



***BRYOPHYLLUM PINNATUM* LEAF EXTRACTS MODERATES
AGEING THROUGH ITS STRESS RESISTANCE EFFICACY IN *C.
ELEGANS***

PARMAR RK^{1,2}, BRAGANZA V² AND HIGHLAND HN¹

1: Department of Zoology, BMT and Human Genetics, Gujarat University, India

2: Loyola Centre for Research and Development, Xavier Research Foundation, Ahmedabad,
Gujarat, India

*Corresponding Author: Prof. (Dr.) Hyacinth N. Highland: E Mail: rparmar2293@gmail.com

Received 16th June 2020; Revised 24th July 2020; Accepted 29th Nov. 2021; Available online 1st July 2022

<https://doi.org/10.31032/IJBPAS/2022/11.7.6234>

ABSTRACT

The present investigation was aimed at evaluating the antioxidative and stress resistance efficacy of *Bryophyllum pinnatum* (BP) extracts using *Caenorhabditis elegans* as the experimental model. The antioxidant activity of the BP extract was evaluated *in vitro* (DPPH) and *in vivo* (DCF-DA assay). Different crude extracts of BP i.e., methanolic, hydromethanolic and aqueous were used to determine impact on lifespan and ROS scavenging activity (20 mM H₂O₂). The present study revealed that BP crude extracts possess significant *in vitro* and *in vivo* antioxidant activities which possibly contributed to its role in enhanced stress tolerance. The crude extracts also proved effective in increasing the mean lifespan of *C. elegans* following exposure to oxidative stress. The study strongly suggests that the *B. pinnatum* extract acts as an antistressor and potent scavenger of reactive oxygen species, and consequently enhances the survival of the worms in different stress conditions.

Keywords: Antioxidative, *Bryophyllum pinnatum*, Crude extracts, *Caenorhabditis elegans*, Antistressor

INTRODUCTION

Ageing is a complex, pleiotropic phenomenon yet to be fully understood, but its related diseases have an ever-increasing economic impact on world health. As a

result, there is an intense interest in developing medicines to delay or prevent the decline in tissue function with age; however to date effective anti-ageing molecules remain elusive.

In nature, cells may encounter both exogenous and endogenous stressors that can alter normal physiological processes, generate reactive oxygen species (ROS), natural by-products of cellular metabolism and play a role in cell signaling and cellular homeostasis. The imbalance between ROS and protective detoxification enzymes can lead to oxidative stress resulting in extensive oxidative damage to macromolecules such as DNA, lipids, and proteins [1, 2] and ultimately results intracellular injury and death, which is recognized to contribute to the aging process and age-related diseases [3, 4].

The oxidative damage (or oxidative stress) theory postulates that accumulation of molecular damage caused by reactive oxygen species (ROS) contributes significantly to the functional decline and increase in mortality that happens in the process of ageing [5]. Ageing has therefore been viewed as the result of an increase in oxidative stress, resulting from increased ROS production and/or a decline in cellular antioxidant defenses. In particular, the superoxide radical, $O_2^{\cdot-}$, generated as a by-product of mitochondrial respiration, has

been viewed as a potential major contributor to aging [5, 6]. Consequently, antioxidants capable of scavenging excessive ROS may help to maintain oxidative homeostasis and prevent related damages.

The major challenge with lifespan extension studies is the longtime span requirement (in months and years) in mammalian systems. This can be circumvented using the Long-lived mutants in *C. elegans* (**Figure1**) which have opened the avenue for healthy lifespan extension research [7, 8] and in addition show fundamental mechanisms and systems similar to the mammalian system [9]. Further, the increase in longevity mechanisms identified in *C. elegans* is shown to follow similar patterns in flies [10], mice [11] and humans [12].

Like mammals, the nematode *C. elegans* has well-defined stress defense systems for protection from toxic compounds [13]. This nematode has a rapid life cycle and a short lifespan with a clearly defined pattern of senescence [14, 15]. It manifests genetic pliability, a fully described developmental program, a well characterized genome, shows ease of maintenance, short and fertile life cycle and small body size. Moreover, *C. elegans* and humans share similar aspects of aging where oxidative stress is considered to be a major limiting

legs. It contains substances such as triterpenoids, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids, and hence it has been selected for this study [21].



