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ANTI-OXIDANT AND ANTI-CANCEROUS PROPERTIES OF *LANTANA CAMARA* AGAINST MCF-7- *IN VITRO*

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ABSTRACT:

Background: Globally, breast cancer is thought to be the primary cause of cancer-related mortality. Many medical preparations, such as those used in Ayurveda and homoeopathy, are recommended in our traditional systems of medicine to address a variety of human health conditions. Plants with antioxidant properties are a great source for discovering anticancer medications against breast cancer. **Objective:** The purpose of the current study was to examine the anti-cancer and antioxidant properties of an etanolic extract of Lantana camara (LC) leaves against MCF-7. **Materials and Methods:** Total phenolic content of the extract was determined by using folin-ciocalteu reagent. The DPPH and H_2O_2 assay were used to measure the free radical scavenging activity of the LC plant extract. The MTT assay was used to evaluate the cytotoxic efficacy of ethanolic extract of the plant against MCF-7 at various concentrations. **Results:** Based on the result it was found the presence of phenolic content in ethanolic extract of LC leaves. **Conclusion:** According to the current study, LC leaf extract significantly possesses antioxidant and anti-cancer properties against breast cancer cell line. This effect may be attributed to the extract's ability to scavenge free radicals.

Keywords: *Lantana camara*, MCF-7, cancer, Antioxidant, Phytochemical

INTRODUCTION

The most common cancer in women and one that has a high death rate is breast cancer [1]. In 2020, breast cancer claimed 685,000 lives worldwide. Women without any particular risk factors other than age and sex account for almost half of all cases of breast cancer. Worldwide, breast cancer affects people in every nation. Men are affected by breast cancer in a range of 0.5–1% [2]. Chemotherapy, the standard cancer treatment, frequently causes side effects. It is difficult to discover new anticancer compounds that are selective for tumour cells and do not damage normal cells [3]. Many natural resources, such as medicinal plants, are now being investigated in an effort to find novel anticancer drugs with the fewest adverse effects. Numerous active substances have demonstrated the ability to induce apoptosis in a variety of cancer cells [4]. Growth in the fields of transcriptomics, metabolomics, proteomics, and genomics has increased the role of natural products in drug discovery. In order to discover new drugs and drug targets, decipher drug action mechanisms, and keep track of developed drugs and their therapeutic outcomes, metabolic studies are increasingly being used. As a result, it is imperative that safer and more potent anticancer medications be developed right away [5].

Lantana camara: LC belongs to family Verbenaceae[6]. The upright, vigorous *Lantana camara* shrub can reach a height of 2-4m. The leaf measures 2–10 cm in length and 2–6 cm in width, giving it an ovate shape. With the aid of support, it can climb up to 15m [7]. The leaves are tough, green in colour with fine hairs. Its smell is strong. When conditions are right, it grows easily, and the months of March and August are when the flowers typically appear. The fruit has two nutlets and is drupaceous, green in colour. A mature plant can yield up to 2000 seeds per year. With numerous tiny side roots and a main taproot, *L. camara* roots are incredibly robust [8]. The plant has been used to treat a wide range of illnesses in many different parts of the world. *Lantana camara* was used in traditional medicine to treat tumours and cancers. The leaves and flowers were used to make a tea that was used to treat fever, influenza, and stomachaches. The leaves were used as a poultice in Central and South America to treat chicken pox, measles, and sores. Preparations made from the plant were used to treat high blood pressure, rheumatisms, colds, and fevers [9]. Among its many biological properties are fungicidal, insecticidal, nematicidal, antipyretic, antimicrobial, and anti-mutagenic properties. Some of these biological activities may be partially attributed to *Lantana*'s secondary

metabolites, which include flavonoids, phenyl ethanoid glycosides, terpenoids, phenolics, iridoid glycosides, furanonaphthoquinones, and other compounds [10].

