occur in association with similar tumors in the urinary bladder. Transitional cell tumors rarely occur and are often reported within the category of other specified epithelial-stromal tumors.

Sex Cord-Stromal Tumors

Sex cord-stromal tumors are ovarian tumors that are believed to originate in theca cells, other stromal cells, and granulosa cells and their testicular sex cord counterparts, the Sertoli and Leydig cells. These tumors often are associated with endocrine manifestations. They account for approximately 8% of all ovarian tumors and approximately 7% of all malignant ovarian tumors.

Granulosa cell tumors

Granulosa cell tumors are rare sex cord ovarian tumors that are formed by cells believed to be derived from those that surround the germinal cells in the ovarian follicles. Two major forms of granulosa cell tumors are recognized: the *adult* form, which primarily occurs in middle-aged and older women, and the *juvenile* form, which typically occurs in children and younger women.

Thecomas

Thecomas are rare, solid ovarian tumors formed by stromal cells that resemble the theca cells that normally surround the ovarian follicles. Most thecomas are unilateral and occur in postmenopausal women. They are uncommon before age 30 years. These tumors commonly have estrogenic manifestations, including postmenopausal uterine bleeding, endometrial hyperplasia, and endometrial cancer. Most thecomas are benign, and surgical excision is curative.

Fibromas

Fibromas are rare, solid ovarian tumors arising from the spindled stromal cells that form *collagen*. On the rare occasions when these tumors are bilateral, they may be associated with *nevoid basal cell carcinoma syndrome*, also known as *Gorlin syndrome*. Fibromas are most common during middle age and rare before age 30 years; the mean age at diagnosis is in the late forties [23, 25, 26]. Unlike other sex cord-stromal tumors, fibromas rarely are associated with hormone production. In almost all cases, they are benign and curable by surgical excision. Fibromas with increased cellularity and cell proliferation (*mitotic activity*) are rare and may follow a malignant course; fibromas of this kind are known as *fibrosarcomas*.

Sertoli cell tumors

Sertoli cell tumors are rare sex cord-stromal ovarian tumors formed by cell proliferations that resemble the rete ovarii and rete testis, which characteristically are arranged in hollow or solid tubules. The mean age at diagnosis is 30 years. These tumors usually do not function, but they may produce hormones that can induce precocious sexual development or, in rare cases, the development of male features (*virilization*) in girls. Most of these tumors are unilateral and Stage I. They form solid, yellow or brown, lobulated masses and rarely metastasize. Surgical treatment often is curative.

Sertoli-Leydig cell tumors

Sertoli-Leydig cell tumors are rare sex cord-stromal ovarian tumors that are formed by variable proportions of cells that resemble epithelial and stromal testicular cells. They can be solid, partially cystic, or completely cystic, and they may or may not have polypoid or vesicular structures in their interior. Most are unilateral and occur in young women. The mean age at diagnosis is in the mid-twenties [8, 10, and 11].

Steroid cell tumors

Steroid cell tumors are rarely occurring, solid, yellow, ovarian sex cord-stromal tumors formed by cells that resemble adrenal gland cells (*stromal luteomas*) or the testicular Leydig cells (*Leydig cell tumors*). Most stromal luteomas occur in postmenopausal women and are associated with estrogenic manifestations, the most common of which is uterine bleeding. The majority of Leydig cell tumors also occur in postmenopausal women but cause virilization (an *androgenic manifestation*) rather than estrogenic manifestations

Germ Cell Tumors

Germ cell tumors are ovarian tumors formed by cells that are believed to be derived from primordial germ cells. These tumors make up approximately one-fourth of all ovarian tumors but only 3–7% of malignant ovarian tumors. In parts of Asia and Africa where the prevalence of surface epithelial-stromal tumors is relatively low, germ cell tumors constitute a larger proportion of all ovarian neoplasms [28].

