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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical Analysis, Antioxidant Activity and Cytotoxic Effect On MCF-7 Human Cancer Cell Line of Ethanolic Extract of *Lantana camara* (Leaves)

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ABSTRACT

Natural products extracted from plant material have immense opportunity to treat various kind of acute and chronic diseases like cancer. Present day treatment like chemotherapy, radiation therapy have many side effects, on the other hand plant's extracted product are less toxic. In present study we are exploring the therapeutic uses of constitute of Lantana Camara's leaves. Total phenolic content and total flavonoids content obtained ethanolic extract of Lantana Camara were56.46±.88 mg GAE/g and 23.16±1.24 mg QE/g of extracted sample respectively. DPPH IC_{50} was found to be131.52 µg/ml using L-ascorbic acid as a standard.MTT assay was performed using Doxorubicin as a standard, IC_{50} value of lantana extract against MCF7 cell line was obtained 317.85±10.70 µg/ml of extract. IC_{50}value calculated using linear regression analysis.This study shows bioactive compound present in Lantana camara may be useful as a drug against Breast cancer.

Keywords- MTT, DPPH, Cancer cell line and cytotoxicity.

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INTRODUCTION

Lantana Camara is perineal herb can be found on roadsides, can grow up to 5-8 feet. It is Verbenaceae's family shrub in the genus Lantana [1].We are well aware of the value of plants. The plant kingdom is a treasure trove of potential medicines, and there has been a growing recognition of their value in recent years. A collection of therapeutic herbs, plant-based medicines are readily available and they are less expensive, safe, and effective, with few adverse effects. The Medicinal plants that have been chosen over thousands of years are the most apparent choice for analyzing the medicinal uses in the present-day that may lead to the quest for novel medications that are medically useful, such as anticancer drugs. There is a necessity to discover alternative safe anti-cancerous medicines from plant sources because, due to high toxicity with major side effects and resistance against malignant cells with highly specific licensed treatments. Some voluminous contributions have been made to ethnopharmacological research prospects regarding plant based drugs[2]. Some research for the therapeutic and anti-neoplastic activities of medicinal plants is underway [3-4]. As a result, herbal medicines have gotten a lot of interest as anticancer alternative drugs [5]. If a scientific foundation could be developed, plant ingredients would be evaluated as possible novel anticancer medicines or lead toward the creation of new anticancer compounds. In developed nations, 80% of the people use medicines which are derived from plant sources[6].These plants should be explored extensively in order to have a better understanding of their qualities, safety, as well as effectiveness. *Lantana camara* plants contains chemical compounds such as flavonoids, glycosides, phenylpropanoid, lantadene [7]. In this study phytochemical analysis and cytotoxicity assay of ethanolic extract of Lantana camara is carried out.

MATERIAL AND METHODS

Collection of plant material

Fresh leaves of *Lantana camara* were collected from the Sanjivini ayurvedic nursery, Bhopal (M.P.).Plant's leaves were subjected to the shade dried, dried leaves crushed well with pestle and mortor, crushed leaves was stored in airtight glass container for further use.

Preparation of plant extract

Crushed leaves were subjected to the extraction in Soxhlet apparatus. 30gm of crushed leaves are packed in thimble tube and 300 ml of ethanol pour in the bottom flask. Extraction procedure should be continue till the siphon tube of Soxhlet become colorless, the extraction procedure took 18 hrs. Pour ethanol from bottom flask to a beaker and keep the beaker in hot water bath at 40°C till the all ethanol get evaporated and dried crude extract was found [8].

Qualitative Phytochemical Analysis

Extract was subjected to the following test for the presence of phytochemicals [9,10]

Test for protein

Millon'stest

2ml of Millon's reagent mixed with crude extract, on heating red colored appeared, which confirmed the protein presence in the crude sample.

Ninhydrin test

2ml of 0.2% solution of Ninhydrin pour in crude extract, mix well and upon boiling violet color appeared which confirmed the presence of amino acids and protein.

Test for carbohydrates

Fehling's test

1 ml of each Fehling A and Fehling B reagents we remixed together in crude extract and upon heating brick red precipitate observed confirmed the presence of reducing sugars.

Benedict's test

2ml of Benedict's reagent add in crude extract upon boiling a red brown color confirmed the presence of carbohydrate.

