



Antioxidant, Cytotoxic and larvicidal Potential in *Oxalis latifolia* Kunth. Methanolic Leaf Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Author RGK did the Lab related work to bioassay test, preparation of the plant extract. Author SM did the data analysis. The experimental work designed and manuscript corrected by authors RGK and SM. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The current work was analyzed in antioxidants, cytotoxicity and larvicidal potential were examined for *O. latifolia* methanolic leaf extract.

Methodology: Antioxidant assay for Hydroxyl radical (H_2O_2). Cytotoxicity potential was studied against colon (HT-29). *Culex quinquefasciatus* (IVth instar) larvae was used for larvicidal test.

Results: Antioxidant and cytotoxicity exhibits significant activities with IC_{50} value of 442.94 μ g/mL and also the cytotoxicity activity IC_{50} values of 50.00 μ g/mL for HT-29. Larval mortality against the larvae of *Cx. quinquefasciatus* showed best activity with LC_{50} 09.50mg/L and LC_{90} 28.72 mg/L.

Conclusion: Thus, this study proved that methanolic leaf extracts of *O. latifolia* have significant biological activities. Hence, it can be used for pharmaceutical applications.

Keywords: Larvicidal potential.HT-29; *Cx. quinquefasciatus*; H_2O_2 .

1. INTRODUCTION

Oxalis latifolia Kunth. (Family: Oxalidaceae) is a most multipurpose medicinal plants & biological activity. It is commonly known as broadleaf as well as native to Mexico, Central and South America. This is a perennial herb. *Oxalis latifolia* excellent plant and also it's required for normal and good health of humans. The leaves are anti-inflammatory, refrigerant and antiscorbutic and anticancer [1].

Colon cancer (HT-29) positions third as far as frequency, yet second as far as mortality and its expanding in Central and South America because of a continuous progress towards more elevated levels of human turn of events [2]. Colon cancer is the second most common cancer in females and also third in males [3]. The treatment of CRC patients is surgery [4] which is performed in ~80% of patients, while half of them will experience a recurrence of the disease [5]. While chemotherapy is one of the most widely used therapeutic strategies some limitations, The discovery of new drugs for use alternative strategies in cancer treatment. The another way for anticancer drugs to produced from plants [6].

Mosquitoes spread number of diseases in worldwide like as, *A. aegypti* is a vector of dengue. Globally, dengue [7]. *A. stephensi* Liston (Diptera: Culicidae) is a carrier of plasmodium which causes malarial disease [8]. Also, *C. quinquefasciatus* Say (Diptera: Culicidae) is responsible for lymphatic filariasis [9]. Chemical pesticides used for mosquito control various effects. So, alternative sources of plant based biopesticides combined as effective for mosquito species control [10]. *Oxalis* spp. Like as, *Oxalis corniculata* its phytochemical investigations have shown the existence of biologically active classes of secondary metabolite. Plant roots removes piles, kapha and vata and to cures diarrhea, dysentery, worms, skin diseases, giddiness and wounds bleeding. Its leaves are used for cough, fever, cold remedies, as anthelmintic, to stop bleeding in wounds and possesses anticancer activity. Its various potent biological activities have already been reported antifungal and insecticidal activities. These effects were due to the presence of several important bioactive compounds [11]. Therefore, this study focused on, to determine the antioxidant potential, larvicidal and anticancer activities of methanolic leaf extracts in *O. latifolia*.

2. MATERIALS AND METHODS

2.1 Materials

Hydrogen peroxide (H_2O_2), Sodium salicylate, Fetal bovine serum (FBS), Dimethyl sulfoxide (DMSO), Dulbecco in modified eagle medium (DMEM), 3-[4,5-Dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT), Phosphate buffered saline (PBS), Penicillin/Streptomycin antibiotic solution, Trypsin-Ethylene diamine tetra acetic acid (EDTA) was purchased from Gibco (USA), Methanol was purchased from Sigma Aldrich (USA). All other chemicals and reagents used in this study were of analytical grade. The experiments in this study were done using sterile distilled water.

2.2 Collection of Plant

The plant was obtained from the Thirumoorthy hills (Fig. 1) Tamil Nadu, India during the month of January and also the plant were authenticated from Botanical Survey of India (BSI) Southern region Coimbatore, Tamil Nadu, India.

2.2.1 Preparation of plant extract

The plant material were washed, shade dried and powdered using mixer grinder. The powdered material (10 g) was extracted with 100 ml of selected organic solvents methanol using soxhlet apparatus and filtered through Whatmann No. 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of the leaves were stored in small vials at $-20^{\circ}C$ and used for further analysis.