Figure 2: *Bryophyllum pinnatum*

Previously, stress and aging related studies [22-26] have been carried out using various stress related parameters with plant extracts and molecules such as FGb761, EGCG, Phycocyanin, Phycoerythrin, *Ocimum sanctum* extracts but have shown less efficacy in the extension of lifespan.

Keeping in view the potent pharmacological properties of *B. pinnatum*, the present investigation was undertaken to assess the efficacy of different crude extracts of *B. pinnatum* in protection against age associated stress and ROS scavenging in *C. elegans*.

MATERIALS AND METHODS

Plant Material

Leaves of *Bryophyllum pinnatum* were collected from Gujarat University campus. The plant material was washed thoroughly under running tap water and was dried in an oven at 37 °C for one week. The dried

plant material was ground to a coarse powder and used for extract preparation.

Extraction

The selection of solvents was based on the available review of literature of various plants, which have extracted the potential phytochemical components such as phenolics and alkaloids [27]. Dried powder (10 g) was kept for defatting with petroleum ether (100 ml) for 24 hours on a magnetic stirrer. Each extract condition was initiated by taking the plant powder and selected solvent to 1:10 [28].

Further, extracts was prepared using with Methanol (M), Hydromethanol (HM) (60:40) and Aqueous (AQ) solvent by the Soxhlet extraction method. The filtrate was concentrated using a rotary evaporator and the crude extract of methanol, hydromethanol and aqueous was stored at 4 °C.

Phytochemical analysis

Estimation of Total Phenolic content

Total phenolic content was estimated by the Folin-Ciocalteu method as described by Herald *et al.* [29] with slight modifications. A volume of 25 µl crude extract was added into the well and to this 25 µl Folin Ciocalteu reagent was added. At the end of the 6th minute, 100 µl of 7.5% Na₂CO₃ was added to the mixture. The plate was incubated in the dark for 90 minutes. The absorbance was measured at 765 nm in a

Multiskan Go plate reader of Thermo Fischer Scientific (Model no. 151002196). Gallic acid was used as a standard to generate a calibration curve (20µg/ml to 100µg/ml). The results were expressed in terms of gallic acid equivalent (mgGAE/g extract).

Antioxidant assay Free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH radical scavenging capacity assay was performed as described by Herald *et al.* [29] with some modifications. A volume of 25µl of crude extract (100-300µg/ml) was added to a 96 well plate and to this 200µl of freshly prepared 0.1 mM DPPH in methanol was added and incubated for 30 minutes. The absorbance was measured at 517 nm at the end of the incubation period using the Multiskan Go plate reader. A standard curve was prepared using Ascorbic acid (10µg/ml to 50µg/ml). The results were expressed as percentage, free radical scavenging activity. Appropriate blank and controls were also used in the experiment. The following formula was used to calculate % DPPH quenching:

$$\% \text{ DPPH quenched} = [1 - (\text{A sample} - \text{A blank}) / (\text{A control} - \text{A blank})] \times 100$$

C. elegans strains cultivation

The wild type *C. elegans* (N2, Bristol) and *E. coli* OP50 were obtained from the

Caenorhabditis Genetic Centre (CGC) at the University of Minnesota, USA. All strains were cultured on *E. coli* OP50 seeded nematode growth medium (NGM) agar plates at 20 °C as described by Brenner [30]. For synchronizing the worm population wild type *C. elegans* strain was given hypochlorite treatment and the resulting eggs were placed on NGM plates spotted with *E. coli* OP50 bacteria. Filter sterilized crude extracts were added to autoclaved NGM medium. For control experiment set, *C. elegans* strains were grown and maintained throughout in normal NGM plates. Age synchronized worms were obtained by sodium hypochlorite treatment as per the method described by Porta-de-la-Riva *et al.* [31].

