

Study the Total Flavonoid, Reductive Ability and Cytotoxic Effects of *Lantanacamar* Ethanolic Extract on Prostate and Breast Cancer Cell Line

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Abstract: *Lantana camara* from the latin lento means to bend. Probably derives from the ancient Latin name of the genus viburnum. Total flavonoids of this plant extract was $141.3 \pm 0.003 \mu\text{g/ml}$ and the reductive ability of this plant was concentration dependent 0.16, 0.32 and 0.64 (0.154 ± 0.022 , 0.300 ± 0.004 and 0.407 ± 0.012) respectively in comparison with trolox. The in vitro cytotoxic activities of *lantana camara* against prostate and breast cell line, the result reveals that *L. camara* was more cytotoxic effect on prostate cancer (PC3) in comparison with breast cancer (MCF7) cell line and results were also concentrations dependents.

Keywords: *Lantana Camara*, total flavonoids, reductive ability, PC3, MCF7.

1. Introduction

Medicinal plants represent an important source of medically essential compounds. Since ancient time, medicinal plants are used to medicine several types of health problems. Systemic analysis of these plants provides a variety of bioactive molecules for the development of newer pharmaceutical products. Recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine (kumar *et al.*, 2010; Priya *et al.*, 2010).

L. camara is an important medicinal plant with several medicinal uses in traditional medication system. It is been used to cure many health problems in different parts of the World. The genus *Lantana*, Verbenaceae, comprises about 150 species. Species of the genus *Lantana* have been used in numerous applications in folk medicine due to its anti-rheumatic, antiseptic, antispasmodic, emetic and antifungal activities. (Srivastava and Singh, 2011).

However, *Lantana camara* it is rich in secondary metabolites possessing that are beneficial biological activities. In India, these plants are used on folk and traditional medicine system like antimicrobial, Fungicidal (Anita *et al.*, 2011), insecticidal and nematicidal activity including hepatotoxicity in animals (Sharma *et al.*, 2011) etc. Verbascoside possesses antimicrobial immunosuppressive and antitumor activities (Anita *et al.*, 2012).

The term cancer is used in the medical sciences for the unregulated cell growth. Cancer cells grow in uncontrollable manner, and caused to malignant tumors which invade on the nearby parts of the body. Numerous active compounds (or their semi-synthetic derivatives) derived from medicinal plants have been evaluated for their efficiency and tolerability in the treatment of breast cancer. (Halliwell and Gutteridge ;2007).

2. Materials and Methods

Collection of *Lantana camara* plant

Lantana camara aerial parts were collected from garden in Al- Nahrain University, Iraq, during the period January - 2017.

They were recognized by Professor Ali Al-Mosawy, Plant Taxonomy, Department of Biology, College of Science, University of Baghdad. The fresh plant was collected and washed to remove traces and dust then let's to dry by air dry. They were grained to be powder.

Preparation of plant extract

Ethanolic extract of *L. camara* was prepared according to Fua *et al.* (2010). Fifty grams of plant leaf powder were extracted with 80% ethanol (250 ml) at 65 °C for 3 hours using the soxhlet apparatus. The extract solution was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried crude extract, which was frozen at -20 °C until use to prepare the required doses and concentrations.

Determination of Total flavonoids

Total flavonoids content was determined in the ethanolic extract of *L. camara* as rutin (flavonoids standard) equivalent by aluminium chloride colorimetric methods as described by sakanaka *etal.* (2005). The ethanolic extract powdered of *L. camara* (3.2 mg) was liquefied in 5 ml of 50% ethanol, followed by addition of 1 ml of a 5% (w/v) aluminium chloride solution was added and the mixture was allowed to stand for a further 5 minute before 10 ml of a 10% (w/v) NaOH solution was added then complete the volume to 50 ml with distilled water and mix well. After that the absorbance was measured at 450 nm with spectrophotometer after 15 min. the same procedure was applied to six concentrations (2.5, 5, 10, 20, 40, 80 μg) of rutin, and from which a standard curve was prepared. The total flavonoids content was determined using the equation $y=0.0012x + 0.1109$ that had been obtained from blotted the standard curve.

Assessment of Reductive Ability in Vitro

This method was described by Fua *et al.*, (2010) to evaluate the reductive ability of *L. camara* plant, in which 1 ml of each concentration of the plant extract (0, 16, 0.32 and 0.64 mg/ml) was mixed with 1 ml of 0.2M phosphate buffer (PH 6.6) and 1.5 ml of 1% potassium ferricyanide, then incubated at 50 °C for 20 minutes. After that, 1 ml of 10% trichloroacetic acid (TCA) was added to the mixture to stop reaction. The mixture was centrifuged for 10 minutes at 3000rpm, and 2.5 ml of freshly prepared 1% ferric chloride (FeCl₃). Then, the absorbance was measured at 700nm. The same procedure was applied to the Trolox solutions (standards). Triplicates concentrations was done for every concentration.

Cancer cell lines

The two cell lines used in this study were: MCF-7 (oestrogen receptor-positive human breast adenocarcinoma cell line) and PC3 (Human prostate cancer cell line) these two cell lines were obtained from American Type Culture Collection (ATCC, Va, USA).

Cytotoxicity assay (MTT assay)

$$\text{Percentage of cell viability \%} = \left(\frac{\text{Sample Absorbance}}{\text{Control Absorbance}} \right) \times 100$$

Statistical analysis

Data were obtained by tabulation in the statistical program Graph-Pad Prism version 5.01 (GraphPad Software, La Jolla, CA, USA) and presented as mean ± standard error.