Dysgerminomas

Dysgerminomas are tumors composed of cells that are similar to primordial germ cells. They display a striking similarity to their testicular counterpart, the *seminoma*. Dysgerminomas are solid, white or grayish-white tumors. Unilateral tumors are more common in the right ovary, and 10–20% of dysgerminomas are bilateral. Most cases occur in the second and third decades of life. Dysgerminomas account for ≤ 2% of all ovarian tumors and only 3–5% of all malignant ovarian tumors; nevertheless, they represent the most common type of malignant ovarian germ cell neoplasm. High prevalence has been reported in Japan and India [23].

Yolk sac tumors

Yolk sac tumors, also known as *endodermal sinus tumors*, are germ cell tumors displaying cellular structures that resemble those of the primitive yolk sac (the *vitelline elements*). These tumors are mainly solid but frequently have cystic spaces. They are highly malignant, frequently invading the surrounding structures and exhibiting extensive spread within the abdominal cavity. Yolk sac tumors metastasize early and do so primarily through the lymphatic system.

Embryonal carcinoma

Embryonal carcinomas are germ cell tumors formed by primitive cells that resemble those of very early embryonic development. They are considered to be the least differentiated type of germ cell tumor. They often are combined with other forms of germ cell tumors, most commonly yolk sac tumors. Embryonal carcinomas are large, predominantly solid tumors with a variegated appearance, and most are unilateral. Involvement of the opposite ovary is commonly considered a manifestation of

Metastatic spread. Embryonal carcinomas occur primarily in children and young adults. These tumors can produce alpha-fetoprotein or human chorionic gonadotropin, the latter may be associated with precocious puberty and abnormal uterine bleeding. Embryonal carcinomas are highly malignant tumors that usually have spread extensively within the abdominal cavity by the time of presentation. They metastasize early and do so primarily through the lymphatic system.

Choriocarcinoma Choriocarcinomas are germ cell tumors formed by placental (namely, trophoblastic) cellular elements. They typically are solid and have a hemorrhagic appearance. Most of these tumors are unilateral. The large majority of primary ovarian choriocarcinomas are not related to pregnancy (nongestational). Some originate after a pregnancy (i.e., they are gestational), in which case, most are metastatic, primarily from the uterus. Choriocarcinomas are rare and often are admixed with other germ cell tumors

Teratoma

Teratomas are germ cell tumors that are formed by cells derived from more than one of the three primitive embryonic layers (ectoderm, mesoderm, and endoderm). Teratomas can be mature (benign) or immature (benign or malignant). Teratomas formed predominantly by endodermal or ectodermal elements are referred to as monodermal or specialized.

Classification of Ovarian Tumors

A significant stride in the direction of a histogenesis-based classification system was made in 1973 with the publication of the World Health Organization (WHO) *Classification of Ovarian Tumors* [29]**.** This classification system was updated in 1999 [25] and was approved by the International Society of Gynecological Pathologists (Table 1). A summarized version of the WHO classification system was proposed in 1998 by the International Agency for Research on Cancer [30] for use in comparative studies; this system was used to classify histologic types of ovarian cancer in the current monograph (Table 2). Two coding systems, the WHO International Classification of Diseases for Oncology [31] And the College of American Pathologists Systematized Nomenclature of Medicine [32] are commonly used to code the histology/morphology of tumor

Table: 01 WHO Histologic Classification of Ovarian Tumors

1 Surface epithelial-stromal tumors
➢ 1.1 Serous tumors: benign, borderline, malignant
➢ 1.2 Mucinous tumors, endocervical-like and intestinal-type: benign, borderline, malignant
➢ 1.3 Endometrioid tumors: benign, borderline, malignant, epithelial-stromal and stromal
➢ 1.4 Clear cell tumors: benign, borderline, malignant
➢ 1.5 Transitional cell tumors: Brenner tumor, Brenner tumor of borderline malignancy, malignant Brenner tumor, transitional cell carcinoma (non-Brenner type)
➢ 1.6 Squamous cell tumors
2 Sex cord-stromal tumors
➢ 2.1 Granulosa-stromal cell tumors: granulosa cell tumors, thecoma-fibroma group
➢ 2.2 Sertoli-stromal cell tumors, androblastomas: well-differentiated, Sertoli-Leydig cell tumor of intermediate differentiation, Sertoli-Leydig cell tumor poorly differentiated (sarcomatoid), retiform
➢ 2.3 Sex cord tumor with annular tubules
➢ 2.4 Gynandroblastoma
➢ 2.5 Unclassified