Lifespan Assay

Lifespan assay of wild type N2 *C. elegans* was performed in 96 well plates in liquid S complete media using the method described by Solis and Petrascheck [32]. In each well of a 96 well plate containing 100µl of S complete medium along with OP50 as food source, around 10 worms of L1 stage were dispensed and allowed to attain adulthood. Later, in order to prevent progeny formation they were treated with 5-fluoro-2'-deoxyuridine (FUDR) on day 0 of adulthood. Then, DMSO as control and the plant extracts were added in the respective wells. Plant extracts and OP50 were

supplied to the worms once in a week to ensure presence of food and crude extracts throughout the experiment. The worms were maintained at 20°C throughout the experimental study. The average number of worms (n) used in the present study was 50-55 in each group. All the experimental studies were performed in triplicates. The survival of worms was observed under inverted microscope every day. Statistical analysis for lifespan was done using Log-rank test and Kaplan Meier survival curve.

Stress resistance assay

A group of age-synchronized L4 stage (5 days old) N2 worms were transferred to fresh NGM plates with and without crude extracts and subjected to oxidative (H₂O₂) stress. Animals were subjected to oxidative stress by soaking them in 10 mM H₂O₂ solution [33]. Soaked worms were allowed to recover for 16 hours by transferring them to fresh NGM plates at 20 °C; live worms were scored following the recovery period.

Intracellular ROS detection by DCFH-DA fluorescence staining

The study was carried out with hydromethanolic extract (100 µg/ml) in three different groups of N2 *C. elegans*: Group I (Control), grown under normal condition; Group II, grown under stress (10 mM Paraquat); Group III, grown under oxidative stress (10 mM Paraquat) with crude extract (100 µg/ml). After 24 hours

of incubation, the worms were stained with 2,7- dichlorodihydrofluorescein diacetate (DCFH-DA) to detect their intracellular ROS level. Staining was performed by soaking the worms in 5 µm (final concentration) DCFH-DA solution for 30 minute at 20 °C. The DCFH-DA stained N2 *C. elegans* were imaged by Leica DMRB fluorescent microscope (USA) in epifluorescence mode with 10X objective lens. The intensity of florescence was quantified by image J software in terms of pixel density of fluorescence [34].

RESULTS

Extract Yield

In the present study, the % yield of methanolic extract was found to be 13.84%, hydromethanolic extract 27.16% and aqueous extract 7.01% (Table 1).

Total Phenolic Content

The results of total phenolic content are as shown in Table 1.

In the present study, the total phenolic content was estimated by folin-ciocalteau method. The total phenolic content of all extracts in BP leaves was found to be 1.63±0.0045 (Methanol), 1.86±0.0097 (HydroMethanol) and 1.57±0.0012 (Aqueous) (Standard Gallic Acid Equivalent).

Antioxidant Capacity (DPPH)

The antioxidant capacity of the plant extract was estimated by DPPH and the results are

as shown in Figure 3. The DPPH test is used to measure the hydrogen atom or electron donor capacity of the extract to the stable radical formed in the solution [35]. A standard curve was created using a range 10 µg/ml to 50 µg/ml of ascorbic acid (average $R^2 = 0.9682$). For the extracts prepared with Soxhlet method, the % free radical scavenging activity ranged from 19.9±0.17% to 65.8±1.10% of methanol extract. For hydromethanol extract it ranged from 56.1±1.19% to 89.6±0.04%. And for the aqueous extract, it ranges from 11.5±1.26% to 22.1±0.96%.

H₂O₂ stress

Bryophyllum pinnatum (BP) treated worm's revealed enhanced tolerance against oxidative stress which was induced by 10mM H₂O₂. The mean survival of aqueous extract of BP (AQ100) treated worms which were subjected to oxidative stress (10mM H₂O₂) was found to be non-significantly altered compared to control. The mean survival of methanolic extract of BP (M100) was found to be significantly increased ($p < 0.033$) after 3 hours compared to control worms. Whereas, hydro-methanolic extract of BP (HM100) treated animals subjected to 10mM H₂O₂ stress up to one hour and two hours showed significant increase in mean survival ($p < 0.002$). Furthermore, after 3 hours the

mean survival was found to be highly significant ($p < 0.0001$) (Figure 4).

Hence, the results of oxidative stress tolerance assay revealed antioxidative potential of BP-HM100 extract to enhance stress tolerance in *C. elegans*.