2.3 Antioxidant

2.3.1 Hydroxyl radical scavenging assay (H_2O_2)

The scavenging activity of the methanolic leaf extract on hydroxyl radicals was measured according to the method [10]. Hydroxyl radicals were generated from $FeSO_4$ and hydrogen peroxide and detected by their ability to hydroxylate salicylate and the hydroxylated salicylate complex is measured at 562 nm. A reaction mixture of 3.0 mL volume contained, 1.0 mL of 1.5 mM $FeSO_4$, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate and 1.0 mL of different concentrations (100-500 $\mu g/mL$) of sample. After incubation for an hour at $37^{\circ}C$, the absorbance of the hydroxylated

salicylate complex was measured at 562 nm. Ascorbic acid was used as positive control. The percentage of scavenging effect was calculated as,

$$\text{Scavenging activity} = \left[1 - \frac{(A_1 - A_2)}{A_0} \right] \times 100$$

Where, A_0 is the absorbance of the control, A_1 is the absorbance in the presence of the sample and A_2 is the absorbance without sodium salicylate.

2.4 Anticancer Study

2.4.1 Cell line

The human colon cancer cell line (HT 29) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.4.2 Cell treatment procedure

The method was followed by Al-Haj Ali [12]. A 13 5-mg/mL solution of MTT (Sigma–Aldrich, USA)

in PBS was produced by dissolving 500 mg of MTT in 100 ml of PBS, then the solution was filtered with 0.45 μ m of cellulose acetate filter paper (VIVID separation and filtration, USA) to ensure sterilisation. After incubation the study materials for 24, h at 37 C in 5 % CO₂ atmosphere, all materials were removed and the PDL cells were washed twice with PBS, then 100 μ l of DMEM and 10 μ l of MTT solution were added to all wells and all plates were incubated for 4 h at 37 C. The supernatant was then eliminated and 100 μ l of DMSO solvent (Sigma– Aldrich, USA) was added to each well. The plates were then placed in a waving shaker machine for 30 min. The absorbance at 570 nm was measured with a microtiter plate reader (Basic Tecan, Austria) with absorbance at 650 nm used as a reference. The in vitro toxicity of the tested materials was calculated as the relative absorbance of tested wells versus untreated (negative control) wells (i.e. mean absorbance of tested wells/mean absorbance of untreated wells 9100).

2.5 Larvicidal Activity

2.5.1 Collection and rearing of mosquito larvae

Cx. Quinquefasciatus was collected (ICMR-VCRC Madurai) and than it kept during 14:10 h photoperiod, 27 °C, 70±5%-RH with provided the fed.



Fig. 1. *Oxalis latifolia* kunth

2.6 Dose Response Bioassay

Larvicidal bioassay was performed as per WHO, [13] standard procedure with some modification as per the method of [14]. The 20 numbers of IVth in star larvae were introduced into each 250 mL capacity plastic cups containing 200 mL of double distilled water. Different dosages of methanolic leaf extracts of *O. latifolia* (5, 10, 15, 20, and 25 mg/L) was added to the cups containing mosquito vector larvae after sonication of methanol extracts. Larval mortality percent was calculated after 24 h of exposure to determine the acute toxicity of *O. latifolia* methanol extracts on *Cx. quinquefasciatus*. The control treatment was also maintained without the addition of methanol leaf extract. The number of dead larvae was calculated after 24 h of exposure and the percent of mortality was described from the average of three replicates. The larval mortality was calculated by using the formula of Abbott [15] and LC₅₀ LC₉₀ values were calculated after 24 h by SPSS using probit analysis.

$$\text{Abbott's percent corrected mortality} = \frac{\% \text{ Mortality in treated} - \% \text{ Mortality in control}}{100\% - \% \text{ Mortality in control}} \times 100$$

2.7 Statistical Analysis

The results were analyzed a Mean ± SD. The larval mortality was calculated by Probit analysis for finding out LC₅₀, and LC₉₀ values. The chi

square values were also calculated by SPSS software version 20.0 (SPSS., USA).

3. RESULTS AND DISCUSSION

3.1 Antioxidant Potential

The Antioxidant study against H₂O₂ was performed using methanol extract in *O. latifolia*. In this study, concentration-dependent manner (Fig. 2). The methanol extract in *O. latifolia* and ascorbic acid (standard) 50% IC₅₀ value of 442.94 and 388.53 µg/mL. Similar study, from *Swietenia mahagoni* extract in H₂O₂ showed the IC₅₀ value of 66.10 µg/mL [16]. In another same study, *Jacaranda mimosifolia* displayed the 50% scavenging value of 45.0 µg/mL [17].