3 Germ cell tumors
➢ 3.1 Dysgerminoma: variant-with syncytiotrophoblast cells
➢ 3.2 Yolk sac tumors (endodermal sinus tumors): polyvesicular vitelline tumor, hepatoid, glandular
➢ 3.3 Embryonal carcinoma
➢ 3.4 Polyembryoma
➢ 3.5 Choriocarcinoma
➢ 3.6 Teratomas: immature, mature, monodermal, mixed germ cell
4 Gonadoblastoma
5 Germ cell sex cord-stromal tumor of nongonadoblastoma type
6 Tumors of rete ovarii
7 Mesothelial tumors
8 Tumors of uncertain origin and miscellaneous tumors
9 Gestational trophoblastic diseases
10 Soft tissue tumors not specific to ovary
11 Malignant lymphomas, leukemias, and plasmacytomas
12 Unclassified tumors
13 Secondary (metastatic) tumors
14 Tumorlike lesions

WHO: World Health Organization

a Source: Scully R, Sobin L. Histological typing of ovarian tumours, volume 9. New York: Springer Berlin, 1999.10

Table: 02 IARC Histologic Groups of Ovarian Tumorsa

Histologic type	WHO ICD-O morphology code
1. Carcinoma	8010–8570,b 9014–9015, 9110
1.1 Serous carcinomac	8441–8462, 9014
1.2 Mucinous carcinomac	8470–8490, 9015
1.3 Endometrioid carcinoma	8380–8381, 8560, 8570
1.4 Clear cell carcinoma	8310–8313, 9110
1.5 Adenocarcinoma NOS	8140–8190, 8211–8231, 8260, 8440
1.6 Other specified carcinomas	
1.7 Unspecified carcinoma	8010–8034
2. Sex cord-stromal tumors	8590–8671
3. Germ cell tumors	8240–8245, 9060–9102

Histologic type	WHO ICD-O morphology code
4. Other specified cancers (including malignant Brenner tumor, müllerian mixed tumor, and carcinosarcoma)	
5. Unspecified cancer	8000–8004

IARC: International Agency for Research on Cancer; WHO: World Health Organization; ICD-O: International Classification of Diseases for Oncology; NOS: not otherwise specified.

a) Source: Parkin DM, Shanmugaratnam K, Sobin L, Ferlay J, Whelan SL. Histological groups for comparative studies, volume 31. IARC technical report. Lyon: International Agency for Research on Cancer, 1998.15
b) Excludes 8240–8245.
c) Includes tumors of borderline malignancy (low malignant potential). Unlike borderline tumors of other types, borderline tumors of serous and mucinous types are included with carcinomas by ICD-O. This approach remains to be validated fully.

Staging of Ovarian Cancer

The extent of tumoral spread, also known as *stage of disease*, at diagnosis is typically established by radiologic evaluation and surgical excision. Surgical management may include debulking of the tumor resection of one or both ovaries, fallopian tubes, and uterus, as well as sampling of lymph nodes, liver, and suspicious sites within the abdomen. Staging of ovarian surface epithelial-stromal tumors is performed according to the TNM system,33 the set of guidelines established by the American Joint Committee on Cancer, which is comparable to an alternative staging system approved by the International Federation of Gynecology and Obstetrics (FIGO) (Table 3) [34].

Table.3 Ovarian Surface Epithelial-Stromal Tumor Staging Protocols:
AJCC TNM System a and FIGO Staging System b

AJCC	FIGO	Description
TX		Primary tumor cannot be assessed.
T0		No evidence of primary tumor.
T1	I	Tumor limited to ovaries (one or both).
T1a	IA	Tumor limited to one ovary; capsule intact, no tumor on ovarian surface. No malignant cells in ascitesc or peritoneal washings.
T1b	IB	Tumor limited to both ovaries; capsules intact, no tumor on ovarian surface. No malignant cells in ascites or peritoneal washings.
T1c	IC	Tumor limited to one or both ovaries, with any of the following: capsule ruptured, tumor on ovarian surface, malignant cells in ascites or peritoneal washings.
T2	II	Tumor involves one or both ovaries with pelvic extension.
T2a	IIA	Extension and/or implants on uterus and/or tube(s). No malignant cells in ascites or peritoneal washings.
T2b	IIB	Extension to other pelvic tissues. No malignant cells in ascites or peritoneal washings.
T2c	IIC	Pelvic extension (2a/IIA or 2b/IIB) with malignant cells in ascites or peritoneal washings.