Lifespan Assay

Effect of BP on lifespan of *C. elegans* was examined by growing the L4 stage wild type N2 worms at 20 °C under different BP crude extracts (M100, HM100, AQ100). The number of dead animals were scored every second day from young adult stage till complete death of all the animals. The mean lifespan of control animals was found to be 14.91±0.79 (mean lifespan±standard error of mean) days compared to BP-treated animals showing respective values of 15.88±0.92 days at AQ100 µg/ml, 18.5±0.78 days at M100µg/ml and 17.5±1.10 days at HM100 µg/ml BP crude extract ($p < 0.001$, log-rank test) (Figure 5).

Intracellular ROS detection by DCFH-DA fluorescence staining

Furthermore, to confirm whether the lifespan extension effect of crude extract is due to its antioxidant activity, the intracellular-ROS level of N2 *C. elegans* has been checked *in vivo* using DCFH-DA fluorescence stain. Results showed a tremendous increase in the intracellular ROS associated green fluorescence in N2

C. elegans body under paraquat-induced oxidative stress. Whereas, the HM100 supplement was seen to limit the generation of intracellular-ROS and thus the onset of aging under oxidative stress. Thus, HM100 treatment limits the intracellular ROS production under paraquat induced oxidative stress. **Figure 6 (A, B and C)** shows fluorescence microscopic image of DCFH-DA stained control, paraquat

(10mM) treated and paraquat (10mM) + HM100 (100 µg/ml) treated adults N2 *C. elegans*, respectively.

Corrected total cell florescence (CTCF)

To measure the total cell florescence following formula was employed:

CTCF= Integrated Density – (Area of Selected cell × Mean florescence of background readings) (**Figure 7**).

Table 1: Extract Yield and Total Phenolic Content (TPC) of various extract of *Bryophyllum pinnatum*

Extracts	% Yield	Total Phenolic content (TPC) (mgGAE/G)
Methanol	13.84	1.63 ± 0.0045
Hydromethanol	27.16	1.86 ± 0.0097
Aqueous	7.01	1.57 ± 0.0012

Value is Mean ± S.E.