Test for glycosides

In the test tube Crude extract was taken to this add 2ml of chloroform and mixed well. To this solution add 2ml of conc. H_2SO_4 was added carefully and shaken. Ared dish-brown color indicated the presence of the glycoside.

Test for phenols and tannins

Add distilled water in Crude extract and warmed to this add 2ml of 2% solution of FeCl3. A formation of blue-green color confirmed the presence of phenols and tannins.

Test for steroid

2ml of chloroform added in crude extract, then 2ml conc. H_2SO_4 was poured along the side of test tube red colored confirmed the presence of steroids.

Test for alkaloids

2ml of 1% HCl added in crude sample heated then add Mayer's and Wagner's reagents. Turbidity indicates the presence of alkaloids.

Test for saponins

In a test tube 5ml of distilled water add in crude extract and mixed well. It was shaken vigorously; formation of stable foam indicated the presence of saponins.

Test for flavonoids

Alkaline reagent test

On addition of 2ml of 2% solution of NaOH in crude extract sample. Deep yellow color was appeared, to this on addition of few drops of diluted acid solution will be colorless, this confirmed the presence of flavonoids.

Shinoda test

fragments of magnesium ribbon mixed with crude extract sample and to this conc. HCl was added drop wise. After few minutes pink color will appeared which indicated the presence of flavonoids.

Quantitative phytochemical analysis

Total phenolic content

Total phenolic content in crude extract was estimated with the folin- ciocalteu (FC) method. 5 ml of 10% FC reagent and 4 ml of 2% sodium carbonate solutions added to 1 ml (1mg/ml) crude extract incubated for 15 min at 45°C. Taken obserbance at 765nm. Gallic acid used as standard in 1mg/ml concentration. Result was calculated using calibrated plot and expressed as gallic acid equivalent (mgGAE/g of extracted sample) [11].

Total flavonoid content

Total flavonoid content in crude was determined by using the aluminum chloride colorimetric method. 1 ml cure extract(1mg/ml) prepared and add 3ml of methanol, 0.2ml of 10% aluminum chloride, 0.2ml of 1M potassium acetate, and 5.6ml of distilled water and remain at room temperature for 30 minutes. Taken obserbance at 420nm. Quercetin used as standard in 1mg/ml concentration. Result was calculated using calibrated plot and expressed as gallic acid equivalent (mgQE/g of extracted sample) [11].

Antioxidant activity

Determination of free radical-scavenging activity using DPPH

3 ml of 0.1mM of DPPH (2,2 – Diphenyl 1- PicrylHydrazyl) solution added to 1ml of various concentration (12.5µg/ml, 25.µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml.) of extract sample and standard sample. L-Ascorbic acid used as standard. Incubate for 30 min at 37°c. absorbance was taken at 517nm.Percentage pf scavenging calculated using following equation [12]

% of DPPH free radical scavenging activity = [(A0-A1)/A0)] *100

where A0 is the absorbance of the control (3ml of DPPH solution) and A1 is the absorbance of extract/standard taken as Ascorbic acid [13].To determine the IC_{50} value (concentration of sample which scavenge 50% of DPPH), graph plotted between % of scavenging versus concentration of sample / standard.

Cytotoxicity Assay (MTT Assay)

The 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of ethanolic extract of leaves of *L. camara* against the MCF-7 cancer cell lines. The cancer cells were seeded at a density of 1x105 cells/ml in complete media in a 96-well plate for this experiment. When cells reaching confluent, the cells were treated with different concentrations of plant extract (400µg/ml, 200µg/ml, 100 µg/ml, 50 µg/ml , 25 µg/ml, 12.5µg/ml) for 48 hr. As a control, DMSO (the vehicles) were used. The medium discarded and using phosphate buffer solution (PBS) adherent cells were washed. 20 µl of MTT stock solution (5 mg/ml in PBS) were added to each well and the plates were further incubated for 15hr at 37 °C. To solubilize the formazan crystals produced by viable cells 100 µl DMSO added to each well. The absorbance was measured at 570 using Microplate Reader.IC_{50} value determined using equation y=50 (50% value) [14-15].

RESULTS

Qualitative Phytochemical analysis

Table 1 showing results of qualitative phytochemicals. Protein, carbohydrates, glycosides, steroids, saponins are appears in modest concentration, while phenols, alkaloids, flavonoids present in high concentration.

