ejpmr, 2021,8(2), 272-283

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYTO CHEMICAL EVALUATION AND CYTOTOXIC ACTIVITY OF EMILIA SONCHIFOLIA: RESEARCH ARTICLE

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Article Received on 11/12/2020 **Article Revised on 01/01/2021** **Article Accepted on 21/01/2021**

ABSTRACT

Emilia sonchifolia (Compositae), is a widely distributed herb mainly used in indigenous system of medicine. This study confirmed to assess therapeutic efficacy of *Emilia sonchifolia* against Human Breast Adenocarcinoma. Aqueous extract of Emilia sonchifolia, a folklore medicinal plant, was found to be cytotoxic to MDA MB (Human Breast Adenocarcinoma) cells. The raw material is subjected to phytochemical standardization. The extraction technique used is soxhletion and the results suggests that *Emilia sonchifolia* is a showing a good cytotoxity against Human Breast Adenocarcinoma cell lines by MTT assay.

KEYWORDS: Breast cancer, Breast cancer cell lines like MDA (M.D. Anderson) and MB (Metastasis breast cancer), cytotoxicity, therapeutic efficacy.

INTRODUCTION

1.1 Breast cancer

Breast cancer is a disease in which malignant (cancer) cells form in the tissues of the breast. Breast cancer is the second leading cause of cancer mortality in women. It is both sex-dependent as well as hormonal malignancy whose development is influenced by a myriad of hormones and growth factors.[1,2] The identified breast cancer susceptibility genes that can be inherited play a minor role in most cases. The geographical variation, environment and dietary factors play a significant role in incidences of breast cancer. The oncogenes that are frequently deregulated in breast cancer are ErbB2, PI3KCA, MYC, and CCND1 (encodes cyclin D1).[3] Approximately, ten tumor suppressor genes are involved in the regulation of genomic integrity associated with hereditary breast cancer.[4] BRCA1 and BRCA2 mutations are associated with a significantly elevated risk for breast cancer. Germ-line mutations in ATM, CHK2, NBS1, RAD50, PALB2 and BRIP, all moderately increase breast cancer risk. Among these tumor suppressors, ATM and CHK2 are kinases involved in the DNA damage response.[5,6] Various pathways are deregulated in breast cancer as a consequence of mutations in several genes. These include pathways involving protein and lipid kinases that function in cell growth and survival, the cell cycle machinery, DNA damage response pathways and apoptosis.[4] Several pathways critical to breast tumorigenesis have been identified and effective pharmacological interventions are now available. New strategies such as targeting of DNA repair pathways, reactivating p53 and inhibiting cancer stem cells are being developed to prevent cancer re-occurrence.[7,8]

1.2 Requirements of new molecular targets for breast cancer chemoprevention

Many molecular pathways and the correlated drug targets are actually involved in the development of advanced cancer therapy, and have the potential activity and tolerability even in cancer chemoprevention setting. The identification of new potential molecular targets and the development of agents aimed at these targets within cancer have already had a significant impact on advanced cancer therapy and provide a wealth of opportunities for chemoprevention.[9] The transcription factors and their associated regulatory proteins are always an ideal target of chemoprevention, and in particular three attractive pathways as the nuclear factor –κB (NF-κB), hypoxia-inducible factor 1α (HIF-1α), and PI3κ- mTOR are well studied.[10-12] Experimental studies have shown that natural antioxidant compounds including isoflavones, indole-3-carbinol (I3C), 3,3-diindolylmethane (DIM), curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, curcumin and others seems to be able to inhibit the activity of NF-κB and the growth of cancer cells and also to induce apoptosis, suggesting that NF-κB could be a target for cancer prevention.[13-15]

Oral bioavailability has been encountered for many naturally occurring chemo preventives e.g. EGCG, resveratrol, curcumin and ellagic acid. These drugs possess poor biopharmaceutical properties with low oral bioavailability, limited either by poor aqueous solubility and/or permeability for absorption into the systemic

circulation.[16,17] Thus advanced drugs have been required to circumvent their bioavailability issues.[18]

In conclusion, the success of chemo preventive approach depends on a tumor-specific risk model for identifying high risk subjects, increasing preclinical drug test over the development of novel and more safety chemo preventive agents, and identifying new surrogate endpoint by using molecular pathways and new targets of drugs activity. Safety is a very important point to take into account, because several large randomized prevention trials in several cancers have shown that major adverse events can prevent widespread public acceptance of active chemoprevention agents.

Despite the success of action showed, for example; endocrine intervention is a promising starting point in order to continue to evolve with the rapid integration of molecular approaches into research and clinical practice. It is urgent to find active agents in other fields as non-hormone responsive lesions. The personalized approaches in advanced cancer therapy and the evolution of molecularly targeted will streamline chemoprevention research and facilitate the development of rational, effective, and safe preventive drugs, involving different pathways and with the ability to modify carcinogenesis in early phases.

1.3 Bioactive compounds

More than half of the clinically used anti-cancer drugs are either direct bioactive compounds or derived from bioactive compounds. Some of the well-known bioactive compounds and their potential use in varieties of cancer are stated below.