AJCC	FIGO	Description
T3 and/or N1	III	Tumor involves one or both ovaries, with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph node metastasis[d]
T3a	IIIA	Microscopic peritoneal metastasis beyond pelvis.
T3b	IIIB	Macroscopic peritoneal metastasis (2 cm or less in greatest dimension) beyond pelvis.
T3c and/or N1	IIIC	Peritoneal metastasis (more than 2 cm in greatest dimension) beyond pelvis and/or regional lymph node metastasis.
M1	IV	Distant metastasis (excludes peritoneal metastasis)[e]

AJCC: American Joint Committee on Cancer, FIGO: International federation of Gynecology and Obstetrics.

- a. Source: Fleming ID, Cooper JS, Henson DE, Hutter RVP, Kennedy BJ. American Joint Committee on Cancer (AJCC) cancer staging manual. Philadelphia: Lippincott-Raven, 1997.18
- **b.** Source: Heintz AP, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. *J Epidemiol Biostat*. 2001;6:107–138.19
- **c.** *Ascites* is the accumulation of excessive fluid within the abdominal (peritoneal) cavity. The presence of nonmalignant ascites is not classified. The presence of ascites does not affect staging unless malignant cells are present.
- **d.** Lymph nodes located in the pelvis or in the back of the abdomen on either side of the aorta (*para-aortic*). Liver metastases confined to the capsule are T3/Stage III. Liver parenchymal metastases are M1/Stage IV.
- **e.** Pleural effusion must have positive cytology for M1/Stage IV malignancy.

Anticancer activity of Euphorbia Heterophylla

Euphorbia heterophylla shows cytotoxicity on Human Ovarian cancer cell lines in this research work.

Materials and Methods

Project Requirement

• To determine the cytotoxicity study on Pa-1 cell lines, 1 sample is received.

• The sample details as to be

Details of Sample for the study

Sl. No.	Sample Name/Code	Concentrations	Cell lines
1	EHE	5 (12.5, 25, 50, 100, 200ug/mL)	Pa-1

Background of the Study

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on the reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibit purple color, the intensity Of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm [38].

Materials

1. Cell lines:

a) Pa-1-Human ovarian adenocarcinoma cell line (From NCCS, Pune)

2. Cell culture medium: DMEM- High Glucose - (#AL111, Himedia)

3. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)

4. Fetal Bovine Serum (#RM10432, Himedia)

5. MTT Reagent (5 mg/ml) (# 4060 Himedia)

6. DMSO (#PHR1309, Sigma)

7. Doxorubicin (#D1515, Sigma)

8. D-PBS (#TL1006, Himedia)

9. 96-well plate for culturing the cells (From Corning,USA)

10. T25 flask (# 12556009, Biolite - Thermo)

11. 50 ml centrifuge tubes (# 546043 TORSON)
12. 1.5 ml centrifuge tubes (TORSON)
13. 10 ml serological pipettes (TORSON)
14. 10 to 1000ul tips (TORSON)

Equipments

1. Centrifuge (Remi: R-8oC).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted biological microscope (Biolinkz, India).
4. 37°C incubators with a humidified atmosphere of 5% CO2 (Healforce, China).
5. 96 well Microplate absorbance reader (ELX-800, Bio-TeK, CA, USA).