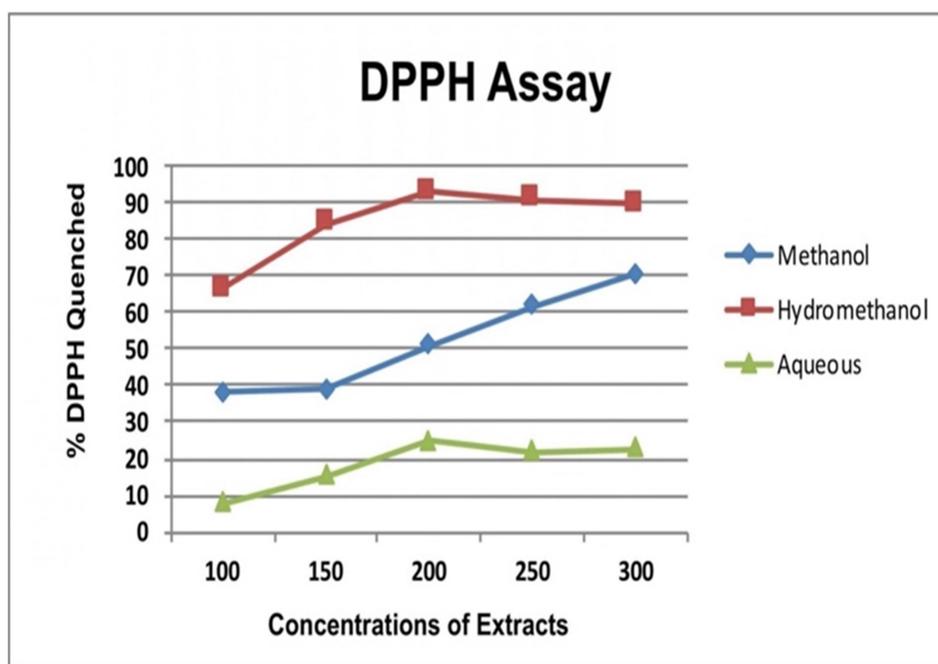
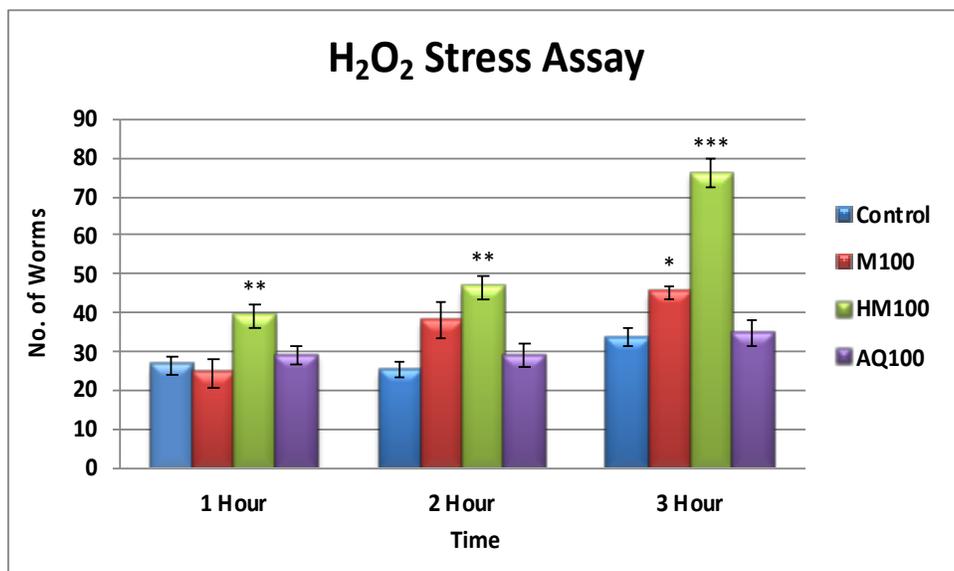
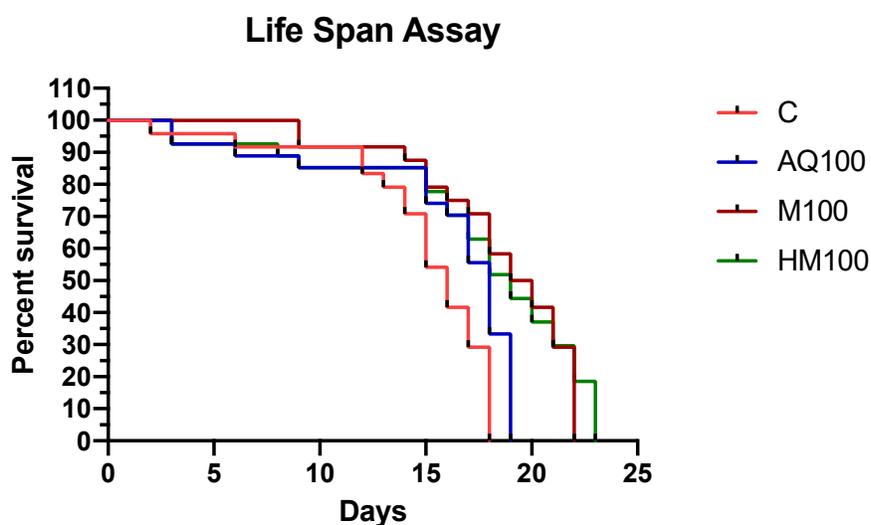


Figure 3: DPPH scavenging activity of *Bryophyllum pinnatum* extracts



*p<0.033; **p<0.002; ***p<0.0001
 (M100=Methanol extract; HM100=Hydromethanol extract; AQ100=Aqueous extract)
 Figure 4: Oxidative stress resistance by various *Bryophyllum pinnatum* extracts



(M100=Methanol extract; HM100=Hydromethanol extract; AQ100=Aqueous extract)
 Figure 5: Lifespan assay of *Caenorhabditis elegans*