1.3.1 Lycopene

An acyclic, non-provitamin, a carotene, lycopene is the red pigmented carotenoid found in tomatoes and in some other edible fruits such as watermelon and papaya.[19] Lycopene possesses potent antioxidant activity due to its extended conjugated hydrocarbon chain and has been known to induce apoptosis and inhibit cell cycle progression in various cancer cells like prostate, breast, lung etc.[20-22] Inhibition of cell cycle progression in breast and endometrial cancer cells was associated with reduction of cyclin D1 levels and retention of p27Kip1 in the cyclin E-cdk2 complexes. The efficacy of lycopene was also reported against xenograft tumors in a number of in vivo studies including prostate, lung and mammary tumors.[23] In vivo animal studies and phase I and II clinical trials have shown that lycopene supplements are non-toxic and also orally bioavailable. Further clinical trials are required to establish the efficacy of lycopene supplementation.

1.3.2 Curcumin

Curcumin, a dietary polyphenol, isolated from turmeric (Curcuma longa) has anti-inflammatory, anti-oxidant activity and studied as a chemoprevention agent in several cancer models including breast cancer. Curcumin can target the genome (DNA) messengers (RNA) and enzymes (Proteins) within the cells either sequentially or simultaneously. Curcumin exhibits pleiotropic properties that involve the modulation of nuclear factor-kappaB (NF-κB), transcription factor activator protein-1 (AP-1), mitogen-activated protein kinase (MAPK), tumor protein 53 (p53), nuclear β-catenin signaling, serine/threonine protein kinase (AKT) signaling and also the Wnt signaling pathways demonstrated that curcumin targets the breast stem/progenitor cells, as evidenced by suppressed mamosphere formation and by a decrease in ALDH-positive cells (a breast stem cell marker aldehyde dehydrogenase). Curcumin in clinical studies is hindered largely by its poor solubility, rapid metabolism or a combination of both, resulting in poor bioavailability upon oral administration.

1.3.3 Resveratrol

In last decade, polyphenolic stilbene compound, resveratrol from a wide variety of plants such as grapes, berries, plums and peanuts has been shown to possess chemopreventive and chemotherapeutic potential against human cancers. It exhibited inhibitory effect on the proliferation of various human cancer cells and on the carcinogenesis in animal models. In breast cancer cells the phosphorylation of Akt was significantly reduced followed by decreased pro-caspase-9 activation. Also, resveratrol-induced apoptosis in human breast cancer cells, MCF-7 and MDA-MB-231, occurs concomitant with ERK1/2 and AP-1-dependent nuclear accumulation of COX-2 which is co-localized with Ser(15)-phosphorylated p53 and p300 a co-activator for p53-dependent gene expression. The abrogation of ERK1/2 activity leads to inhibition of interaction of COX-2/p53/p300 and subsequent resveratrol induced apoptosis. A significant decrease in extracellular levels of vascular endothelial growth factor (VEGF) has been associated with apoptosis following resveratrol treatment in breast cancer cells. This suggests that resveratrol affects cancer cell progression by acting on inflammation-related proteins in a cell context-dependent manner. Resveratrol inhibits cancer metastases both in vitro and in vivo by decreasing matrix metalloproteinases (specifically MMP-9, gelatinase-B) activity that degrades

1.4 Emilia sonchifolia

Plants are invaluable in the search for new drugs. There is a tremendous historical legacy in folklore uses of plant preparations in medicine. Scientific studies on plants used in ethno medicine led to the discovery of many valuable drugs. Some examples of plant derived drugs are taxol, camptothecin, vincristine and vinblastine. Emilia sonchifolia (L.) DC (Compositae), a herbaceous plant, is found in India and other countries in Asia. It is used as folklore medicine against inflammation, rheumatism, cough, cuts and wounds in India.[26] In China, the leaves were used in fever and dysentery. It is also used as an analgesic agent and antibiotic. The aqueous extract of this plant showed antimicrobial

activity. The aerial part of the plant contains alkaloids, and flavanoids. Plants belong to Compositae are reported to have anticancer properties. In the present project, we describe the cytotoxic and antitumor properties of E. sonchifolia.

Literature survey

Review of literature is backbone of research, through literature we get many ideas and knowledge of work done and recent researches also many literature were referred but these are the summary of some important literature.

Satoskar. D *et al.,* (2011) reported Chemical preservatives have been used in the food industry for many years. However, with increased health concerns, consumers prefer additive-free products or food preservatives based on natural products. This study evaluated antimicrobial activities of extracts from Emilia sonchifolia L. (Common name: lilac tassel flower), Tridax procumbens L. (Common name: tridax daisy) and Vernonia cinerea L. (Common name: Sahadevi), belonging to the Asteracea family, to explore their potential for use against general food spoilage and human pathogens so that new food preservatives may be developed. Three methanol extracts of these plants were tested in vitro against 20 bacterial species, 3 yeast species, and 12 filamentous fungi by the agar diffusion and broth dilution methods. The V. cinerea extract was found to be most effective against all of the tested organisms and the methanol fraction showed the most significant ($p < 0.05$) antimicrobial activity among all the soluble fractions tested. The minimum inhibitory concentrations (MICs) of extracts determined by the broth dilution method ranged from 1.56 to 100.00mg/mL. The MIC of methanol fraction was the lowest in comparison to the other four extracts. The study findings indicate that bioactive natural products from these plants may be isolated for further testing as leads in the development of new pharmaceuticals in food preservation as well as natural plant-based medicine.[24]