3.2 In-vitro Cytotoxicity Assay

Cytotoxic activity of *O. latifolia* in methanolic leaf extract against colon cancer (HT-29). In this study showed concentration dependent cytotoxic activity and it's displayed a significant increase the %inhibition of HT-29 when the concentration of methanolic leaf extract was increased from 18.75-300µg/mL (Fig. 3) as well as treated images shown in Fig. 4. Similar findings were reported by Sivaskathi et al. [18]. The 50% (IC₅₀) of inhibition activity against HT-29 at 139.71 µg/mL. Similar documented by Turan et al. [6] who reported that the *Rosa canina* extract showed the significant activity and the IC₅₀ value of 405.4 µg/mL.

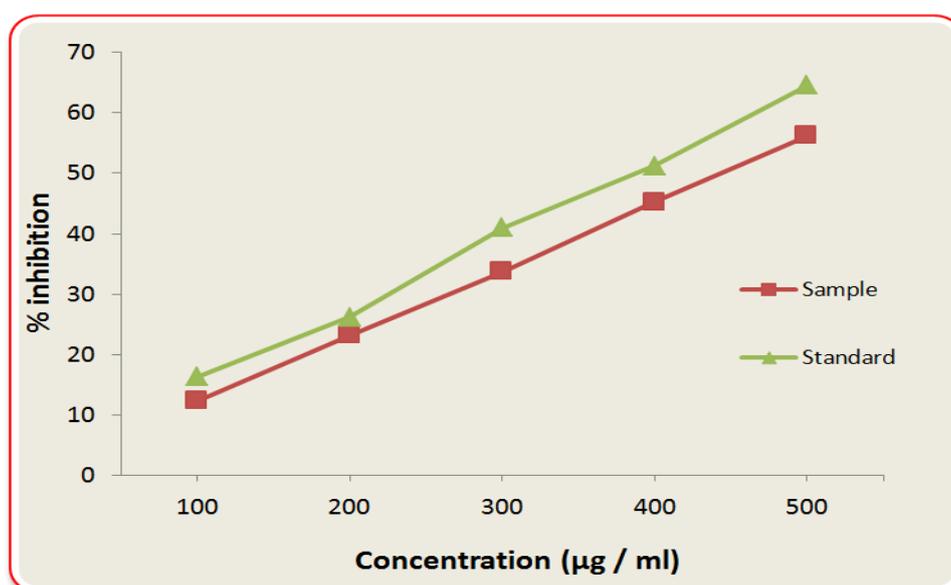


Fig. 2. Antioxidant study in methanolic leaf extract of *O. latifolia* against H₂O₂

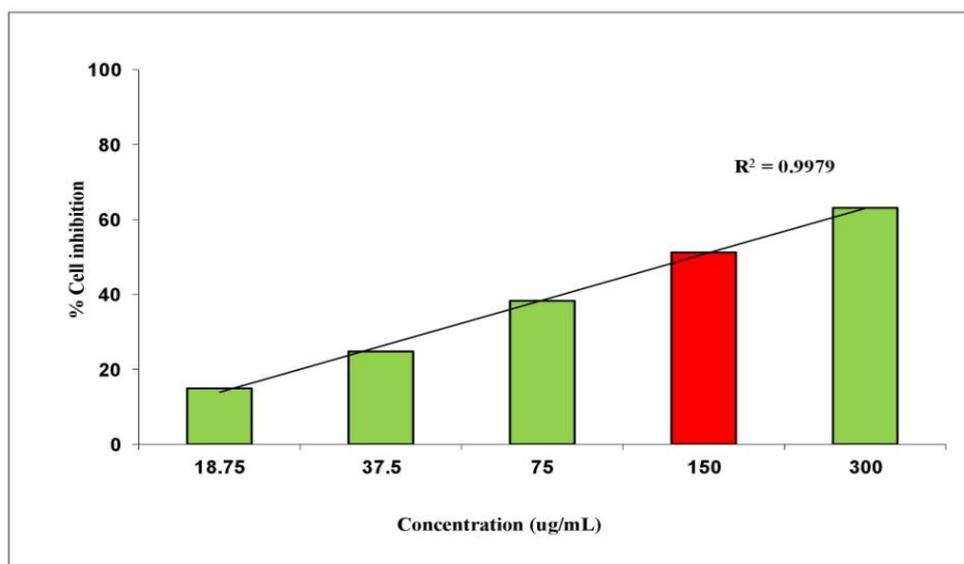


Fig. 3. Cytotoxic effects of methanolic leaf extract of *O. latifolia* against colon cancer (HT 29) cell line

