1. INTRODUCTION

Breast cancer may be defined as a malignant proliferation of a cellular constituent of the breast.[1] It continues to be the most frequently and top most occurring cancer in women around the world.[2] The increased incidence, mortality, economic costs is a burden shared among women globally. It continues to be a major public health problem in developed as well as developing countries. The report of National Cancer Registry Programme published by Indian Council of Medical Research stated breast cancer to be in list of top most cancers in Indian female.[3, 4] The gynecological cancers accounts for over 50 to 60 percent of all cancers.[4, 5] By 2026, the incidence of cancer might increase to 0.935 million in Indian women, with 2,35,490 (0.235 million) cases of breast cancer.[4]

Epidemiologic studies have identified a number of risk factors that are associated with an increased risk of a woman developing breast cancer. Women who have a first-degree relative (mother or sister) with breast cancer have a twofold to three fold increased risk of developing it. Factors like early menarche, late first pregnancy and menopause, use of oral contraceptives and hormone replacement therapy, alcohol consumption have all been consistently associated with an increased risk of breast cancer.[1] Researchers have also documented extensive genomic abnormalities and aberrations in expression of genes like BRCA 1 and BRCA 2, EGFR/ErbB1 (Epidermal Growth Factor Receptor), HER-2/neu (Human EGFR Related), TNF-α (Tumor Necrosis Factor- α), IGF (Insulin like Growth Factor) and VEGF (Vascular Endothelial Growth Factor).[6] Tumor-specific mutations, DNA amplifications and translocations are also known to distort the normal programs of gene expression and function, resulting in unregulated activity of apoptosis, angiogenesis and cell proliferation.

Primary breast cancer starts with solitary cell which has escaped normal physiology of proliferation and differentiation but they are confined inside the cell layer of duct or lobule. The breast cancer till this stage is known as *in-situ* breast cancer. As the cell multiples, at some time point they will penetrate through the basement membrane of duct or lobule and eventually metastasize to other organs. The cancer of this stage is now called invasive or infiltrating breast cancer. Anatomically, the breast cancer is divided into two subtypes.[7] The cancer arising from duct is ductal carcinoma. 65-90% carcinomas are ductal carcinoma. The ductal carcinoma *in-situ* (DCIS) can go unnoticed and at the time of diagnosis it may have become invasive, known as invasive ductal carcinoma (IDC). The IDC is most common type of cancer, accounting for 8 out of 10 invasive breast cancers. The cancer arising from lobes is known as lobular carcinoma. It accounts for 10% of breast cancer. It can be lobular carcinoma *in-situ* (LIS) or lobular carcinoma invasive (LIC). About 1 in 10 invasive breast cancer is LIC. The less common variants of breast cancers are paget disease of the nipple, inflammatory breast cancer, angiosarcoma and phyllodes tumor. Genetically, the cancer may be subdivided into Luminal or hormone positive breast cancer (i.e. estrogen and progesterone positive), HER-2 positive and Basal or triple negative breast cancer.[8]

Based on the stage of diagnosis, breast cancer is treated with a multidisciplinary approach involving surgery, radiation and systemic therapy including chemotherapy or hormonal therapy.[1, 9] The biology and behavior of breast cancer affects the treatment plan. For both DCIS and early-stage invasive breast cancer, surgery is recommended.[10] Surgical procedures for breast cancer can cause short-term pain and tenderness in the treated area. Also, the skin in the breast area may feel tight, and the muscles of the arm may feel stiff or weak. Surgery involving lymph nodes may cause lymphedema in

later stage of life. Surgery alone may increase the chances of relapse.[11] For larger cancers, or those that are growing more quickly, systemic treatment is given before surgery to shrink the tumor size or after surgery to prevent recurrence.[9, 11] Generally, combination of chemotherapeutic agents are used for early stage and locally advanced breast cancers. The side effects of chemotherapy depend on the individual, the drug(s) used, the schedule and dose used.[1, 9] These side effects can include fatigue, risk of infection, nausea, vomiting, mouth sores, hair loss, anorexia, diarrhea and bone marrow suppression. Hormonal therapy, also called endocrine therapy, is an effective treatment for most tumors that test positive for either estrogen or progesterone receptors.[1] It is a valuable option for the treatment in postmenopausal women. Tamoxifen is the most commonly used drug. Despite the fact that Tamoxifen has been the set up treatment for over 20 years its long haul utilize is related with a few decency concerns and may prompt expanded danger of endometrial malignancy and thromboembolic complexities.[1, 12, 13] Moreover, numerous patients who at first react to treatment with endocrine operators in the long run backslide with safe ailment and develop resistance.[1] Aromatase inhibitors are also used in postmenopausal women but longer use increased odds of developing cardiovascular disease and bone fractures.[14] Radiation therapy often helps to lower the recurrence risk. It can cause side effects, including fatigue, swelling of the breast, redness and/or skin discoloration/hyperpigmentation and pain/burning in the skin where the radiation was directed, sometimes with blistering or peeling.[15]