MCF-7: It is a human breast cancer cell line having estrogen, progesterone and glucocorticoid receptors. It was developed in 1970 by Dr. Soule of the Michigan Cancer Foundation in Detroit from the pleural effusion of a 69-year-old Caucasian woman who had metastatic breast cancer (adenocarcinoma)[11,12]. MCF-7 cells have been used as models for both in vitro and in vivo studies of the oestrogen response. The precise process by which oestrogen promotes the growth of MCF-7 cells is still being investigated. One important aspect of how oestrogen controls the cell cycle is undoubtedly its regulation of growth factor signalling and action, which was the subject of early reports[13]. The MCF-7 cell line was utilised as a breast cancer model in this study. Because of its long history and oestrogen receptor expression, it is one of the most frequently used breast cancer cell lines in cancer research. Human epidermal growth factor receptor + progesterone receptor + oestrogen receptor indicates that most patients (72.7%) with a known HR/HER status are HR+/HER2- [14]. MCF-7 cells are a highly relevant model for studying invasive breast cancer because they are one of the few HR+/HER2-positive breast cancer cell lines. Post-GWAS analyses based on experimental manipulation in ER+ systems, such as MCF-7, are currently lacking in the literature [15]. Overall, on the basis of these results, here bring into focus that MCF-7 can be used as a model to study the effects of phytochemicals on it.

MATERIALS AND METHODS

Plant material

Lantana camara leaves in good condition were collected from Hamirpur, Himachal Pradesh, India. Leaves were identified and authenticated by Department of Agriculture, Abhilashi Group of Institutions, Mandi, HP.

The preparation of *Lantana camara* leaf ethanolic extracts (LC-LE):

After being cut off from the plant, the LC leaves were shade-dried. The dried leaves then powdered by using mortar and pestle. The powder is stored between 2 and 4 °C until needed. Each of the examined LC's 500 g of powdered leaves was extracted using 95% ethanol through cold maceration until exhaustion. In each instance, the combined ethanol extract was evaporated at 40 °C under reduced pressure until it was completely dry [16].

Cell line culture

The breast cancer cell line MCF-7 was sourced from the National Centre for Cell Science (NCCS), located in Pune, India. The cells (10000cells/well) were grown in DMEM medium supplemented with 10% FBS and 1% antibiotic solution for 24 hours in a 96-well plate. The cells were kept in a humidified incubator with 5% CO_2 at 37ºC in 95% air [17].

Chemicals

Folin-ciocalteu reagent, Na_2CO_3, Gallic Acid, DPPH, Methanol, Ascorbic Acid, Ethylenediaminetetraacetic acid (EDTA), H_2O_2. All the chemicals and reagents used were of analytical grade.

Total Phenol Content Estimation:

Principle:

The Foiln-Ciocalteu phenol reagent is a mixture of hetero-poly phosphomolybdic and phosphotungstic acid, where the tungsten and molybdenum are in 6+ states. When specific reducing agents are used to reduce the material, tungsten blue and molybdenum blue are produced.

Procedure:

The Folin-Ciocalteu reagent was used to determine the phenolic compounds. The test sample dilutions were combined with 40 µl of aqueous Na2CO3 (1.0 M) and 50µl of diluted folin ciocalteu reagent. The reaction mixture was prepared in accordance with the setup table for reaction mixtures, allowed to stand for 15 minutes, and then the double beam JASCO V-630 spectrophotometer was used to measure absorbance at 760 nm. Gallic acid was prepared as a standard curve in a 50:50 v/v methanol:water mixture at concentrations ranging from 25 µg/mL to 300 µg/ml. [18].

DPPH Scavenging Assay:

Principle:

The presence of the picryl group in DPPH solutions causes the deep violet colour of the 1,1-diphenyl-2-picrylhydrazyl molecule to disappear when combined with a material that can donate a hydrogen atom, instead turning it into a pale yellow colour. The powder has a red colour and is stable [19].

$$(DPPH) + H\text{-}A \rightarrow DPPH\text{-}H + A$$

(Purple) (Yellow)

Procedure:

In a 96-well plate, 0.1 ml of 0.1 mM DPPH solution was mixed with 5µl of a distinct stock of the test compound. The reaction was set up in triplicate, and blanks with 0.2 ml of DMSO/methanol and 5 µl of a compound at various concentrations were made in duplicate.The plate was left in the dark for thirty minutes. Using a micro plate reader (iMark, BioRad), the decolorization was measured at 495 nm at the conclusion of the incubation. The control was a reaction mixture that contained 20µl of deionized water. In comparison to the control, the scavenging

activity was expressed as "% inhibition". Utilising Software Graph Pad Prism 6, IC-50 was computed [20]. **Calculations**

