INTRODUCTION

Cancer is a disease that knows no geographic boundaries. Despite researchers investing decades of research and trials in pursuing promising new targets, cancer still remains a cause of mortality. The statistics gathered by American Cancer Society reveals a 2-3% increase in death due to cancer worldwide translating into 3.5 million people annually affected (Karthiresan, 2006). The war against cancer is far from over. The post genomic era has now opened new avenues in cancer treatment, which is contemplated to be more effective and specific for tumor cells. The sequencing of the human genome is likely to speed up the identification of factors involved in cancer pathogenesis and lead to an age of individually tailored anticancer drug therapy. However, about half of the global cancer burden is carried by developing countries that ironically have access to only 5% of the resources available to fight the disease (Pal *et al*., 2003).

Breast cancer as the most frequent tumor disease in women is of special relevance. It is currently found that one woman in every eight will be faced with the diagnosis of breast cancer in her lifetime. The National Cancer Institute estimated that the chance of an American woman developing invasive breast cancer during her life time is about 1 in 8. This risk was about 1 in 11 in 1975

(DeSantis *et al*., 2014). Indian studies reveal a steady increase in breast cancer with over 100,000 new cases reported and detected (Zeleniuch and Roy, 2005). The increase reported by the cancer registries is nearly 12% from 1985-2001, representing a 57% rise in Indians affected with cancer (Farooq and Coleman, 2006; Yip *et al.*, 2006).

The rise of incidence over time also indicates a statistically significant cases of population in India (Yeole and Kiekue, 2003). With particular emphasis on breast cancer, government agencies evaluated breast and cervix cancer in populations based cancer registries which revealed a decrease in cervical cancer but alarming incidence of breast cancer (Yeole, 2008).

The development of breast cancer involves activation and deactivation of several types of genes to protect malignancy (Ingrasson, 2001). At the molecular level, free radicals and aldehydes, produced during chronic inflammation, can induce deleterious gene mutation and posttranslational modifications of key cancer-related proteins.

Among the options to treat cancers, surgery, radiation therapy and chemotherapy are the established therapies. However, hormone and biological therapies are also in use. It is the prerogative of the doctor to either use one or a combination of these methods as the treatment established by the doctor aims on a life-saving. In cases where cancer cannot be completely removed from the patient, controlling the spread of cancer cases is undertaken. Over the time of treatment, radiotherapy is dominant and effective mode although chemotherapy is used in the advanced stages of cancer for rendering metastasis (Somkumar *et al*., 2003; Pandey *et al*., 2006). A major problem with present cancer chemotherapy is the serious deficiency of an active drug without a side effect (Valeriote *et al*., 2002) and tissue damage.

The protection of healthy tissues during chemotherapy has been one of the strong motivations for continuing research on natural products. The development of such anticancer agents provided convincing evidence that plants could be a source of novel chemotherapeutic agents. Search of natural products containing anticancer activities dates back to 1550 B.C., but scientific search has begun only recently with the investigations by Hartwell and co-workers in late 1960s on the application of podophyllotoxin and its derivatives. Over the years, a number of approaches have been developed for clinical use and a number of anticancer drugs have thus been introduced. More than 60% of currently used anticancer agents have been derived in one way or another from natural products (Cragg *et al*., 2003; Balanus *et al*., 2005).

Indian systems of medicine such as Ayurveda, Unani and Siddha have largely depended on a number of plant derived extracts that are used and tested against diseases. This plant based approach mainly is accountable by the

immune modulatory and antioxidant potential leading to anticancer derivatives from medicinal plants. This immune modulatory activity is stimulated by both non-specific and specific immunity (Pandey *et al*., 2005). Dietary natural occurring substances that are absorbed by the human body due to consumption of large amounts of vegetables and fruits are also targeted and identified as potential chemopreventive agents (Vecchia and Tavani, 1998).

The plant-derived natural compounds such as flavonoids, terpenes and alkaloids have been an important source of medicines for various diseases. Studies also reveal antitumour activity of plant based or plant derived products in rodents and cancer cell lines (Lin *et al*., 1996). Several plant products have been tested for anticancer activity and some of them such as vincristine and taxol are now available as a drug of choice.

The advantage of using phytochemical compounds for cancer treatment is their relatively non-toxic nature and availability in an ingestive form. Many of the phytochemicals present in human diet have been identified as potential chemopreventive agents. Although a good number of anticancer agents have been developed from plants or their derived agents, development of a safe, economic and site-specific drug is still a challenge. The searches of anticancer agents' ethno botanically have been based on practice based approach by folklore practitioners that guide the protocols for testing the efficacy of selected plants for modern science.

Acorus calamus is a plant, rich in alkaloids, phenolics and flavonoids and a potential source of compounds possessing beneficial biological activities. It has a long history of medicinal, cultural and ritual use and hence was spread outside its indigenous areas in Asia and now found across Australia, Europe and North America (Motley, 1994). Historical ethno botanical review of *A. calamus* dates back possibly to the time of Moses in Old Testament of the Holy Bible and in early Greek and Roman medicine. It is generally used from ancient and Vedic periods because of its ability to rejuvenate brain and nervous system and to normalise the appetite.