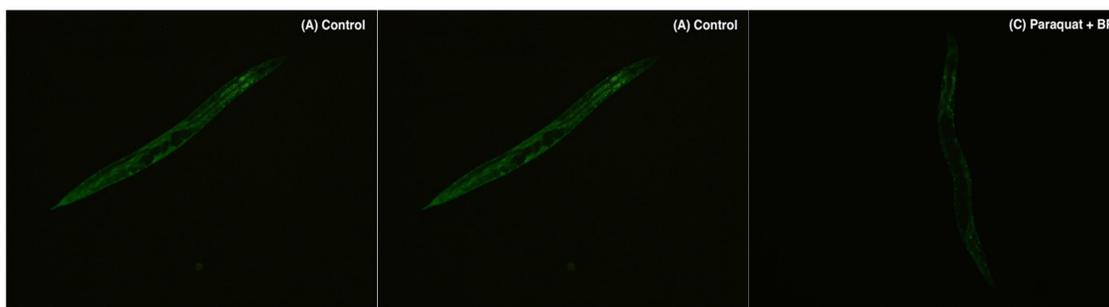


Figure 6: DCFH-DA stained Control (A), Paraquat treated (B) and Paraquat+BP (HM100) treated (C) adult *C. elegans*

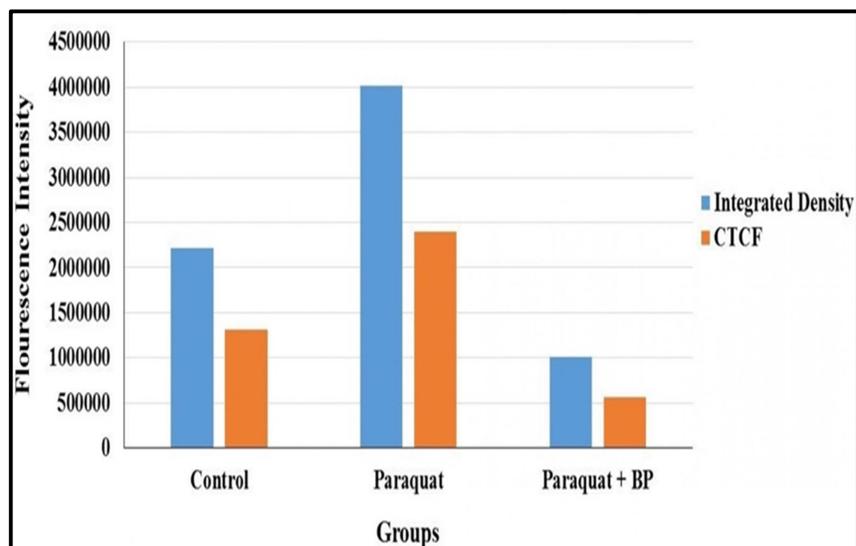


Figure 7: Corrected Total Cell Fluorescence (CTCF) of *C. elegans*

DISCUSSION

Aging pattern between *C. elegans* and mammals has a considerable degree of similarity and hence *C. elegans* has turned out to be a potential model organism for ageing related studies to explore potential anti-ageing drugs. Reactive oxygen species (ROS) has been reported as a major cause of ageing, and antioxidants have been reported to curtail the ageing effects of ROS. Antioxidants reduce the extent of free radicals-mediated oxidative damage and hence result in increased lifespan and healthy ageing. Several studies are being undertaken to explore possible anti-ageing wonder drugs. We too investigated the potency of *B. pinnatum* as a possible candidate for curtailing ageing and age-related disorders.

The results of the present investigation revealed that *B. pinnatum* significantly enhanced stress tolerance and also

increased the mean lifespan of the worms during oxidative stress (H_2O_2) and paraquat induced oxidative stress. Similar to other herbal formulations, our results also suggest that *B. pinnatum* reduces the ROS level which might be the possible explanation for the worms' survival under stress conditions.

The phytomolecules in plant extracts efficiently ameliorate disease conditions of various ailments caused by different stressors due to their metal chelating and free radical scavenging properties. The *in vitro* antioxidant activities depend on the total polyphenolic contents and in the present study we have observed that the hydromethanolic extract showed highest total phenolic content (1.86 ± 0.0097 mg GAE/g) among the various extracts of *B. pinnatum*, which may explain its anti-ageing and stress resistance activity. Moreover, the result indicated that in the *B.*

pinnatum leaf extract the total polyphenolic content was increased in a concentration dependent manner.