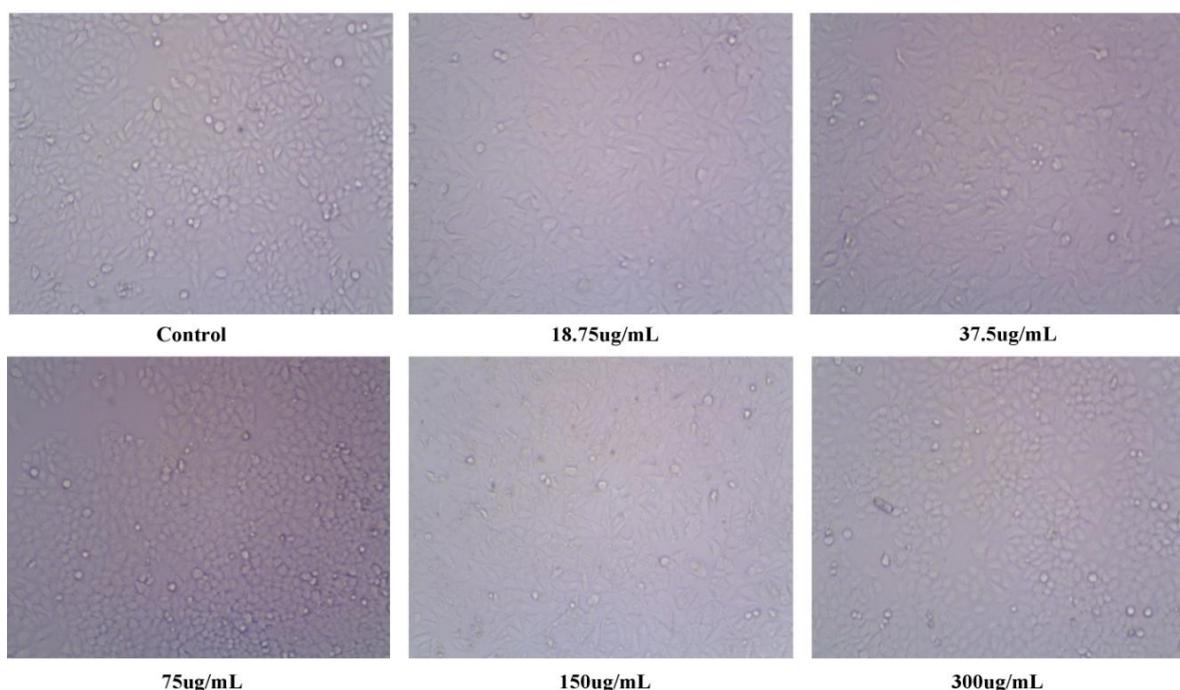


Fig. 4. Cytotoxicity observed from bright field microscope: Control and various concentrations in methanolic leaf extract of *O. latifolia* treated on colon cancer (HT-29) cell line

3.3 Larvicidal Efficacy

O. latifolia in methanolic leaf extract were tested against 4th instar larvae of in *Cx. quinquefasciatus* at 24h was investigated. The concentration of *O. latifolia* in methanolic leaf extract were 5,10,15,20 and 25 mg/L and its shown better

larvicidal activity with LC₅₀ 09.50mg/L and LC₉₀ 28.72 mg/L. In this study dose depended larvicidal activity. The similar, dose depended activity was observed by Swargiary et al. [19]. In recent report by Loganathan et al. [20] the methanolic leaf extracts of *Knoxia sumatrensis* against *Cx. quinquefasciatus* larvae was best

activity. Thus larvicidal activity results clearly showed that the larval mortality could directly related to ethanol leaves extracts concentration.

4. CONCLUSION

O. latifolia of methanolic leaf extract exhibited potential antioxidant activities against H₂O₂. Further, the extracts showed significant cytotoxicity activity against the colon (HT-29) cell line were using MTT assay at 24h. Moreover, the laricidal activiyt of *O. latifolia* methanol leaf extract was more toxic to *Cx. quinquefasciatus*. Further study on fractionations and isolation of active compounds for physicochemical stability is required to develop cost-effective formulations from the active fractions and for making substantial representation of plantbased insecticides in global insecticide market.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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