Assay Controls

1. Medium control (medium without cells)
2. (ii) Negative control (medium with cells but without the experimental drug/compound)
3. (iii) Positive control (medium with cells and 5uM/ml of Doxorubicin)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

Our Research Work Done In Laboratory at: Stellixir Biotech Pvt Ltd,

Maintenance of Cell Lines

The Pa-1 cell lines were purchased from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic- antimycotic solution in the atmosphere of 5% CO2, 18-20% O2 at 370C temperature in the CO2 incubator and sub-cultured every 2 days**.**

Steps Followed

1. Seed 200µl cell suspension in a 96-well plate at the required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours.
2. Add appropriate concentrations of the test agent
3. Incubate the plate for 24hrs at 37°C in a 5% CO2 atmosphere.
4. After the incubation period, take out the plates from the incubator and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
5. Wrap the plate with aluminum foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
7. Remove the MTT reagent and then add 100µl of solubilization solution (DMSO).
8. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals, especially in dense cultures.
9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as the reference wavelength.
10. % Cell viability is calculated using the below formula: % cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] x 100
11. The IC50 value was determined by using a linear regression equation i.e. Y =Mx+C.

Here, Y = 50, M, and C values were derived from the viability graph.

Results and Discussions

Table 04: Histochemical color reactions of Euphorbia heterophylla leaf powder.

S.No	Reagents	Constituent	Colour	Histological Zone	Degree Of Intensity
1.	Aniline so4+H2SO4	Lignin	yellow	xylem	++
2.	Phloroglucinol + HCl	Lignin	Pink	Xylem,Sclerenchyma	+++
3.	Conc. H2SO4	Cellulose	Green	Mesocarp	+
4.	Weak Iodine solution	starch	– –	– –	– –
5.	Millons reagent	protiens	white	Spongy paraenchyma	+
6.	Dragendorffs reagent	Alkaloids	– –	– –	– –
7.	H2SO4	Ca. Oxalate	Needles	Mesophyll, and midrib parenchyma	+
8.	SbCl3	Steroids/ Triterpenoids	Reddish pink	Mesocarp	+ + +

9.	5% Aq. KOH, Anthra-quinone	An-thra-qui-none glyco-sides	– –	– –	–

+++ High, ++ Moderate, + Slight, - Negative.

Table 05: Fluorescence analysis of Euphorbia heterophylla leaf

Color reaction	Day light	Uv light 365nm
Powder + NaOH		Dark greenish fluorescence.
Powder + Methanol + nitrocellulose.	Green color	Yellowish green fluorescence.
Powder + nitrocellulose	Purple green	Strong yellow green fluorescence.
Powder + NaOH in water	Yellowish green	Faint yellowish green Fluorescence.
Powder + nitrocellulose +HCl	Green	Faint green fluorescence.
Powder + Hcl	Grayish green	Dark grey with faint yellow fluorescence.
Powder + H2SO4	Yellowish green	Black
Powder + HNO3	Blackish Black	Black
Powder	Brownish black Green	Greenish yellow florescence

Table 06: Behaviour of *euphorbia heterophylla* leaf powder with different reagent

Reagents	colour/ppt	constitutions
Picric acid	No ppt	Alkaloids absent
Conc.H2SO4	Reddish brown	steroids/triterpinoids present
Aq.FeCl3	No change	Tannins absent
Iodine solution	No change	Starch absent
Ammonia present	No change	Antraquinone glycoside absent

Table 07: physicochemical analysis of *euphorbia heterophylla* leaves

Types of ash value/extractive values	%w/w
Ash value	
Total ash	11.50
Acid insoluble ash	1.50
Water soluble ash	2.50
Sulphated ash	12.50
Extractive values	
Pet- ether	12.0
Ethyl acetate	2.4
Ethanol	28.0
Water	20.0

Phytochemical screening of *Euphorbia heterophylla* extracts

The results of phytochemical screening were obtained as follows

Phytochemical screening of ethanol soluble fraction

Table 08: Phytochemical screening of methanol soluble fraction of *Euphorbia heterophylla*

