

## Evaluation of Antioxidant Activity of *Clitoria ternatea* and *Alternanthera sessilis* Plant Extracts Using Model System for Yeast Cells

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**Abstract:** To evaluate the antioxidant effects and apoptotic study of the leaves of *Clitoria ternatea* and *Alternanthera sessilis* by using the yeast cell. The yeast cells were isolated from the sugar factory effluents and isolated the yeast cell DNA. The leaves extract from different solvents were tested for their scavenging activity against the stable free radical DPPH (2, 2'-diphenyl-1-picryl hydrazyl) in dot plot rapid screening assay method and quantified using a spectrophotometric assay method. Oxidative damage was induced *in vitro* by treating yeast DNA and analyse the effects of the leaf extracts. Genomic DNA samples were isolated from YBD broth culture. DPPH scavenging activity was highly elicited by the methanol extract of *C. ternatea* and aqueous extract of *A. sessilis*. The *C. ternatea* and *A. sessilis* leaf extracts treatment effectively decreased the extent of DNA damage. *C. ternatea* and *A. sessilis* leaves have the potential antioxidant activity. Thus validating it is a source of valuable antioxidant drugs.

**Key words:** Apoptosis • Cancer • Herbal plants • Medicine • Oxidative stress • Scavenging activity

### INTRODUCTION

Apoptosis is a type of programmed cell death, is a process that can occur normally, during tissue remodeling and embryogenesis, or abnormally during certain pathologies, such as cancer [1]. Yeast can be killed by expression of mammalian proapoptotic genes or in response to oxygen stress, which model for cellular aging [2]. Medicinal plants have pharmacological activities and thus may be a source for novel anti-tumor agents. The mechanisms of action of anti-tumor agents include anti-proliferate and anti-oncogenic effects, induction of apoptosis, oxidative stress, oncogenes and tumor suppressor genes [3]. The herbal medicines are commonly used by patients in an attempt to prevent cancer and also treat cancer. They allowed as adverse effects associated with conventional cancer treatments [4]. Extracts of medicinal plants are believed to contain different chemo-preventive or chemotherapeutic compounds [5]. Plants are the source of medication for preventive, curative, protective or promote purposes [6]. About 80%

of the world's population depends on traditional medicines for primary healthcare. Herbal medicines are in great demand in both developed and developing countries because of their great efficacy and no side effects [7].

*Clitoria ternatea* (Linn), commonly known as Shankupushpam, belongs to the plant family *Fabaceae* and is propagated through seeds. It is a perennial twinning herb with blue and white flowers. The parts used for medicinal purposes include roots, leaves and seeds. The plant contains tannins and glucose [8]. *C. ternatea* is widely used in traditional systems of medicine as a brain tonic [9]. The blue flowered bearing plant of *C. ternatea* is used for my study and the second medicinal plant is taken for my study is the green leaf variety of *Alternanthera sessilis* (Linn.), belongs to the plant family *Amaranthaceae*. *A. sessilis* is a prostrate or procumbent, annual or perennial herb. A decoction is recommended as an herbal remedy to treat wounds, flatulence, nausea, vomiting, cough, bronchitis, diarrhea, dysentery and diabetes. Its root can relieve inflamed

wounds [10]. *A. sessilis* is used as a local medicine often in mixtures with other medicinal plants, to treat hepatitis, tight chest, bronchitis, asthma and other lung troubles. The leaves and shoots boiled and drunk as an antihypertensive remedy [11].

The model system used for our study is *Saccharomyces cerevisiae*. [12]. Such machinery represents a simple and valuable model, which will assist in the future understanding of the complex connections between apoptotic and non-apoptotic mammalian programmed cell death pathways [13].

## MATERIALS AND METHODS

**Yeast Sample Collection:** Yeast sample was collected from the Sugar factory effluent in Madurai- Alanganallur sugar factory, Madurai Dt, in Tamilnadu, India. Sample was collected in sterile plastic bottles and brought to the Laboratory.

**Plant Sample Collection:** The *Clitoria ternatea* were purchased from Horticulture Research Institute, Periyakulam, Tamilnadu and *Alternanthera sessilis* leaves collected from agricultural land in Kattivayal, Ramnad, Tamilnadu, India. They were grown in a pesticide-free area. Fresh leaves were used to estimate all the parameters. They were washed free of surface contaminants in running tap water and blotted dry between the folds of soft tissue paper.

**Extract Preparation:** The leaves were washed free of surface contamination with tap water, rinsed with distilled water and blotted gently with filter paper. 1g of fresh leaf was homogenized and the residues were percolated through the different solvents (Methanol, Ethanol and Water) in 5ml. The homogenates were centrifuged at 2000 rpm for 5 minutes and the supernatant was used [14].

**Isolation of the Yeast:** The sample was serially diluted using sterile saline. The sample was mixed thoroughly and streaked on the YPD agar plates then incubated at 37°C in an incubator in inverted position. Duplicates were maintained for 2 days incubation and the plates were examined for colonies formation. A single colony from a plate was picked out and it was sub cultured in YPD broth and it was used for further studies [15].