Table 1 Qualitative phytochemical analysis of leaf extracts of *L. camara*

Constituents	Presence
Test for protein	+
Test for carbohydrates	++
Test for glycosides	++
Test for phenols and tannins	+++
Test for steroid	+
Test for alkaloids	+++
Test for saponins	++
Test for flavonoids	+++

+++: Strongly positive, ++: Moderately positive, +: Weakly positive

Quantitative phytochemical analysis

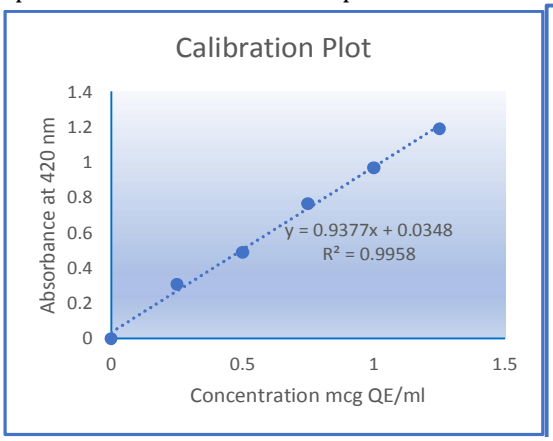
Total phenolic content(TPC)

Result shown in Table3Total phenolic content estimated using gallic acid as standard. Results were calculated using calibration equation y=0.001x+0.0112. TPC value for leaves extract was 56.46±.88(mean ± S.E.M., n=3) mg GAE/g of extracted sample(Graph 1).

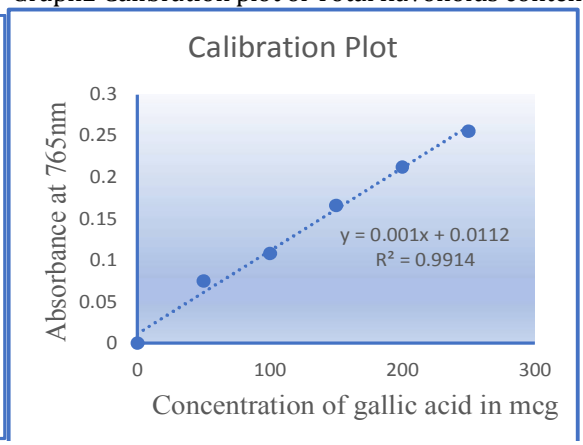
Total flavonoids content (TFC)

Result shown in Table 3Total flavonoids content estimated using Quercetin as standard. Result was calculated using calibration equation y=0.9377x+0.0348. TFC value for leaves extract was 23.16±1.24 (mean ± S.E.M., n=3) mg QE/g of extracted sample (Graph 2).

Graph 1Calibration Plot of Total phenolic content



Graph2 Calibration plot of Total flavonoids content



Determination of free radical-scavenging activity using DPPH

Results were shown in Table 2 express Relative % of scavenging activity of Ascorbic acid and leaf extract of *Lantana camara*. IC_{50} value calculated using equation y=0.2235x+20.605, where y=50 (50%value), (Graph 3). IC_{50} value of scavenging was 131.52 µg/ml(Table 3).

Graph 3
DPPH assay calibration plot

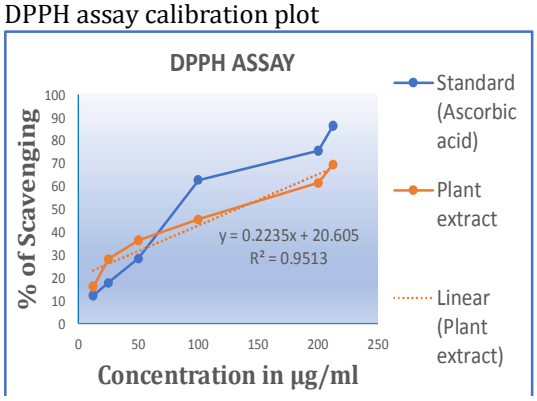


Table 2:
DPPH scavenging activity of leaf extract of L.camara

Conc. (µg/ml)	% of Scavenging of L.Camara extract	% of Scavenging of Ascorbic acid
12.5	12.5	16.4
25	17.96	28.35
50	28.51	36.4
100	62.81	45.54
200	75.62	61.48
400	86.48	69.53

Table 3: Quantitative analysis, antioxidant activity and cytotoxicity assay of leaf extract of L.camara