Shao RG *et al.,* (1997) Emilia sonchifolia (Lin) DC (compositae) has found various medicinal uses in folkloric medicine, as a cure for various ailment such as sore-throat, tonsillitis, styptic, vulnery, wounds healing, stomach ache, conjunctivitis, depurative, infantile tympanitis, anticonvulsants, bowel complaints and sores; aqueous extract is used to treat internal heat among pregnant women. The need to pharmacologically establish these claims stimulated this investigation of the plant. The LD50, preliminary phytochemical screening, anti-inflammatory and analgesic potentials of the methanolic extracts of Emilia sonchifolia (ES) were investigated in mice using carragenin, egg albumin, capsaicin-induced paw oedema, formalin-induced paw licking, acetic acid induced writhing and hot plate nociception in mice. The LD50 i.p. was calculated to be 2874.02mg/kg; phytochemical screening revealed the presence of terpines, flavonoids, tannins, saponins and alkaloids. The extract (287.4, 574.8, 862.2, ASA (100mg/kg) and 574.8 + ASA (mg/kg) (i.p.) produced a dose dependent ($p<0.05$-0.001) inhibition carragenin, egg-albumin, capsaicin, formalin-induced paw licking, acetic acid-induced writhing and hot plate nociception in mice. Preliminary phytochemical screening revealed the presence of flavonoids, tannins and alkaloids. Results from this study show that ES may be useful as anti-inflammatory and analgesic agent.[25]

Tang L *et al.,* (2005) designed to investigated the comparative study of the thrombolytic activity and phytochemical analysis of the methanolic extract of leaves of Wedelia chinensis Osbeck. Merr, Eclipta alba (L) Hassk., Emilia sonchifolia (L.) DC. and Spilanthes paniculata Wall. (All of which have the same family-asteraceae) were determined. From our study we also found that Wedelia chinensis Osbeck. Merr., Emilia sonchifolia (L.) DC., Eclipta alba (L) Hassk. and Spilanthes paniculata Wall. Showed 24.48%, 28.71%, 15.19% and 42.77% clot lysis activity respectively and they showed significant % of clot lysis effect with reference of Streptokinase (71.43%) and water (2.96%). The phytochemical analysis showed the presence of different phytochemicals of different fractions of solvents. It is concluded that the in-vitro thrombolytic activity showed by the plants were due to the presence of these phytochemicals. Further studies are highly needed for further drug development.[23]

Lian F *et al.,* (2007) investigated the Whole plant genuine extract of E.sonchifolia (25mg/kg body weight) was administered intraperitoneally to C57BL/6mice. Animals were sacrificed on 21stday after tumour induction and the lung tumour nodules were counted. Various lung and serum biochemical parameters along with major cytokine levels were recorded. Survival rate was monitored. Histopathology of the lung tissue andexpression studies of the major genes involved in metastasis was also carried out. E. sonchifolia significantly inhibited pulmonary tumour formation and increased the lifespan of animals. Lung collagen hydroxyproline, uronicacid, hexosamine, serumsialicacid, γ-glutamyltranspeptidase, vascularendothelialgrowthfactor (VEGF), granulocyte mon cytecolony stimulating factor and other cytokine levels were significantly lowered in the treated group of animals. Histopathological analysis was also correlated with these findings.[22]

Nahum A *et al*., (2001) had isolated flavonoid fraction from E. Sonchifolia (Whole plant) and reported TBARS, SOD, GPx, GR, GST, GSH levels. The results obtained show that the flavonoid fraction from E. Sonchifolia is a potent inhibitor of peroxidative damage and can be used as a therapeutic agent.[21]

Van Breemen RB *et al.,* (2008) had investigated the effect of an active fraction from Emilia sonchifolia, belonging to the family Asteraceae, a plant well known

for its anti-inflammatory and antitumor effects, on the inhibition of tumor-specific angiogenesis. Administration of the active fraction from E sonchifolia (AFES; 5 mg/kg, body weight, intraperitoneally) containing the major compound γ-humulene significantly inhibited B16F10 melanoma-induced capillary formation in C57BL/6 mice. The level of serum vascular endothelial growth factor and serum proinflammatory cytokines such as interleukin-1β, interleukin-6, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor were also reduced significantly. At the same time, administration of AFES significantly enhanced the production of antiangiogenic factors such as tissue inhibitor of matrix metalloproteinase-1. Dose-dependent reduction can be seen in the budding and expansion of microvessels from rat thoracic aorta by AFES treatment. Inhibition of the activation of proenzyme to active enzyme of matrix metalloproteinase along with a successful reduction of proliferation, invasion, and migration of human umbilical vein endothelial cells demonstrated the antiangiogenic effect of AFES in vitro. To date, no study has examined the antiangiogenic activity of this plant with already well-known anti-inflammatory and antitumor effects. Results obtained in the present study by using both in vivo and in vitro angiogenic models altogether proved the inhibitory effect of AFES on tumor-specific neovessel formation.[19]