S.No	Phytochemical Test	Reagents	Interferance	Result
1.	Alkaloid test	1.Mayer's test 2. Hager's test	Appearance of yellow cream ppt. Formationof yellowish white ppt	positive
2.	Carbohydrate test	1.Molish's reagent 2.Benedict's reagent 3.Fehling's reagent	Formation of violet ring. Formation of orange red ppt. Formation of red ppt.	positive
3.	Saponin test	Foam test	Produce foam that lasts for more than 10 minutes.	positive
4.	Glycoside test	1. Modified Bron-trager's test 2. Legal's test	No formation of rose pink colour. No formation of pink/blood red col-our.	positive
5.	Sitosterol test	Salkowski's test	No golden colour was observed.	positive
6.	Fats and fixed oil test	Filter paper press test	No oily stain was observed.	positive
7.	Resin test	Acetone water test	No appearance of turbidity.	Negative
8.	Phenol test	Ferric chloride test	Appearance of bluish black ppt.	positive
9.	Tannin test	Gelatin test	No formation of white ppt	Negative
10.	Diterpines test	Copper Acetate test	No formation of bright green colour.	Negative
11.	Flavanoids test	Alkaline reagent test Lead acetate test	No intense yellow colour obtained. No yellow ppt. obtained	Negative
12.	Proteins and amino acid test	Xanthoproteic test	Formation of yellow colour	positive

Phytochemical screening of chloroform soluble fraction

Table 09: Phytochemical screening of chloroform soluble fraction of *Euphorbia heterophylla*

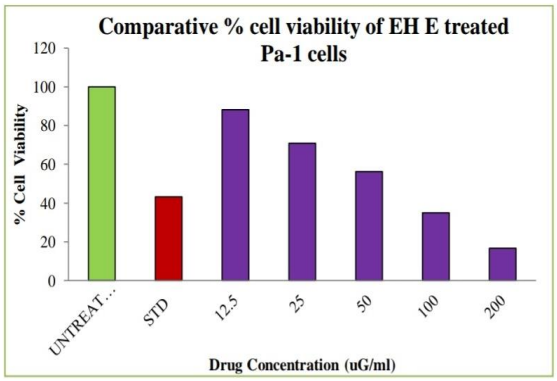
S.No	Phytochemical Test	Reagents	Interference	Result
1.	Alkaloid test	1.Mayer's test 2. Hager's test	Appearance of yellow cream ppt. Formation of yellowish white ppt	Positive
2.	Carbohydrate test	1.Molish's reagent 2.Benedict's reagent 3.Fehling's reagent	Formation of violet ring. Formation of orange red ppt. Formation of red ppt.	Positive
3.	Saponin test	Foam test	Produce foam that lasts for more than 10 minutes.	Positive
4.	Glycoside test	1. Modified Bron-trager's test 2. Legal's test	No formation of rose pink colour. No formation of pink/blood red col-our.	Positive
5.	Sitosterol test	Salkowski's test	Appearance of golden colour was ob-served.	Positive
6.	Essential oil test	Filter paper press test	No oily stain was observed.	Positive
7.	Resin test	Acetone water test	Appearance of tur dity.	Negative
8.	Phenols test	Ferric chloride test	Appearance of bluish black ppt.	Positive
9.	Tannins test	Gelatin test	No formation of white ppt	Positive
10.	Terpeniods test	Salkowski test	No formation of bright green colour.	Negative
11.	Flavanoids test	1. Alkaline reagent test. 2. Lead acetate test 3. shinoda test 4.Zinc HCl test	Formation intense yellow colour. No yellow ppt. obtained. Formation of magenta colour. Formation of magenta colour.	Positive
12.	Proteins and amino acid test	Xanthoproteic test	Formation of yellow colour	Positive

Table showing the IC50 concentration of the Test compound, EHE against the Pa-1cells observed after 24hrs and results plotted in Bar graph as below.