**Isolation of Genomic DNA from the Yeast Cells:** 10ml of yeast cultures were grown overnight by saturation in YPD media at 30°C. Spun the cells down for 5 min at 1,500 rpm (Beckmann) and suspended the cells in 0.5 ml sterile distilled water. Transfer the cells to 1.5ml tube and spin them down for 5 sec in the Centrifuge at the maximum speed (14,000 rpm). Decant the supernatant and briefly vortex the pellet in the residual water. In the following order, add 200µl Yeast lysis Buffer, 200µl phenol: chloroform: isoamyl alcohol (25:24:1). Vortex for 3-4 min and add 200 µl TE (pH 8). Spin for 5 min in a microfuge and transfer aqueous phase to a new tube. Add 1 ml of 100% Ethanol and mix by inversion. Spin for 1 min in a microfuge and aspirate the supernatant. Suspend the pellet in 400 µl TE buffer and 4 µl of RNase A and incubate until the pellet was dissolved at 37°C. Add 10 µl of 4M ammonium acetate and 1 ml 100% Ethanol. Spin for 1 min in a microfuge and discard the supernatant. Wash with 1ml 70% Ethanol. Air dry the pellet and suspend in 50 µl TE. The reaction mixture was then mixed with 6µl of gel loading dye, loaded into 1% agarose gel and run at 100V for 15 minutes in a submarine gel electrophoretic apparatus. The DNA was visualized and photographed using an Alpha Digidoc digital gel documentation system (USA) [16].

### Rapid Screening of Antioxidant Activity

**Dot Plot Assay:** Aliquots (3µl) of *C. ternatea* and *A. sessilis* extracts were spotted on a TLC plate and allowed to dry. The TLC plate bearing the dry spots was placed upside down for 10 seconds in the solution of DPPH. The spots exhibiting radical scavenging, antioxidant activity showed up as yellow spots in a violet background. The intensity of the yellow colour depends on the amount and nature of the radical scavenger present in the spot [17].

**DPPH Photometric Assay:** An exact amount (0.5ml) of the methanol solution of DPPH was added with 20 µl of the leaf extracts in the different solvents and the crude aqueous extract (corresponding to 4mg) and 0.48ml of methanol and allowed to stand at room temperature for 30 minutes. Methanol served as the blank [18]. After 30 minutes, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows:

$$\text{Scavenging activity (\%)} = \frac{A_{518} [\text{sample}] - A_{518} [\text{blank}]}{A_{518} [\text{blank}]} \times 100$$

**Effect of Leaf Extract on Oxidative Damage:** The reaction was conducted in a total volume of 30µl containing 5µl of tris buffer, 5µl of Yeast cell DNA and 5µl of plant extract prepared in tris buffer. Then 10µl of H<sub>2</sub>O<sub>2</sub> and 5µl of FeCl<sub>3</sub> were added and incubated at 37°C for 30 minutes. The reaction mixture was then mixed with 6µl of gel loading dye, loaded into 1% agarose gel and run at 100V for 15 minutes in a submarine gel electrophoretic apparatus [19]. Yeast cell DNA with H<sub>2</sub>O<sub>2</sub> using for negative control. The DNA was visualized and photographed using an Alpha Digidoc digital gel documentation system (USA).

## RESULTS

**Isolation of the Yeast and Yeast Cell DNA:** White coloured colonies were observed using YPD agar medium plates (Fig. 1) and the yeast genomic DNA was isolated for YPD broth culture.

### Rapid Screening of Antioxidant Activity

**Dot Plot Assay:** The maximum DPPH scavenging activity was expressed by the methanol extract, followed by the ethanol extract of *C. ternatea* leaf extract shown in (Fig. 2). *A. sessilis* leaf extract showed the DPPH scavenging activity in aqueous extract, followed by ethanol and methanol extract Fig. 3).

**DPPH Photometric Assay:** The radical scavenging effects of *C. ternatea* and *A. sessilis* leaves were also quantified using the photometric assay. To confirm the observations in dot plot rapid screen, the photometric quantification of the extent of DPPH scavenging by different solvent extracts was followed. The results, expressed as percent scavenging, are represented in (Table 1). The results revealed that the maximum extent DPPH scavenging activity was in methanol extract, followed closely by ethanolic extract and then by the aqueous extracts. This trend was observed in *C. ternatea* leaves. But *A. sessilis* leaves have maximum DPPH scavenging activity in aqueous extract, followed by ethanol and methanol. The *Alternanthera sessilis* leaves exhibited more DPPH scavenging activity than the *Clitoria ternatea* ones. The extracts of *Clitoria ternatea* and *Alternanthera sessilis* leaves effectively scavenged hydroxyl radicals. It can be observed from the results that *Alternanthera sessilis* leaves rendered better protection than *Clitoria ternatea* leaves.



Fig. 1: Yeast cell colonies in YPD agar medium plates

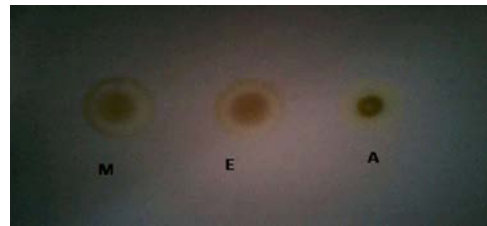


Fig. 2: Dot plot assay DPPH free radical Activity of extracts of *Clitoria ternatea*.