Bansal SS *et al.*, (2012) reported Methanolic extract of *Emilia sonchifolia* (Compositae), a folklore medicinal plant, was found to be cytotoxic to Daltons lymphoma (DL), Ehrlich ascites carcinoma (EAC) and mouse lung fibroblast (L-929) cells, but not toxic to normal human lymphocytes, under in vitro conditions. Oral administration of the extract (100 mg:kg, b. wt) to mice reduced the development of both solid and ascites tumors and increased the life span of these tumor bearing mice. Further, the extract inhibited DNA synthesis as judged from a reduction in tritiated thymidine incorporation into DL cells under in vitro conditions.[18]

Theoritical analysis

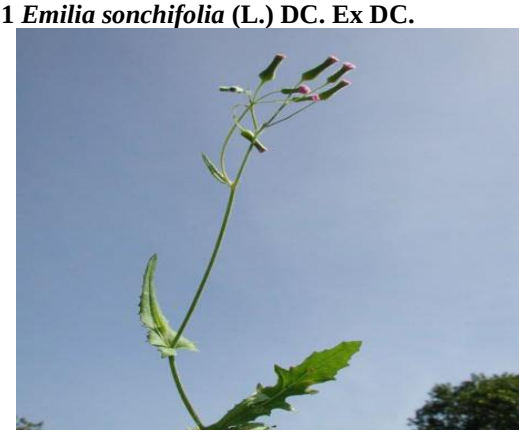
3.1 Scope

Emilia (family Compositae, about 100 species) plants are distributed widely in tropical and subtropical regions, especially in Asia, Africa and America. Some of them have long been used as medicinal herbs, such as Emilia sonchifolia (L.) DC. The annual herb, known as ‘‘Yi Dian Hong’’ or ‘‘Zi Bei Cao’’ in Chinese, is usually found in south China. In folk medicine, E. sonchifolia has been used for the treatment of inflammation, rheumatism, dysentery, urinary infections, etc. Modern pharmacological investigations showed the herb had cytotoxic, anti-inflammatory, antioxidant, antinociceptive and anticonvulsant effects, etc. Previous phytochemical studies on this plant led to isolation of some flavonoids, alkaloids, coumarins, phenylpropanoids, fatty acids and a few sterols. As a part of our work on searching for cytotoxicity of whole plant of E. sonchifolia towards breast cancer cell lines.

3.2 Plant profile

Botanical information of the selected plants as follows

3.2.1 *Emilia sonchifolia* (L.) DC. Ex DC.



Taxonomy

Kingdom	: Plantae
Order	: Asterales
Family	: Asteraceae
Genus	: Emilia
Specific epithet	: sonchifolia
Botanical name	: *Emilia sonchifolia (L.)DC. Ex DC.*

Vernacular names

Sanskrit-Sasasruti
Bengali– Sadimodi
Hindi–Hirankuri
Tamil– Muyarsevi
Telugu –Sadamandi
English – Purple Sow Thistle

Habitat

Common in moist places like bunds of paddy fields, road sides and cultivated lands. Yerpedu, Chandragiri, Tirumala, SVU Campus in Tirupati.

Parts used Whole plant

Chemical constituents

Onion bulbs contain a volatile oil with sulphurous constituents, including allylpropyldisulphide; sulphur containing compounds, including allicin, alliin; flavonoids; phenolic acids and sterols.

Pharmacological action

In India it is used in folklore medicine, against inflammation, rheumatism, cough, cuts and wounds. In China, the leaves were used in fever and dysentery. It is also used as an analgesic agent and antibiotic. The aqueous extract of this plant showed and microbial activity. The aerial part of the plant contains alkaloids and flavanoids. Plants belonging to Compositae showed antioxidant, antitumor and anticarcinogenic propemes.

3.3 Plan of work

The work was proposed to be carried out on following stages-

Stage 1: Literature Review
Stage 2: Selection and Authentication of Plant material
Stage 3: Standardization of Raw Materials
- Extractive values
- Foreign Matter
- Ash values
- Phytochemical studies

Stage 4: Extraction
Stage 5: *In-vitro* cytotoxicity studies using MTT Assay.

Experimental investigations

4.1 Plant materials

Emilia sonchifolia (L.) Whole plant

4.2 Chemicals and reagents

All chemicals and reagents used were analytical grade.

4.3 Equipments

- Vacuum oven –S.D India of Corporation
- Water bath-S.D India of Corporation
- Electric furnace-A.R.scientific company
- Centrifuge Remi instrument Ltd.
- Microscope – Labomed USA
- Digital Balance - Citizen Ltd.
- Hot air oven - spectra scientific.
- P^H meter – CD instruments, Mumbai.
- Heating Mantels - AR scientific company, Banglore.
- Auto analyzer – Biolis 24i
- BOD incubator - AR scientific company, Banglore.
- Auto clave - AR scientific company, Banglore.

4.4 Collection & Authentification of selected plant materials

The whole plant material *Emilia sonchifolia* was collected from Chandragiri, Andhra Pradesh and authentified by Dr.K.Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi.