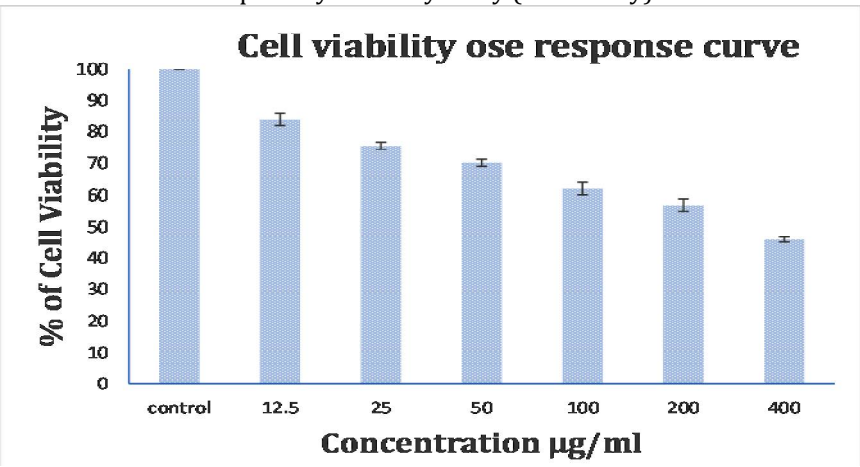
Plant extract	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH assay IC_{50} (µg/ml)	MTT assay IC_{50} (µg/ml)
Ethanolic extract of leaves of L.camara	56.46±.88	23.16±1.24	131.52	317.85±10.70
Logarithm equation	y = 0.001x + 0.0112 R^2 = 0.9914	y=0.9377x+0.034 R^2 = 0.9958	y=0.2235x+20.605 R^2 = 0.9513	y=-0.0854x+77.027 R^2 = 0.8593

Results are shown in Mean ± S.E.M. in triplicate.

Calculation of cell viability

Results of dose dependent % of cell viability shown in Graph 4. IC_{50} value calculate using using calibration plot was 317.85±10.70 µg/ml of extract(Table3). The result was expressed with mean ± S.E.M. (n=3).

Graph 4. Cytotoxicity assay (MTT assay)



DISCUSSION

In this present study phytochemical constitutes were found in ethanolic extract of leaves of *L. camara* , was also reported previously [16-17].Phytochemicals have various pharmological effects on human body [18]. Saponins, are proved to anti-inflammatory, was present in modest concentration. Total phenolic content and total flavonoids content shows the correlation with the scavenging activity which was determined by DPPH assay[19]. DPPH scavenging activity was found in agreement with another study of *L. camara* [20].High scavenging or reducing activity of leaf extract of *L .camara* depends upon the high presence of polyphenolics compounds. Phospholipase A2 and ATPase are membrane bound enzyme these enzyme action inhibited by flavonoids; this may be a reason that flavenoids show antioxidant properties [21]. Extract shows high scavenging activity so this plant may useful for treating cell which damage by radicals [22]. Alkaloids was present in high concentration, alkaloids have cytotoxic effect [23].Free radicals play a major role in diseases related to liver, degenerative diseases and cancer because of deficiency of natural antioxidant defence [24-25].These observation suggest that *L. camara* may have medicinal use.

Leaf extract of *L. camara* shows significant cytotoxicity in dose dependent manner. Dose response curve clearly suggest that cytotoxicity effect on MCF-7 increases by increasing the concentration of leaf extract of *L. camara*, similar kind of finding shown by others . In various studies have been proven that leaf extract of *L. camara* has cytotoxicity effect on different type of cancer cell lines [26,27,28]. Cytotoxic activity of *L. camara* is due to the presence of camaraside and lantadene A, lantaden B and lantadene C. camaraside isolated from *L. camara* by Mehto *et al* [29]. Lantaden A, B and C identified in leaf extract of L.camara by liquid chromatography and mass spectrometry (LC-MS) by Shamsee *et al*, lantadene A and B induce apoptosis in MCF-7 cell. Lantadene A also responsible for arresting cell in G1 phase [30].

Leaf extract of *L. camara* shown good antioxidant activity and cytotoxicity and anti-cancerous activity. These effect due to various biological compound present in L. camara. Present study also support previous finding on *L. camara* which shows it can play a role in against cancer, however further study is required.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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