Several previous studies have reported phytochemical composition of *A. calamus* whole plant and its different parts viz., roots, rhizomes, leaves and essential oil (Raina *et al*., 2003). The major compounds identified from this plant are asarones (alpha and beta), caryophyllene, isoasarone, methyl isoeugenol and safrol (Wang *et al*., 1998). The rhizomes, roots and essential oil of *A. calamus* have been reported to possess several biological activities including antifungal (Lee, 2005), antibacterial (Phongpachit *et al*., 2005) and immunosuppressive activities (Mehrotra *et al*., 2003). Rhizome of this plant is widely used in the Indian systems of medicine, such as Ayurveda, Siddha and Unani (Meena *et al*., 2010).

Oxidation is a metabolic process that leads to production of energy required for key cell activities. However, aerobic metabolism in living cells also leads to unavoidable production of reactive oxygen species (ROS) (McCord, 1994; Adegoke, 1998), which are involved in the onset of many diseases (Ames *et al*., 1993). These free radicals attack the unsaturated fatty acids of bio membranes, which results in lipid peroxidation and destruction of protein and DNA, leading to cell inactivation due to a series of deteriorative changes in the biological system. Thus, identification of antioxidants, which can slow the process of lipid peroxidation by blocking the free radical chain reaction, has gained importance in recent years.

Antioxidants are known free radical scavengers that prevent and cure cancer by protecting cells from the damage caused by highly reactive oxygen compounds. Humans are exposed to free radicals in the environment through radiation and pollution and in the body through various metabolic reactions. Antioxidants scavenge these free radicals and enable the cells to rejuvenate or stabilise for the process of life (Murali *et al*., 2011).

A number of reports have shown that flavonoids, alkaloids, phenolics, triterpanoids and steroids of natural products exert multiple biological effects because of their antioxidant and free radical scavenging abilities. These

phytochemical constituents produced protective effects against cancer, tumor, heart diseases and certain other pathologies (Gonzales and Valerio, 2006).

Flavonoids are large family of aromatic plant secondary metabolites synthesised in plants for the protection from photosynthetic stress, ROS and wounds. Studies of flavonoids have produced most compelling data for the anti-tumour activities of plant secondary metabolites in various types of cancers (Yang *et al*., 2001). Due to their antioxidant activity, several flavonoids have been shown to inhibit cancer development in various animal models (Barnes *et al*., 1995; Kuo, 1997; and Lahiri *et al*., 1999).

Dalton's Ascites Lymphoma (DAL) tumorigenesis model in Balb/C/Swiss albino mice provides a convenient model system to study antitumor activity within a short time (Shanker *et al*., 2000). Dalton's Ascites Lymphoma is referred as an undifferentiated hyper diploid carcinoma with high no-regression, transplantable capability, shorter life span, rapid proliferation, 100% malignancy and functions without the tumor specific transplantation antigen (TSTA) (Ozaslan *et al*., 2011). Following transplantation of DAL cells into the abdominal cavity of healthy recipient mice, tumor genesis begins immediately and aggressively. So far, no reports are available on antitumor and anticancer activities of the rhizome of *A. calamus* in DAL-bearing mice. Hence, this study was carried out to gain an insight into the anticancer and antioxidant potential of the methanolic extract of *A. calamus* (MEAC) against DAL cells.

Identification of medicinal plants with significant cytotoxic potential can helps the development of cancer therapeutics and has gained increasing importance in the last decade, and research in this field is expanding (Al-Kalaldeh *et al*., 2010). Human cancer cell lines have aggregated an easily usable set of biological models to examine cancer biology (Green, 2003). The utility of cell lines acquired from tumor allows the investigation of tumor cells in a simplified and controlled environment (Arya *et al*., 2011).

MCF -7 is one of the most frequently used human breast cancer cell lines. It was isolated from pleural effusion of a 69 year old Caucasian woman with

metastatic breast cancer. Several mechanisms of action were detected in MCF-7 cells. To our knowledge, there is no previous reports on the cytotoxic potential of *A. calamus* against MCF -7 cells. Therefore the present study attempts to find the cytotoxic potential of *A. calamus* against MCF- 7 cells.

Traditional herbal medicines are perceived by the public as (relatively) safe. Due to various factors such as adulteration, contamination, plant misidentification, use of substitutes there is a rise in interactions that indicates toxicity of herbs (Zhang *et al*., 2012). Therefore it is important to evaluate the safety and efficacy of *A. calamus* against genotoxic hazards.

Human lymphocytes in culture constitute an ideal test system to evaluate the cytogenetic damage induced by environmental factors (Rossner *et al*., 2005). The use of chromosomal alterations as markers of early biological effects is well established in genotoxicity studies. A relationship between chromosomal damage and cancer development has been suggested since the beginning of the 20th century, but only since 1960 have extensive data been gathered on the frequency of chromosomal alterations (CAs) in Peripheral Blood Lymphocyte Culture (PBLC) of humans exposed to known or suspected genotoxic carcinogens. The idea of causal association between chromosomal alterations and cancer risks based on the concept that genetic damage in lymphocytes reflects similar damage in cells undergoing carcinogenesis.