4.5 Standardization of raw materials

4.5.1 Determination of Extractive values[27]

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists. The extraction method involves, hot extraction and cold maceration. Different solvents like water, alcohol and ether were used.[28]

This technique determines the amount of active constituents in a drug when extracted with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents.

Types of extractive values

- Water soluble extractive
- Alcohol soluble extractive

Significance

- It is used to give preliminary information on quality of drugs.
- Water soluble extractive gives information about the drugs containing tannins, sugars and plant acids.
- Alcohol soluble extractive gives information about the drugs containing resins and tannins.
- Ether soluble extractive gives information about the drugs containing fixed oils and coloring matter presents.
- Hexane soluble extractive gives information about the drugs containing fatty materials.

4.5.1.1 Water soluble extractive

5 g of the air dried coarse drug powder taken in a Stoppard flask and macerated with 100 ml of water for 24 hours, shaking frequently during every six hours and allowed to stand for 24 hours. It was then filtered rapidly taking precaution against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom dish and dried at 105°C to constant weight and weighed. The percentage of the water soluble extractive was calculated with reference to the air dried drug.[27]

4.5.1.2 Ethanol soluble extractive

5g of the air dried coarse drug powder taken in a stoppered flask and macerated with 100 ml of ethanol of the specified strength, in a closed flask for 24 hours, shaking frequently during every six hours and allowed to stand for 24 hours .It was then filtered rapidly taking precaution against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom dish and dried at 105°C to constant weight and weighed. The percentage of the ethanol soluble extractive was calculated with reference to the air dried drug.[27]

4.5.2. Determination of foreign matter

Weigh 100 to 500 gms, or the quantity specified in the individual monograph, of the original sample and spread it out in a thin layer. Inspect the sample with the unaided eye or with the use of a 6x and separate the foreign organic matter from the weight of the drug taken. Use the maximum quantity of the sample for coarse or bulky drugs.

Significance

To identify moulds, insects or other animal contamination

4.5.3 Ash value[28]

The ash remaining after ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash.

- Total ash
- Acid insoluble ash
- Water soluble ash.
- Sulphated ash

Significance

- Find out the excess of calcium oxalate crystals present in a drug.
- To find out adulteration and determine the silica impurities admixed with the drug.

4.5.3.1 Total ash

Weighed accurately 2 g of the air dried crude drugs in tarred silica dish and incinerated at a temperature not exceeding 450°C until free from carbon then cooled and weighed. This was repeated till the constant weight was obtained. The percentage of ash with reference to the air dried drug was calculated.[28]

4.5.3.2 Acid insoluble ash

The ash obtained in the total ash was boiled with 25 ml of dil.Hcl for 5 minutes. The insoluble ash was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred to a tarred silica crucible incinerated at the temperature 650°C until free from carbon. The crucible was cooled and weighed. The entire procedure was repeated till a constant weigh is observed. The percentage of the total ash was calculated with reference to the weight of the air dried drug.

4.5.3.3 Water soluble ash

The ash obtained in the total ash was boiled with 25 ml of water for 5 minutes. The insoluble ash was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred to a tarred silica crucible incinerated at the temperature 650°C until free from carbon. The crucible was cooled and weighed. The entire procedure was repeated till a constant weigh is observed. The percentage of the total ash was calculated with reference to the weight of the air dried drug.[27]

4.5.3.4 Sulphated ash

The obtained in the total ash was treated with Conc. Sulphuric acid and kept at 850°C, until free from phosphates and silicates. the crucible was cooled and packed. The entire procedure repeated until constant weight. The percentage of sulphated ash was calculated with reference to air dried drug.

4.6 Phytochemical evaluation

Qualitative analysis of extracts

Phytochemical studies[28]

Qualitative Phytochemical tests were carried out for all prepared extracts.

4.6.1 Test for alkaloids

To the solvent free extract, 50 mg is stirred with few ml of dilute HCl then shake well and filtered. The filtrate was used for the following tests

4.6.1.1 Dragendorff's test

To the filtrate (0.5 ml), few drops Dragendorff's reagent was added, appearance of orange brown precipitate indicates the presence of alkaloids.

4.6.1.2 Hager's test

To the filtrate (0.5 ml), few drops Hager's reagent was added, appearance of yellow precipitate indicates the presence of alkaloids.

4.6.1.3 Mayer's test

To the few ml of filterate, a drop or two of Mayers reagent added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

4.6.1.4 Wagner's test

To the few ml of filtrate, a drop or two of Wagners reagent added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

4.6.2 Test for glycosides

General test for glycosides

Coarsely powdered plant material 1 gm was introduced in to two different beakers. To one of the beaker sulphuric acid (5 ml) was added while water (5 ml) was added to the other beaker. The two beakers were heated for 3 minutes and the contents filtered in to labeled test tubes. The filterate was made alkaline with sodium hydroxide (0.5 ml) and allowed to stand for 3 minute. The presence of reddish brown precipitates in the filterate was taken as positive for glycosides.

For detection of glycosides

50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hrs. On a water bath, filtered and the hydrolysate was subjected to the following test.