DPPH Scavenging activity = ((Abs $_{Control}$- Abs $_{Sample}$)/Abs$_{Control}$)×100

Hydroxyl Free Radical Scavenging Assay:

Principle:

This colorimetric H_2O_2assay includes a full kit for measuring hydrogen peroxide in cell culture supernates, serum, plasma, urine, and other biological fluids. This kit's objective is to quantify low-level H_2O_2 concentrations in biological matrices. A colour reagent reacts with xylenol orange dye in an acidic solution containing sorbitol and ammonium iron sulphate to produce a purple colour that is directly proportional to the amount of H_2O_2 in the sample being tested.

Procedure:

In the 96-well plate, the following were added in order: 10µl of plant extract (concentration as specified in the excel sheet), 24µl of phosphate buffer (50 mM, pH 7.4), 10µl of ascorbic acid (-SD Fine- F13A/0413/1106/62) and 24.14 mg of deoxyribose (SRL-84384), 88µl FeCl3 (Fischer Scientific-Cat no.-23585) (10mg/ml), 28 µl H2O2 (Neurochem Laboratories-HP6520) (6%), water up to 33 ml, and 10µl of plant extract (Concentration as per mentioned in excel sheet). The standard was gallic acid (SRL-Cat no.-5995-86-8) with a concentration as indicated in the excel sheet. Following incubation, 50µl of 1% TBA (HiMedia-Cat no. RM1594) and 10% TCA (Fischer Scientific-Cat no. 28444) were added to each well. A chromogen in pink was created. The absorbance was then measured at a wavelength of 540 nm. [19].

Calculation:

Scavenging activity was calculated by the following formula…

$$\frac{A(control)-A(sample)}{A(control)} x\,100$$

A (control): Absorbance of the control and

A (Sample): Absorbance of the extracts/standard.

In vitro Cytotoxicity Evaluation of ethanoilc extract of LC leaves on MCF-7

Principle:

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, also known as the MTT reagent, is a mono-tetrazolium salt made up of three aromatic rings—two of which are phenyl moieties and one of which is thiazolyl—encircling a positively charged quaternary tetrazole ring core with four nitrogen atoms. The core tetrazole ring is disrupted upon reduction of MTT, and formazan, a violet-blue water-insoluble molecule, is formed [20]. The MTT reagent is reduced to formazan by metabolically active cells and can cross both the cell membrane and the inner membrane of the mitochondria in viable cells, most likely

because of its positive charge and lipophilic structure [21].

Procedure:

MTT Solution (a final concentration of 250µg/ml) was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 660 nm.IC-50 Was calculated by using software Graph Pad Prism -6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS) [22, 23, 24].

RESULTS

Total Phenol Content Estimation:

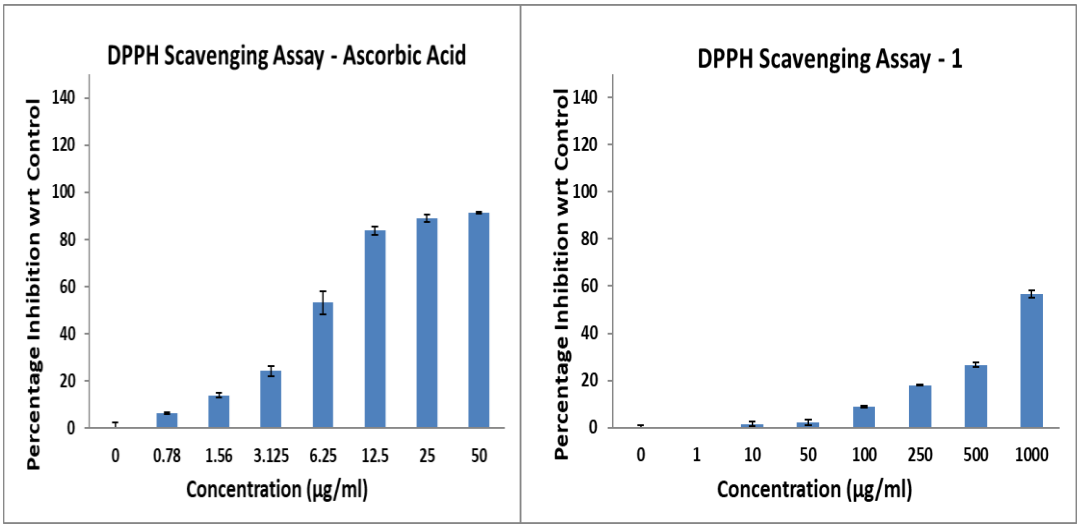
Phenolic compound determination was carried out using Folin ciocalteu reagent. Based on the results obtained from the experimental work it appears that Ethanolic extract of *Lantana camara* (LC) leaves has the content of 352.73µg/mg.