Table 10: IC50 concentration of the Test

Sl.No	Cell line	IC50 (ug/ml)
1	Pa-1	57.08

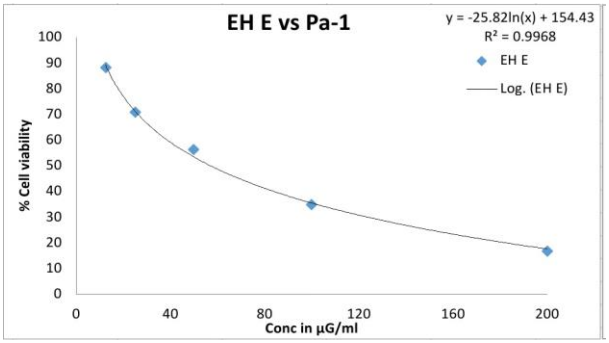
Bar graph depicting the % cell viability of the Test compound, EHE against the Pa-1cells observed after 24hrs.



MTT Assay Calculations

Table 11: Table showing the ASSAY calculations

Concentration Unit: uG/ml		**Incubation:24hrs**						
Parameter	**Blank**	**Untreted**	**Standard**	12.5	25	50	100	200
Abs Reading1	0.048	0.835	0.384	0.735	0.604	0.482	0.335	0.174
Abs Reading2	0.041	0.852	0.396	0.764	0.608	0.506	0.313	0.182
Mean Abs	0.0445	0.8435	0.39	0.7495	0.611	0.494	0.324	0.178
Mean Abs [sample-Blank]		0.799	0.3455	0.705	0.5665	0.4495	0.2795	0.1335
Standard Deviation		0.012020815	0.008485281	0.020506097	0.009899495	0.016970563	0.015556349	0.005656854
Standard Error		0.0085	0.006	0.0145	0.007	0.012	0.011	0.004
Cell viability		100	43.24155194	88.23529412	70.90112641	56.25782228	34.98122653	16.70838548



Graph depicting the % cell viability of the Test compound, EHE against the Pa-1cells.

IC 50 VALUE= 57.08µG/ml

Discussion

The fluorescence analysis and behaviors of powder with different chemical reagents were studied [Tables 2 and 3]. The physicochemical standards are important to check the quality, purity, and adulteration of given crude drug. The foreign matter, loss on drying (LOD), ash, and extractive values were determined and summarized in Table 3. The fluorescence analysis being specific chemical reaction of chemical present in the plant part and different chemicals and combination of chemical. Certain biological active constituents gives the fluorescence, is its marked characteristic. The distinct color appearance in day light and UV light can be used for preliminary identification of specific plant. The ash values of a drug give

an idea of the earthy matter or inorganic composition and other impurities present along with the drug. Extractive values are useful for determination of exhausted or adulterated drug. Thus ash, extractive values, fluorescence analysis will be helpful in the identification and authentication of plant material. The preliminary phytochemical analysis of extract showed the medicinally potential constituents contain alkaloids, flavanoids, carbohydrates, saponins, glycosides, sitosterols, fats and fixed oils, phenols, proteins, aminoacids, tannis terpenoids. The Observations in Statistical data of MTT cytotoxicity study suggesting that in Pa-1 cells, the Test Compound namely EHE showed IC50 value at 57.08ug/ml respectively after the incubation period of 24hrs. The direct Microscopic Observations of Drug Treated Images of Pa-1 cell lines by Inverted Biological Microscope at the magnification of 10x.

Conclusion

Cytotoxicity Study of the Test Compound, EHE against Pa-1 cell lines by MTT Assay was Observations in Statistical data of MTT cytotoxicity study suggesting that in Pa-1 cells, the Test Compound namely EHE showed IC50 value at 57.08ug/ml respectively after the incubation period of 24hrs. The direct Microscopic Observations of Drug Treated Images of Pa-1 cell lines by Inverted Biological Microscope at the magnification of 10x. The Test compound –EHE showed significant cytotoxicity on Human ovarian cancer cell lines, viz., *Pa-1 cells with the IC50 concentrations* (The Concentration of the Compound has the capacity to kill 50% of Viable Cells) at 57.08ug/ml respectively after the incubation period of 24hrs. The obtained results suggesting us that the EHE may have effective anticancer potential against the Human ovarian cancer (Pa-1) cells due to its low IC50 value. However further studies need to be evaluated to confirm the molecular mechanism of action behind the anti-cancer potency of the test compound on Human ovarian cancer (Pa-1) cells.

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Conflict of Interest

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Author Contribution

All authors are contributed equally.

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