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

[1]Balachandar Balakrishnan, [2]A. Jayachitra, [1]S. Paramasivam and [1]A. Arul kumar

[1]Department of Oceanography and Coastal Area Studies, School of Marine Sciences,
Alagappa University, Thondi Campus-623 409, Tamilnadu, India
[2] Department of School of Biotechnology, Madurai Kamaraj University, Madurai, Tamilnadu, India

Abstract: To evaluate the antioxidant effects and apoptotic study of the leaves of *Clitoria ternatea* and *Alternanthra sessilis* by using the yeast cell. The yeast cells were isolated from sugar factory effluent 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 and quantified using a Spectrophotometric assay. Oxidative damage was induced *in vitro* by treating 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, such as 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 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 chemical composition includes 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 this study and the second medicinal plant is taken for present study is the green leaf variety of *Alternanthera sessilis* belongs to the 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,

Corresponding Author: Balachandar Balakrishnan, Department of Oceanography and Coastal Area Studies,
School of Marine Sciences, Alagappa University, Thondi Campus-623 409, Tamilnadu, India

bronchitis, asthma and other lung troubles, to stop bleeding and as a hair tonic. The leaves and shoots are boiled and drunk as an antihypertensive remedy [11].

The model system used for our study is *Saccharomyces cerevisiae*. *S. cerevisiae* has been reported to die under certain conditions, from programmed cell death with apoptotic markers [12]. Such machinery represents a simple and valuable model, which wills assists 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 which is in Madurai-Alanganallur sugar factory, Madurai Distric, Tamilnadu. Sample was collected in sterile bottles and brought to the Laboratory, Dept of Biotechnology, Sourashtra College and Madurai.

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 district, Tamilnadu. They were grown in a pesticide-free area. The leaves were procured fresh for the estimation of each parameter. 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 leaves was homogenized and the residues were percolated the different solvents (Methanol, Ethanol and Water) in 5ml. The homogenate was centrifuged at 2000 rpm for 5 minutes and the supernatant was used [14].

Isolation of 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 Yeast Cells: 10 ml of yeast cultures were grown overnight by saturation in appropriate media at 30°C. Spanned 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_2O_2 and 5µl of $FeCl_3$ 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. Yeast cell DNA with H_2O_2 using for negative control. The DNA was visualized and photographed using an Alpha Digidoc digital gel documentation system (USA) [19].

RESULTS

Isolation of 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).

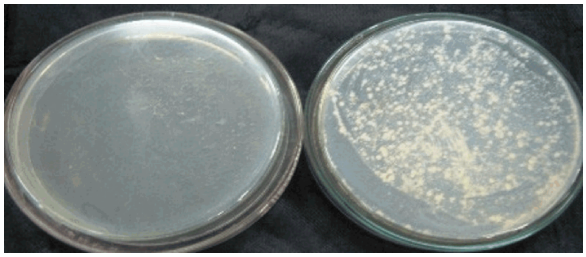
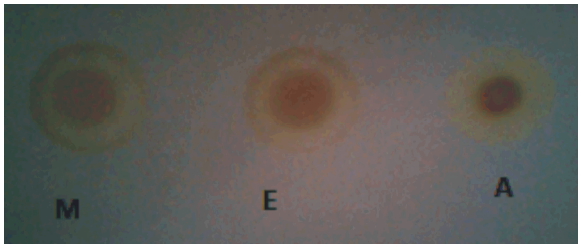
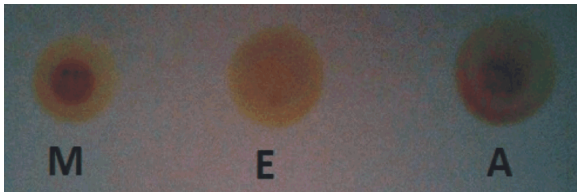


Fig. 1: Yeast cell colonies in YPD agar medium plates.