DPPH Scavenging Assay:

Antioxidant property was observed in the ethanolic leaves extract of *Lantana camara*. Antioxidant property (DPPH scavenging) was observed (IC50= 888.1 ± 0.038µg/ml), as compared to standard Ascorbic Acid (IC50=5.662 ± 0.031µg/ml).

Table 1: Antioxidant property of LC using DPPH assay compared to Ascorbic acid.

Sample	IC50 value (µg/ml)
Ascorbic Acid	5.662 ± 0.031
LC	888.1 ± 0.038



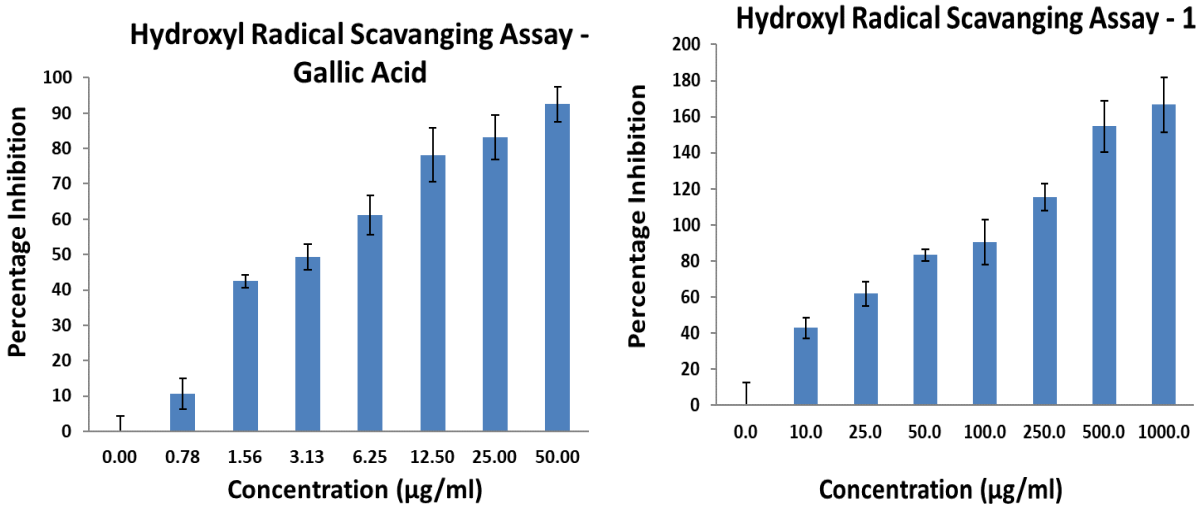
Graph 1&2: Comparative account of Antioxidant property of LC using DPPH to Ascorbic acid.

Hydroxyl Free Radical Scavenging Assay:

Based on the results obtained from the study it was found that *Lantana camara* (IC_{50} = 14.49 ± 0.3655 µg/ml) having hydroxy radical Scavenging ability with respect to standard gallic Acid (3.373 ± 0.06296 µg/ml).

Table 2: Antioxidant property of LC using H_2O_2 assay compared to Gallic acid.