3. Results and Discussions

3.1 Determination of Total Flavonoids

Total flavonoids content were determined spectro photochemically in 80% ethanolic extract of *L. camara* as rutin equivalent. The plant extract was found to contain 141.3 ± 0.003 µg mL⁻¹ flavonoids. Such finding belong to Rabia and Asghari, 2013whom demonstrate that the total flavonoids was (53.112± 0.199) mg g⁻¹ dry weight of plant methanolic extract. In which it was demonstrated that *L. camara* grown in Iraq is a rich source of flavonoids in comparison with flavonoids grown in Pakistan.

3.2 Reductive ability

In the three concentrations tested (0.16, 0.32 and 0.64 mg/ml), the absorbance of *L. camara*ethanolic extract was significantly higher than trolox (vitamin E), and such findings suggest that the plant extract is more effective than trolox in the reductive ability, which was depended on the concentrations. It was 0.154 ± 0.022 at the concentration 0.16 mg/ml of the ethanol extract, and increased significantly to 0.407 at the concentration 0.64 mg/ml (Table 3-1).

The MTT assay was used for to determine the activity of *L. camara* plant extract on cell viability on MCF7 and PC3 cell lines. Concisely, the cells were seeded at 10, 000 cells per well (100 µL) in 96-well dish and then incubated overnight. Different concentrations of plant extract (15.625, 31.250, 62.5, 125, 250, and 500 µg mL⁻¹) were used and incubated for 24 hrs.

MTT (dissolved in phosphate buffered saline (PBS)) was added to each well and incubated for 60 min in the dark.

MTT was detached, and 100 µL of acidified isopropanol (0.1% 0.1N acidic isopropyl alcohol) was added to each well to dissolve the formazan crystals. After 5 min of incubation, absorbance was measured at 570 nm in a Biotexmicroplate reader. The intensity of color produced is directly proportional to the number of viable cells. Each experiment was performed in triplicate number Ferrari, 1990. The relative cell viability (%) of the control wells containing the cell culture medium only without extractand the results of cell viability was calculated as the equation below:

Table 3.1: Reductive ability of *L. camara* ethanolic extract and trolox (vitamin E)

Concentration (mg ml ⁻¹)	Reductive Ability Absorbance (Mean ± SE)	
	<i>L.camara</i> Extract	Trolox (Vitamin E)
0.16	0.154 ± 0.022	0.114 ± 0.004
0.32	0.300 ± 0.004	0.132 ± 0.007
0.64	0.407 ± 0.012	0.211 ± 0.015

3.3 Cell growth and viability assay

Lantana camara (*L. camara*) is mainly used as herbal medicine and in some areas as firewood and mulch Saraf *et al.*, 2011. It is also used for the treatment of cancers, measles, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus and malaria Mandal *et al.*, 2011.

In this experiment the results reveals that prostate cell line (PC3) was more sensitive than Breast cell line (MCF7) in the same concentrations.from the result at concentration 500 µg ml⁻¹for PC3 and MCF7(50.183 ± 0.068) and (62.487 ± 0.504) respectively, we observe PC3 was more sensitive than MCF7 at the same concentration. In addition to other active compound, this plant containing flavonoids which have reductive ability and its antioxidant activity this means its anticancer activity (Al-Anee *et al.*, 2015). As we mention before PC3 was more sensitive than MCF7, the result reveals that at lower concentration15.625µg ml⁻¹ for PC3(89.205 ± 0.068) and MCF7 (99.333 ± 0.088), We observe there are no significant effects at concentration 15.625µg ml⁻¹ for MCF7 cell line in comparison with PC3 at the same concentrations (Table 3.2).

Table 3.2: Mean of cell Viability of PC3 and MCF7 cell lines treated with different concentrations of the *L.camara* ethanolic extract.

Concentrations µg ml ⁻¹	PC3	MCF7
	% viability ± S.E	% viability ± S.E
500	50.183 ± 0.068	62.487 ± 0.504
250	63.287 ± 0.223	69.797 ± 0.439
125	77.703 ± 0.102	84.117 ± 0.829
62.5	81.480 ± 0.154	91.700 ± 0.208
31.250	87.747 ± 0.099	96.647 ± 0.231
15.625	89.205 ± 0.068	99.333 ± 0.088

The result obtained appeared that the *L.camara* plant ethanolic extract was concentrations dependent.

Breast cancer is one of the most commonly diagnosed cancers in women, and is associated with a high fatality rate. Breast cancer therapies, including chemotherapy, monoclonal antibody therapy and radiation, are associated with tolerance and side effects (Tempfer *et al.*, 2009; Weis *et al.*, 2009). Breast cancers without hormone receptors, cancers that have spread to the lymph nodes in the armpits, or those associated with specific genetic characteristics, pose a higher risk than other cancers and are therefore treated more aggressively (Bentzon *et al.*, 2008).

Han *et al* 2015 found that the apoptosis induced by treatment with the *L. camara* extract was regulated by the Bcl-2 family. Bid and Bax was increased and Bcl-2 was decreased by *L. camara* extract. *L. camara* extract modulated cleavage of caspase-8, and caspase-9, as well as poly (ADP-ribose) polymerase (PARP). Our results support the potential use of the *L. camara* extract as an anti-breast cancer drug.

4. Conclusion

This study demonstrates that *Lantana camara* ethanolic extract exerts potent reductive ability and cytotoxic effects on PC3 and MCF7 cells. The result reveals that PC3 was more sensitive than MCF7, and its/their potential as antitumor agent(s) should be investigated in the nude mice xenograft model system.

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