M = Methanol, E = Ethanol and A = Aqueous

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

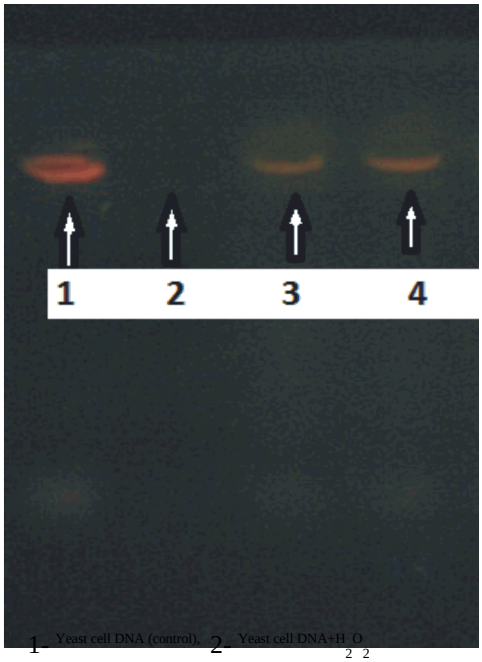


M = Methanol, E = Ethanol and A = Aqueous

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

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

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



1- Yeast cell DNA (control), 2- Yeast cell DNA+H_2O_2

3- Yeast cell DNA+H_2O_2 + *C. ternatea* leaf extract and

4- Yeast cell DNA+H_2O_2 + *A. sessilis* leaf extracts

Fig. 4: Effect of leaf extract on oxidative damage

DPPH Photometric Assay: The radical scavenging effects of *C. ternatea* and *A. sessilis* leaves were also qualified 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 methanol extract, followed closely by ethanolic extract and then by the aqueous extract. 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.

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 *in vitro* assays were applied to evaluate the free radical scavenging and antioxidant effects of *C. ternatea* and *A. sessilis*.

Clitoria ternatea and *Alternanthra 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 presence 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 *Mensona 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 very effective in revers 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.

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REFERENCES

1. Subhash Basu, Rui Ma, Joseph R. Moskal, Manju Basu, Sipra Banerjee, Biochemical Roles of Eukaryotic Cell Surface Macromolecules Advances in Experimental Medicine and Biology, pp: 233-256.
2. Frank Madeo, Eleonore Fröhlich, Martin Ligr, Martin Grey, Stephan J. and Sigrist, Kai- Uwe and Frohlich, 1999. Oxygen Stress: A Regulator of Apoptosis in Yeast. The Journal of Cell Biology., 145(4): 757-767.