4.6.2.1 Borntrager's test

The filtrate (0.5 ml) was boiled with (0.5 ml) dilute H_2SO_4 and filtered. To the cold filtrate an equal volume of benzene-chloroform was added. To the separated organic layer ammonia solution (0.5 ml) was added, appearance of pink or red colour in the ammonia layer indicates the presence of glycosides.

4.6.2.2 Legal's test

50 mg of the extract was dissolved in pyridine; sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycosides is indicated by the pink color.

4.6.3 Test for the steroidal glycosides

Preparation of test extract solution

The extract was prepared with water and 1 volume of 10% v/v sulphuric acid solution was added. The mixture was heated on the water bath for half an hour and the hydrolyzed extract was extracted with the $CHCl_3$. The $CHCl_3$ layer was separated and concentrated. The test for steroid /phytosterol was carried out on the concentrated fraction.

4.6.4. Test for Steroids / Phytosterol

Preparation of the test extract solution

The extract was refluxed separately with alcoholic solution of pottesium hydroxide till complete

saponification. The saponified extract was diluted it water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in chloroform.

4.6.4.1 Salkowski reaction
To the 0.5 ml of test sample solution 0.5 ml of chloroform and con.H_2SO_4 was added and shaken well. Appearance of red color in the chloroform layer and appearance of yellow fluorescence in the acid layer indicates the presence of steroids.

4.6.4.2 Liebermann –Burchard reaction
To the test sample solution, 1 ml of chloroform and 1 ml of acetic anhydride was added and mix well. To the above solution 2 drops of conc.H_2SO_4 was carefully added along the side of test tube. Appearance of red, then blue and finally green color indicates the presence of steroids.

4.6.5. Test for phenolic compounds
4.6.5.1 Ferric chloride test
To the samples solution, few drops of ferric chloride solution were added. Appearance of green colour indicates the presence of phenolic compounds.

4.6.5.2 Lead acetate test
To the 0.5 ml of sample solution, 0.5 ml of 10% lead acetate solution was added. Appearance of white precipitate indicates the presence of phenolic compounds.

4.6.6. Test for flavonoids
4.6.6.1 Shinoda test
To the sample solution (0.5 ml), 0.5 ml of 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings were added. Appearance of pink color indicates the presence of flavonoids.

4.6.6.2 Zinc test
To the sample solution (0.5 ml), 0.5 ml of dilute HCl & zinc dust was added. Appearance of pink color indicates the presence of flavonoids.

4.6.6.3 Alkaline reagent test
To the sample solution (0.5 ml), few drops of 10% ammonium hydroxide solution was added. Appearance of yellow fluorescence indicates the presence of flavonoids.

4.6.7. Test for carbohydrates
The extract is dissolved in 5 ml of water and filtered. The filterate is subjected to the following test:

4.6.7.1 Molish test
To 2 ml of filterate, two drop of alcoholic solution of α-napthol was added, this mixture is shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tubes and allowed to stand. A violet ring indicates the the presence of carbohydrate.

4.6.7.2 Fehling's test
1 ml of filterate was boiled on the water bath with each 1 ml of Fehling solution A and B. A red precipitate indicate the presence of sugar.

4.6.7.3 Benedict's test
To 0.5 ml of the sample solution, 0.5 ml of Benedict's reagent was added. The mixtures were heated on boiling water bath for 2 min. A characteristic red color precipitate indicates the presence of sugars.

4.6.7.4 Barfoed's test
To 1 ml of filterate, 1 ml of Barfoed's reagent was added and heated on the boiling water bath for 2 min. Red precipitate indicates the presence of sugar.

4.6.8. Test for Saponins
1 ml of the sample solution was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

4.6.9. Test for Protein /Amino acids
The extract is dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filterate is subjected to test for protein and amino acids.

4.6.9.1 Ninhydrin test
Two drops of freshly prepared 0.2% ninhydrin reagent was added to the extract and heated. Development of blue color indicates the presence of proteins, peptides or amino acids.

4.6.9.2 Biuret test
An aliquot of 2 ml of filterate was treated with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol was added, followed by excess of KOH pellets. Pink color in the ethanolic layer indicates the presence of protein.

4.6.9.3 Millon's test
To the 2 ml of filterate, few drops of millon's reagent are added. A white precipitate indicates the presence of proteins.

4.6.10 Test for triterpenoid
Preparation of test extract solution: The test solution was prepared by dissolving the extracts in $CHCl_3$.

4.6.10.1 Salkowoski test
Few drops of concentrated H_2SO_4 was added to the test solution of the extract, the solution was shaken and on standing lower layer turns golden yellow indicating the presence of triterpenoid.

4.6.10.2 Liebermann – Burchard reaction
To the test sample solution, 1 ml of chloroform and 1 ml of acetic anhydride was added and mixed well. To the above solution 2 drops of Con.H_2SO_4 was carefully added along the side of test tube. Appearance of red, then blue and finally green color indicates the presence of steroids.

4.6.11 Test for Saponin
Preparation of sample
The solution was prepared by dissolving the extract in water.

4.6. 11.1 Foam test
The test solution was shaken vigorously. The formation of foam, which is stable for 15 min, was considered as positive for saponin.