Thus, surgery, chemotherapy and radiation are effectively managing breast cancer patients. However, plants and plant-derived compounds have furnished tremendous backing in conventional medication framework since antiquated times, and also have been used as source of new potential drugs in modern

pharmaceutical industries. Several new studies have discovered that most patients on cancer therapy are concurrently self-medicating with one or several complementary and alternative medicines.[16] There are approximately more than 3000 plants possessing anticancer potential.[17, 18] Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products (Vincristine, Vinblastine, Paclitaxel, Etoposide etc.) and the plant kingdom has been the most significant source.[19]. Phytoconstituents obtained from the herbs such as *Vinca rosea, Allium sativum, Panax pseudoginseng, Taxus wallichiana, Tinospora cordifolia, Viscum album, Zingiber officinale* have been utilized as a part of various arrangements to help the body to fight malignancy all the more effectively and furthermore diminish the hurtful symptoms of chemotherapy and radiotherapy.[20] All of these documented evidences establish the superiority of a well-chosen drug combination over a single drug in the management of cancer patient.

In light of the above facts it is also observed that chemotherapy has seen a gradual transition from the long and passionately advocated mono-substance therapy toward a multidrug therapy.[21] It is becoming increasingly obvious through observation that many diseases possess a multi-causal etiology and a complex pathophysiology, which can be treated more effectively with well-chosen drug combinations than with a single drug.[22] Parallelism can be drawn from this and can be extended to plant and plant therapy. A living plant is a complex system with thousands of interacting chemicals, and many of them work together in *synergy* – creating a greater impact than the effect of consuming isolated components in single molecule pharmaceuticals.[22] Numerous components present in herbs when given as a whole plant/ plant extracts, forestall harm through multiple actions like advancing detoxification, altering the action of endogenous hormones and chemicals, decreasing deadly

reactions and inconveniences of chemotherapy and radiotherapy. They may help stabilize each other, potentiate or enhance each other, or modify the impact of certain elements. The individual components may help make another constituent present in the plant extract more water soluble or protect it from stomach acids. All of these are ways that the synergies created in taking in a whole plant can produce radically different results than taking the chemically isolated ingredients.[22] These advantages can be extrapolated to toxicity also. Generally substantial decrease in toxicity is observed when whole plant extracts are compared with individual molecules derived from plant. For example, non-glycyrrhizin components of Liquorice extract reduces intestinal absorption of glycyrrhizin thus attenuating toxicity.[22] Pure drugs that are industrially produced or isolated from plants rarely have the same degree of activity as the unrefined extract at comparable concentrations or dose of the active component. This phenomenon is attributed to the absence of positive interacting substances present in the extract.[21, 23] Furthermore, many plants contain substances that inhibit multi-drug resistance (MDR).[23] A further disadvantage is that pure drugs are often more expensive to produce and distribute, and so are often unavailable and/or unaffordable to the poorest populations in remote areas who need them most. In contrast, herbal medicines can sometimes be grown and produced locally, at lower cost, by or close to those who need them. Therefore, using whole plant extract should be approached rather than using isolated constituents.[21-26]

Thus, an attempt was made in the current study to identify an easily available common herb and evaluate its potential in the treatment or prevention of breast cancer. The present study focuses on three plants namely *Butea monosperma* flowers, *Lycopersicon esculentum* fruit and *Cassia fistula* pods.