M- Methanol  
E- Ethanol  
A- Aqueous



Fig. 3: Dot plot assay DPPH free radical Activity of extracts of *Alternanthera sessilis*.

M- Methanol  
E- Ethanol  
A- Aqueous

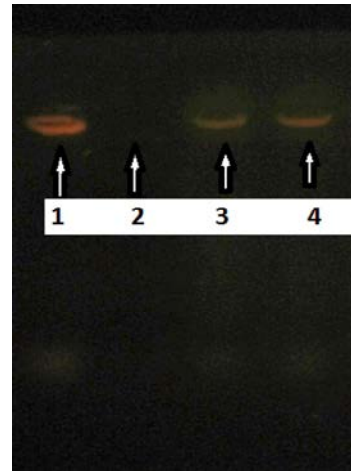


Fig. 4: Effect of leaf extract on oxidative damage

1- Yeast cell DNA (control)  
2- Yeast cell DNA+H<sub>2</sub>O<sub>2</sub>  
3- Yeast cell DNA+H<sub>2</sub>O<sub>2</sub> + *C. ternatea* leaf extract  
4- Yeast cell DNA+H<sub>2</sub>O<sub>2</sub> + *A. sessilis* leaf extracts

Table 1: DPPH free radical Activity of extracts of *Clitoria ternatea* and *Alternanthera sessilis*

S. No.	Test components	Inhibition (%)	
		<i>C. ternatea</i>	<i>A. sessilis</i>
1	Methanol Extract	43.11	33.44
2	Ethanol Extract	27.26	48.30
3	Aqueous Extract	16.63	68.04

**Effect of Leaf Extract on Oxidative Damage:** Lane first shows the yeast cell DNA then second lane show the DNA was damaged by  $H_2O_2$ . Last two lanes showed the Yeast DNA damaged using  $H_2O_2$  and then prevented from *C. ternatea* and *A. sessilis* leaf extracts to yeast DNA in (Fig. 4).

## DISCUSSION

Plant extracts and their isolated constituents have always been an important part of various therapeutic systems [20]. The use of different parts of the medicinal plants not only helps to decrease the cost of medication but is also locally available with lesser side effects as compared to chemical based formulation [5]. The use of plant extract can be great significance in therapeutic treatments, such plants are *C. ternatea* and *A. sessilis* [21]. Various cell free systems and invitro assays were applied to evaluate the free radical scavenging and antioxidant effects of *C. ternatea* and *A. sessilis*.

*Clitoria ternatea* and *Alternanthera sessilis* leaves, extracted serially into solvents of increasing polarity (Ethanol, Methanol and water) were tested for their free radical scavenging activity against DPPH. In the present study, the maximum extend of DPPH scavenging activity was elicited by the methanol extract followed by the ethanol extract and then by the aqueous extract. *A. sessilis* leaves exhibited more DPPH scavenging activity than the *C. ternatea*. Any oxidant entering the biological system can maximally manifest its damaging effects only after entry into the cellular environment and by passing the endogenous antioxidant system. For the entry into the cell or its organelles, an oxidant needs to cross the membrane barriers (both plasma membrane and internal membranes). Yeast DNA was damaged significantly by  $H_2O_2$ . *C. ternatea* and *A. sessilis* leaf extracts significantly reduced the extent of DNA damage in the Yeast DNA.  $H_2O_2$  is a strong oxidant that can result in extensive damage to biomolecule, ultimately causing cellular death [22].

DNA contains reactive groups in its bases that are highly susceptible to free radical attack.  $H_2O_2$  plays an

important role in the generation of free radical-induced DNA damage, inducing mutation [23]. Similar DNA protective effects have been reported for *Polygonum aviculare* extract *Satoreja hortensis* extract and *Menisona procumbens* extract [24, 25, 26]. Plant antioxidant components like grape seed polyphenols and anthocyanins have been reported to protect DNA against oxidant induced damage [27].

In the present study *C. ternatea* and *A. sessilis* leaf extracts were found very effective in reverse the DNA damage induced by  $H_2O_2$ . *C. ternatea* and *A. sessilis* leaf extracts treatment effectively decreased the extent of DNA damage from baseline DNA damage. The effects of *A. sessilis* leaf extract were more efficient than that of *C. ternatea* leaf extract. The present study highlights the protective effects rendered by *C. ternatea* and *A. sessilis* leaf extracts under oxidative stress conditions. Oxidative stress is the major causative factor under laying the pathogenesis of several disease conditions. The outcome of the present study, thus, scientifically validates and strengthens the candidature of *C. ternatea* and *A. sessilis* leaves in the preparation of medicinal aids to combat the wide spectrum of myriad diseases arising due to oxidative stress.

## ACKNOWLEDEMENTS

Authors are thankful to Sourashtra College, department of Biotechnology, Pasumalai, Madurai, Tamilnadu, India for providing necessary facilities.

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