4.7 Extraction
Definition[28]
It is a process of separation of soluble materials from an insoluble residue, either liquid or solid, by treatment with suitable liquid solvent. The process of extraction is controlled by mass transfer. Mass transfer is a unit operation, which involve the transfer of mass of soluble material from solid to a fluid.

There are many different methods of processing medicinal plants for therapeutic uses. Some are extremely basic simply involving the crushing of the fresh whole plants or parts of the plant and using it directly as medicine.

Extraction of herbs[27]
Herbs for decoctions were powdered, with tougher herbs powdered to moderately fine and softer herbs powdered to moderately coarse. The powdered parts of all the raw material were taken in 1 liter round bottom flask and macerated with hydro alcohol Water and Methanol (1:1) by hot decoction extraction method.

4.8 Determination of Percentage yield[27]
The raw material to be extracted is been weighed accurately and after the crude extract is obtained, and its evaporated at as low a temperature as possible until the solvent is removed and its heated on a water bath until the residue is apparently dry. Transfer to an oven and dry to constant weight at 105°C and the product obtained is been weighed. Owing to the hygroscopic nature of certain residues, it may be necessary to use dishes provided with well-fitting covers and to cool in a dessicator.

4.9 *In-vitro* antiproliferative effect determination by MTT assay[29]
MDA MB (Human Breast Adenocarcinoma) cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma aldrich, USA). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS (Foetal Bovine Serum), L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

4.9.1 Cells seeding in 96 well plate
Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

4.9.2 Preparation of plant extracts and compound stock
1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

4.9.3 Antiproliferative evaluation
After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

4.9.4 Antiproliferative assay by direct microscopic observation
Entire plate was observed after 24 hours of incubation in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.[40]

Anticancer assay by MTT method
15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals.

The absorbance values were measured by using microplate reader at a wavelength of 540 nm.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

RESULTS AND DISCUSSION

5.1 Extraction

The extraction was carried out for the *Emilia sonchifolia (L.) Whole plant*, by aqueous Soxhletion method. The yield of individual extract as follows. The percentage yield of plant is given in table no: 1

Table no: 1. Percentage yield of plant extracts.

Extract	Percentage yield	Colour and nature
Emilia sonchifolia	14.05 % w/w	Green solid

5.2 Determination of extractive values

The water soluble extractive, alcohol soluble extractives were performed for all crude drugs and results are tabulated in Table No.2.

Table no. 2: Extractive values.

S No	Parameters	*Emilia sonchifolia*
1	Alcohol soluble Extractives	12.06
2	Water soluble extractives	11.76

5.3 Determination of foreign matter

The Determination of Foreign matter was performed for crude drug and results are tabulated in Table No.3

Table no. 3: Foreign matter.

S. No	Parameters	*Emilia sonchifolia* (%w/w)
1	Foreign matter	2.68

5.4 Determination of Ash values

The ash value viz total ash, acid insoluble and water soluble ash were performed for all the crude drugs and results are reported in Table No.4.

Table no. 4: Ash values.

S. No	Parameters	*Emilia sonchifolia* (%w/w)
1	Total ash	4.35
2	Acid insoluble ash	1.38
3	Water soluble ash	4.23
4	Sulphated ash	3.36

5.5 Phytochemical evaluation

The extracts were subjected to various quantitative phytochemical tests and reports are shown in table no.5.

Table no. 5: Quantitative phytochemical tests.

S. No	Tests	*Emilia sonchifolia (%w/w)*
1	Alkaloids	+
2	Carbohydrates	-
3	Glycosides	-
4	Saponins	+
5	Triterpenes	+
6	Fats & Oils	+
7	Resins	-
8	Phenols	+
9	Tannins	+
10	Flavanoids	+
1	Proteins and Aminoacids	+
12	Gums and Mucilages	-
13	Steroids	+
14	Monoterpenes	+
15	Diterpenes	+

\+ = Present - = Absent

Table no. 6: Determination of cytotoxicity by using MTT Assay.

Sample Concentration (µg/mL)	OD value I	OD value II	OD value III	Average OD	Percentage Viability
Control	0.9576	0.9523	0.9547	0.9549	100.00
Sample					
6.25	0.9097	0.9031	0.9178	0.9102	95.32
12.5	0.8785	0.8524	0.8617	0.8642	90.50
25	0.8368	0.8538	0.8428	0.8445	88.44
50	0.3681	0.3356	0.3229	0.3422	35.84
100	0.2543	0.2874	0.2674	0.2697	28.24

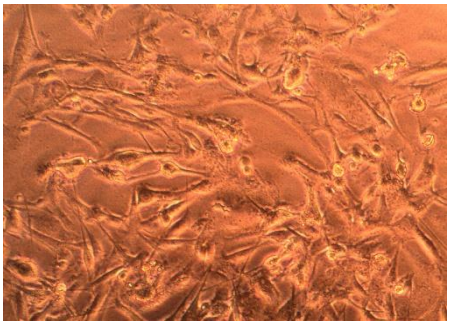


Figure 1.1: 6.25 µg/ml.