Sample	IC50 value (µg/ml)
Gallic Acid	3.373 ± 0.06296µg/ml
LC	14.49 ± 0.3655µg/ml



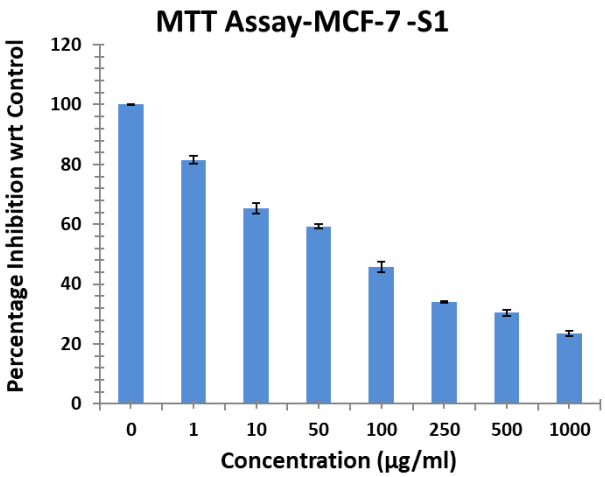
Graph 3&4: Comparative account of Hydroxyl Scavanging of LC using H_2O_2 to Gallic acid.

In vitro Cytotoxicity Evaluation of ethanoilc extract of LC leaves on MCF-7

Cytotoxicity of the provided samples on MCF-7 cell line was determined by MTT Assay. The cells (10000cells/well) were cultured in 96 wells plate for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. Next day cells were treated from of the formulations (different concentrations were prepared in incomplete medium). After incubation for 24 hours, effect of ethanolic extract on MCF-7 cell line was determined by MTT Assay. Based on the results obtained from the MTT assay, it was observed that when the MCF-7 cell line was exposed to different concentrations of the sample, cytotoxic activity was observed (IC_{50} = 94.53 ± 0.1572 µg/ml).

Table 2: Cytotoxic activity of LC extract against MCF-7

Sample	IC50 value (µg/ml)
LC	94.53 ± 0.1572



Graph 5: MCF-7 cell line exposed to different concentrations of LC.

DISCUSSION

One of the main causes of death and multistep development, cancer causes unchecked and fast cell division. There are over 3000 plant species that have been used as anticancer , alkaloids, flavonoids, terpenoids, and saponins [26]. The majority of these metabolites are tiny organic molecules that are frequently very good starting points for medication development. Every year, a large number of new cytotoxic secondary metabolites are discovered; these could be a valuable resource for research into the prevention of cancer [27]. *Lantana camara* L. is used in folk medicine in many parts of the world and is considered both a weed and an ornamental garden plant. The Lantana plant has several parts that are used as carminative, diaphoretic, antiseptic, and antispasmodic. Additionally, it has been reported that *L. camara* leaf extracts have anti-inflammatory, antipyretic, and analgesic properties. Isolated treatments in various countries; plants are a common source of cancer treatment. In the past, plants have been a plentiful supply of reasonably priced secondary metabolites, including phenols, steroids from *L. camara* leaves, verbascoside demonstrated antitumor activity and protein kinase C inhibition [28]. Immune cell activation in the biological system produces a lot of hydroxyl radicals, which are extremely toxic radicals that severely damage all molecules found in living cells. By destroying DNA nucleotides, these radicals can cause mutagenesis and cell toxicity [29]. Consequently, assessing the H_2O_2 radical scavenging activity of *L. camara* leaf extracts prepared using various solvents can yield useful information about their antioxidant potential. The most popular technique for estimating the AOA (anti-oxidant activity) of natural extracts is the DPPH assay, which measures the degree of free radical inhibition

by observing the decolorization of the DPPH solution from deep purple to pale yellow in the presence of a hydrogen donor [30]. The results section reveals that the ethanolic extract of *Lantana camara* contains phenolic components as well as also exhibits that noteworthy antioxidant potential and H_2O_2 scavenging activity. The most popular method for determining the effectiveness of any medication is the MTT assay. Based on the IC_{50} value, **the current research data clearly explains why the *Lantana camara* leaf ethanolic extract has potential anti-cancer activity, as indicated in the results section.**

CONCLUSION

The result of the current study suggests that ethanolic extract of LC leaves has antioxidant and anti-cancerous efficacy against MCF-7 which might be due its free radical scavenging activity. Thus, in conclusion, this study indicates that extract may be considered as an alternative medicinal plant in the treatment of breast cancer.

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CONFLICTS OF INTEREST

All authors declared no conflict of interest.

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