Essential oils are complex mixtures of volatile organic compounds produced as an end product of secondary metabolism. These oils with standardized content of components have to contain certain chemicals which determine the therapeutic quality. Each component of the essential oils contributes to the beneficial or adverse effects of these oils because the component of each essential oil has different properties and bio availabilities. In this study an attempt was made to screen the volatile constituents of the rhizome of *A. calamus* using gas chromatography and mass spectrometry in order to reveal the scope and applications of *A. calamus* in pharmaceutical field to bring about more drugs and natural products out of it.

Introduction of new drugs and novel therapeutic solutions is a long and costly process (Myers and Baker, 2001; DiMasi *et al*., 2003). Traditionally, pharmacologists strive to optimize and accelerate this process by developing new *in vivo* and *in vitro* investigation strategies. However, the last decades have been witnessing the rise of alternative research models, the so-called *in silico* approaches, using computational environments as their experimental laboratories. Imitating the common biological terms *in vivo* and *in vitro*, the term *in silico* refers to performing experiments using computers.

The majority of the *in silico* methods are primarily used in parallel with the generation of *in vivo* and *in vitro* data for accurate modelling and validation of a wide range of applications from the ligand design and optimization to the characterization of fundamental pharmacological properties of molecules such as absorption, distribution, metabolism, excretion and toxicity (Ekins *et al*., 2007).

Despite the common belief that phytocompounds are safe, they all have inherent risks similar to synthetic compounds. Thus it is within the scope of the phytosciences to elucidate side-effects, appropriate doses, identify bioactive phytocompounds and ways of extraction and conservation. With this regard, the plant extract from *A. calamus* have been taken for this study to inhibit breast cancer diseased proteins such as BRCA1, BRCA2, PTEN, HER2, BRBb2, ATM and CHEK2.

BRCA1 and BRCA2 are human genes that produce tumor suppressor proteins by repairing the damaged DNA and play a role in ensuring the stability of the cell's genetic material. When either of these genes is mutated, or altered DNA damage may not be repaired properly resulting in cellular development of additional genetic alterations that can lead to cancer. The PTEN protein is a lipid phosphatase with putative tumor suppressing abilities, including inhibition of the PI3K/Akt signaling pathway. Inactivating mutations or deletions of the PTEN gene resulting in hyper-activation of the PI3K/Akt signaling pathway are

increasingly being reported in breast cancer and have been related to features of poor prognosis and resistance to chemotherapy and hormone therapy.

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer is highly aggressive and has higher risk of recurrence than HER2-negative cancer. CHK2 is a multiorgan tumor susceptibility gene that encodes for a serine/threonine protein kinase involved in the response to cellular DNA damage. ATM, the protein kinase mutated in the rare human disease Ataxia Telangiectasia (A-T), has been the focus of intense scrutiny over the past two decades as investigating ATM signaling has yielded valuable insights into the DNA damage response, redox signaling and cancer, ATM alterations have been revealed both in the germ line as a predisposing factor for cancer target (Lavin, 2008). ErbB2-overexpressing cancer cells derived from a primary mouse ErbB2 tumor also show HSF1 inactivation and HSP90 client destabilization in response to ErbB2 inhibition. ErbB2-positive breast cancer is characterized by highly aggressive phenotypes and reduced responsiveness to standard therapies.

Docking studies reveal phytochemicals as the long searched anticancer drugs for breast cancer (Ferdous *et al*., 2013). Ligand based drug designing for breast cancer target was analyzed from isolated compounds of *A. calamus* rhizome. Further the study has progressed as Interaction profiling, post docking validation and MD simulation of protein ligand complex. This will impact to know that which protein has higher influence to target and design the drug and also it will pave the way to find the criteria of derivative groups to be interacted with the protein

Considering all the above information, the present investigation has a broader objective of comprehensive evaluation of the anticancer activity of the rhizome of *A. calamus* in three different model systems. The *in vitro* assays were done on the cultured breast cancer cell lines and the cells obtained from the cancer patients. *Mus musculus* was the animal model for the *in vivo* studies on the effects of MEAC. Further, using molecular docking studies an attempt has

been made to identify the active principle that could be used as a potential drug against breast cancer.

Thus, the specific objectives of the present study are to

- Evaluate the *in vitro* cytotoxic activity of MEAC against Dalton's Ascites Lymphoma (DAL) cell lines.
- Carry out *in vivo* studies on the antitumor activity of MEAC on *M. musculus*.
- Estimate the cytotoxic potential of MEAC in breast cancer cell lines (MCF -7) using MTT assay.
- Study the DNA repair proficiency of MEAC on cultured lymphocytes of breast cancer patients using CBMN assay and chromosome sensitivity analysis.
- Evaluate the secondary active constituents from *A. calamus* by GC-MS.
- Identify the active breast cancer targets if any from the isolated secondary compounds of *A. calamus* rhizome by the ligand based drug designing.