3. Efferth, T., 2007. Antiplasmodial and antitumor activity of artemisinin- from bench to bedside. Planta medica., 73(4): 299-309.
4. Heather Boon and Jacqueline Wong, 2004. Botanical medicine and cancer: a review of the safety and efficacy. Journal of Expert On Pharmacotherapy. 5(12): 2485-2501.
5. Ranga, R.S., S. Sowmyalakshmi, R. Burikhanov, M.A. Akbarsha and D. Chendil, 2005. A herbal medicine for the treatment of lung cancer. Molcular Cell Biochemistry., 280: 125-133.
6. Sidhu, K., J. Kaur, G. Kaur and Pannu, 2007. K. Prevention and cure of digestive disorders through the use of medicinal plants, Journal of Human Ecology., 21: 113-116.
7. Trivedi, M. and J. Rawal, 2001. Alteration of hepatic glutathione and release into serum after treatment with bromobenzene and CCl_4, Biochemistry Pharmacology., 39: 4239-4244.
8. Aruna, R.V., B. Ramesh and V.N. Kartha, 1999. Effect of beta carotene on protein glysylaion in alloxan induced diabetic rats. Indian Jornal of Experimental Biology., 37: 399-401.
9. Gomez, S.M. and A. Kalamani,, 2003. Butterfly pea (*Clitoria ternatea*): A nutritive multipurpose forage legume for the tropics. An Overview, Pakistan Journal of Nutrition., 2: 374-379.
10. Hosamani, K.M., S.S. Ganjihal and D.V. Chavadi, 2004. Alternanthera triandra seed oil: A moderate source of ricinoleic acid and its possible industrial utilization. Indian Crop Production, 19: 133-136.
11. Acharya, E. (Siwakoti) and B. Pokherl, 2006. Ethno-medicinal plants used by Bantar of Bhaudaha Morang, Nepal. Our Nature., 4: 96-103.
12. Gabriela, F. Ribeiro, Manuela Coˆrte-Real and Bjo¨rn Johansson, 2006. Characterization of DNA Damage in Yeast Apoptosis Induced by Hydrogen Peroxide, Acetic Acid and Hyperosmotic Shock. Molecular Biology of the Cell., 17: 4584-4591.
13. Ludovico, P., M.J. Sousa, M.T. Silva, C. Leao and M. Corte-Real, 2001. Saccharomyces cerevisiae commits to a programmed cell death process in response to acetic acid. Microbiology., 147: 2409-2415.
14. Chenielle Delahaye, Lois Rainford, Alison Nicholson, Sylvia Mitchell, John Lindo and Mohammed Ahmad, 2009. Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. Journal Medical and Biological Sciences., 3(1): 1-7.
15. Haley, L.D., 1971. Identification of yeasts in clinical microbiology laboratories. American Journal Medical Technology, 37: 125-131.
16. Namjin Chung. DNA Preparation from 10-mL Yeast Culture. Web: https://web.duke.edu/ceramide/ protocols/0002.html. April20, 1996.
17. Mensor, L.L., F.S. Menezes, G.G. leitao, A.S. Reis, T. Dossantos, C.S. Coubes and G.S. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotheraphy Research, 15: 127-130.
18. Solar- Rivas, C., J. Carlos- Espin and H.J. Wichers,, 2000. An easy and fast test to compare total free radical scavenger capacity of food stuff. Phytochemical Analysis., 11: 330-338.
19. Chang, T.S., W. Jeong, S.Y. Choi, S. Yu, S.W. Kang, and S.G. Rhee, 2002. Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation. Jounal of Biological Chemistry., 277: 25370-25376.
20. Vanitha, C. and M. Kathiravan, 2006. Importance and scope of medical plants, Agriculture Herbal Vision., 15: 30-31.
21. Kumar, K., S.C. Gupta, S.K. Baidoo, Y. Chander and C.J. Rosen, 2005. Antibiotic uptake by plants from soil fertilized with animal manure. Journal of Environmental Quality., 34: 2082-2085.
22. Madeo, F., E. Herker, C. Maldener, S. Wissing, S. Lachelt, M. Herlan, M. Fehr, K. Lauber, S.J. Sigrist, S. Wesselborg and K.U. Fro¨hlich, 2002. A caspase-related protease regulates apoptosis in yeast. Molecular Cell., 9: 911-917.
23. Ferrer, J., G. Paez and Z. Marmol, 2001. Agronomic use of biotechnologically processed grape wastes. Bioresource Technology, 76: 39-44.
24. Hsu, C.Y., 2006. Antioxidant activity of extract from *Polygonum aviculare L*. Biological Resarch., 39(2): 281-288.
25. Fatemeh Mosaffa Javad Behravan, Gholamreza Karimi and Mehrdad Inanshahi, 2006. Antigenotoxic effects of *Saturja hortensis L*. on rat lymphocytesexposed to oxidative stress. Archives Pharmacal Research., 29(2): 159-164.
26. Min Yang, MD., Zhengping Xu PHD, Ronghua Zhang MD, Pianhong Zhang MPH, Yan Weng MD and Yueliang Shen MD, 2006. Protection of myocardium in streptozotocin-induced diabetic rats by water extracts of Hsian-tsao (Mesona procumbens Hemsl). Asia Pacific Journal of Clinical Nutrition., 17(1): 23-29
27. Weisel, T., M. Baum, G. Eisenbrand, H. Dietrich, F. Will, J.P. Stockis, S. Kulling, C. Rufer, C. Johannes, and C. Janzowski, 2006. An anthocyanin/ polyphenolic-rich fruit juice reduces oxidative DNA damage and increases glutathione level in healthy probands. Biotechnology Journal., 1: 388-97.