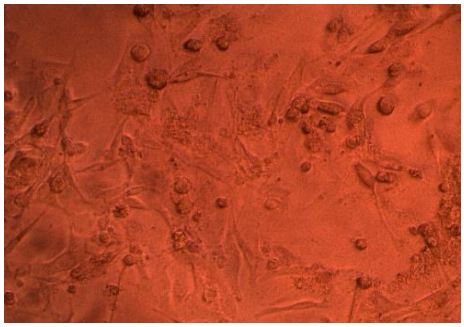


Figure 1.2: 12.5 µg/ml.

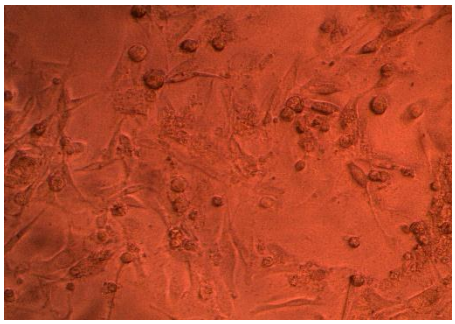


Figure 1.3: 25 µg/ml

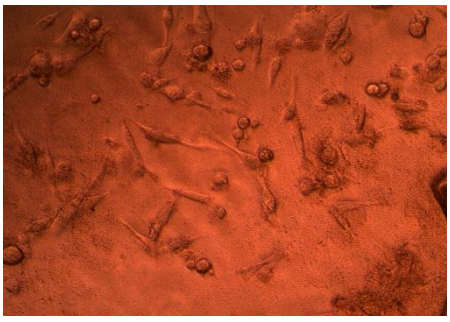


Figure 1.4: 50 µg/ml

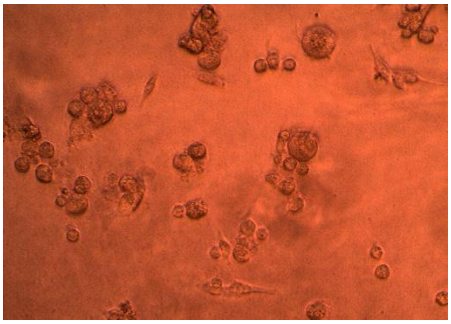


Figure 1.5: 100 µg/ml

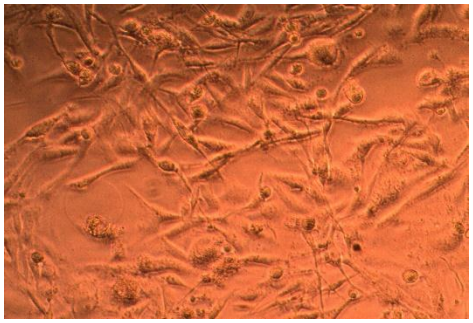


Figure 1.6: Control
MDA MB (Human Breast Adenocarcinoma) cells

SUMMARY AND CONCLUSION

Breast Cancer is one of the most dreaded diseases of the 20th century and spreading further with increasing incidence in 21st century. It is considered as an adversary of modernization and advanced pattern of socio-cultural life. Multidisciplinary scientific investigations are making best efforts to combat this disease, but perfect cure is yet to be brought into the world of cancer medicine. Cancer is an ailment that affects more or less 200 types of cells. There are many difficulties in treatment and more important are of them drug resistance, toxicity, and low specificity. Despite the extensive application of established cancer therapies and the new wave of biotherapies, deaths from cancer are projected to continue rising; an estimated 9 million people are expected die from cancer in 2015. In recent years, a greater emphasis has been given towards research on complementary and alternative medicine that deals with cancer management. Several studies have been conducted on herbs under a multitude of ethno botanical grounds. For example, Hartwell has collected data on about 3000 plants, those of which possess anticancer properties and several of them have been used as potent anticancer drugs. Several of the anticancer drug that are in use are derived from plants. Ayurveda, the traditional Indian medicine has been successful from very early times in using plant drugs for preventing or suppressing various tumors using various lines of treatment. Plant derived extracts containing antioxidant phytoconstituents have been reported to exhibit cytotoxicity towards tumor cells and antitumour activity in experimental animals. There is also supportive evidence that antioxidants enhance antitumor effects of chemotherapy, both in vitro and in vivo. *Emilia sonchifolia* is widely used in folk medicine. Pharmacological studies carried out on these members have reported several activities including anticancer activity but not on breast cancer.

The following are some of the important conclusions made from the present study; Whole plant, *Emilia sonchifolia* were evaluated for its quality by performing foreign matter, ash values and extractive values. It was found that plant is of good quality as seen from their ash values and extractive values and foreign matter. Preliminary phytochemical studies reveal that Alkaloids, carbohydrates, glycosides, proteins are not present in any

of the extract, flavanoids, terpenoids, Steroids and Phenols are present.

The cytotoxicity of *Emilia sonchifolia* after 72 h of cell exposure in medium on MDA MB (Human Breast Adenocarcinoma) cells were studied using MTT assays. The MTT Assay revealed the concentration dependent cytotoxic effects on nanoparticles in concentration range of 6-100 µg/ml. Data reported in Fig. 1 showed a reduction of cell viability after treatment with all samples that increase with sample. In terms of cytotoxicity for HeLa cells lines. The Lethal Dose LD 50 value– 40.0311µg/ml.

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