Butea monosperma (Palash) of Leguminosae family is traditionally employed intensively as folklore remedy for a wide spectrum of liver diseases in India. The century old healing system, Ayurvedic medicine, has utilized flowers, bark, leaves, gum and even the seeds of *B.monosperma* to prepare herbal remedies. Practically every part of *Butea monosperma* have been reported to be associated with various remedial properties such as, anti-diarrhoeal,[27] antiestrogenic activity, [28-31] anti-implantation and anti-ovulatory activity,[32] anthelminthic, bactericidal and fungicidal influence[33] and antitumor property against hepatic carcinoma.[28] The methanol extract of powdered *Butea monosperma* has shown activity against tumor promotion related events of carcinogenesis in rat liver and the protective activity of plant might be due to two major constituents *viz.* isobutrin and butrin.[28, 34] On hepatic carcinoma, treatment with aqueous extract of flowers of *Butea monosperma* inhibited cell proliferation and accumulation of cells in G1 phase.[30] This was accompanied by induction of apoptotic cell death.[29, 30] The important active principles of *B.monosperma* are butein, butrin, isobutrin and chalcones.[28] Butein shows antiproliferative effect on wide range of human tumor cells including breast carcinoma.[29] The plant polyphenol butein inhibits testosterone-induced proliferation in breast cancer cells expressing aromatase.[35] Furthermore, Butein also possess free radical scavenging activity. [29, 30]

Second plant, *Lycopersicon esculentum* (commonly known as tomato; Family: Solanaceae) are one of the most widely used and versatile fruit. Epidemiologic studies suggest that consumption of tomato and tomato-based products reduces the risk of chronic diseases such as cardiovascular disease and cancer. Carotenoids have been found to inhibit the growth of several cancer cell lines including, prostate, lung, mammary, colon and leukemia. [36-40] A diet rich in tomato-based products may help reduce the risk of pancreatic cancer, according

to a study from The University of Montreal.[41] A number of studies have been conducted which indicate that the high levels of lycopene in tomatoes works to reduce your chances of developing prostate, colorectal and stomach cancer.[37, 38, 41] Aqueous extract of tomato is found to reduce the expression of TNF-α and IL-1β in LPS-activated macrophages.[42] Tomatoes contain four major carotenoids: alpha- and beta-carotene, lutein, and lycopene. [41] Lycopene is the best antioxidant amongst all.[41, 43] Studies suggests that tomato extract and lycopene inhibit doxorubicin-induced cardiotoxicity and might serve as a combination chemotherapeutic agent with doxorubicin to limit its cardiotoxic effects.[44] Dietary lycopene and tomato extract supplementations inhibit nonalcoholic steatohepatitis-promoted hepatocarcinogenesis in rats.[45] It interferes in insulin-like growth factor 1 signaling and inhibits VEGF.[46] Studies suggested it to be potent aromatase inhibitor [47] and anti-estrogenic.[48, 49]

Cassia fistula (commonly known as Garmalo, Family: Fabeceae) was found to be potent anticancer agent on human colon cancer cell line.[50] It is widely used in traditional medicinal system of India and has been reported to possess hepatoprotective, anti-inflammatory, antitussive, antifungal and antibacterial. Oral administration of *Cassia fistula* bark extract to DMBA painted animals completely prevented the formation of oral squamous cell carcinoma.[51, 52] Rhein component from flower is found to be anticarcinogenic.[50] Moreover, *Cassia fistula* possess antiestrogenic activity.[53] It also contain lupeol which is proven to be antitumor agent.[54]

With this background, the present study was aimed to evaluate *Butea monosperma* flowers, *Lycopersicon esculentum* fruits and *Cassia fistula* pods in breast cancer.

The objectives of the study were:

1. Evaluating the effect of various extracts on cell viability in human normal epithelial breast cell line *in-vitro* (MCF-10A).
2. Evaluating the cytotoxicity of various extracts *in-vitro* on human breast cancer cell lines (estrogen positive MCF-7, HER-2 positive MDA-MB-453, triple negative MDA-MB-231).
3. *In-vivo* preventive study of two potent extracts in N-methyl- N-nitrosourea (MNU) induced mammary carcinogenesis.
4. *In-vitro* mechanistic assay for angiogenesis (Chick chorioallantoic membrane assay); apoptosis (Annexin V- FITC binding assay), oxidative stress (DCFH-DA assay) and metastasis (Scratch motility assay)
5. Evaluating the curative proficiency of extracts against syngeneic model (Ehlrich Ascites Carcinoma induced solid mammary tumors).