Also, DPPH radical scavenging activity was found to be increased in a dose dependent manner. DPPH is a stable free radical and loses its deep purple colour and turns to yellow upon reaction with any oxidising compound. The method validates the ability of radical scavengers or hydrogen donors to attenuate free radical induced oxidative stress. In the present study, the highest free radical scavenging activity was observed in the hydromethanol extract (89.6±0.04%) whereas aqueous extract (22.1±0.96%) had the lowest free radical scavenging activity. In accordance to our study, previous study with the methanolic fraction of ethyl acetate extract of *Nyctanthes bortristis* leaf has demonstrated 23% DPPH scavenging activity [36]. Moreover, another study on *Nyctanthes bortristis* also showed 27.8% free radical scavenging activity of its methanolic extract [37]. Hence, it is evident that a significantly higher free radical scavenging activity was obtained in the *B. pinnatum* HM extract used in this study.

Consistent with previous study, our results revealed that *B. pinnatum* has *in vitro* antioxidant activities [38]. Furthermore, our results also corroborate the previous investigation which revealed similar trend

of total phenolic content and DPPH activity in *Bacopa monnieri* [34].

Our results showed that upon treatment of *C. elegans* with various *B. pinnatum* crude extracts, the mean lifespan of treated group was increased as compared to control. This could be attributed to the rich phytoconstituent composition of *B. pinnatum* extracts. Similar findings have also been reported by other researchers where in the phytoconstituents of *Oscimum sanctum* and *B. monnieri* have significantly enhanced the mean lifespan of the worms [24, 34]. However, the extent of survival obtained in our study was significantly higher than that described by these authors. Evidence of the *in vivo* intracellular free radical scavenging activity of the *B. pinnatum* extract in *C. elegans* was obtained by using a non-fluorescent dye HDCF-DA. Cellular ROS levels can be measured in live cells by a technique that converts 2',7'-dichlorofluorescein diacetate (DCFDA) which is oxidized to a fluorescence dye 2',7'-dichlorofluorescein (DCF). The fluorescence generated is directly proportional to the amount of DCFDA oxidized to DCF. Since the emission of the fluorescent dye is at 529nm, it can be measured in the FL-1 green channel by fluorescent microscopy. The non-polar and non-ionic probe, H2DCFDA, can easily penetrate the

cellular membrane and is enzymatically deacetylated by esterases. This biochemical reaction turns H₂DCFDA into the non-fluorescent compound H₂DCF which is then rapidly oxidized to highly fluorescent 2',7'-dichlorofluorescein (DCF) in the

presence of ROS. This non-fluorescent dye after interaction with intracellular ROS is converted into a fluorescent compound 2',7'-dichlorofluorescein (DCF) [39] (Figure 8).

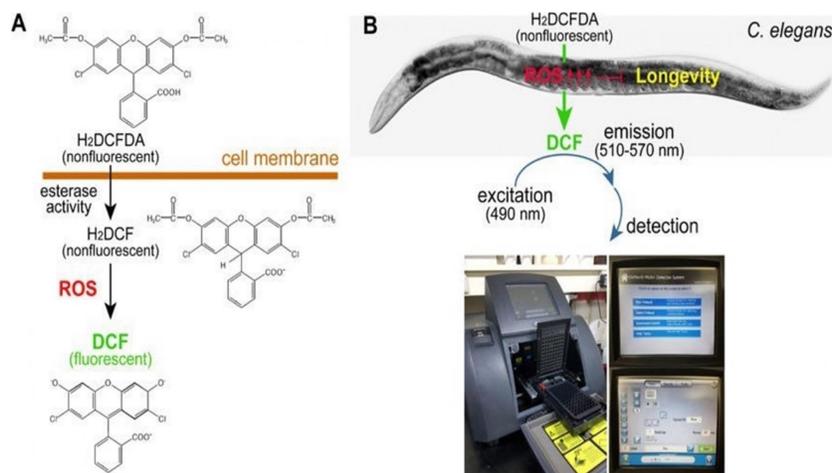


Figure 8: Mechanism of DCFH-DA stain on *C. elegans* (Source: Yoon et al., 2018)

A. Production of fluorescent DCF by intracellular ROS

B. Measurement of intracellular ROS using molecular probe (H₂DCFDA) in *C. elegans*.

Our results indicated that hydromethanolic extract of *B. pinnatum* reduce the intracellular ROS level in *C. elegans*. These results demonstrated the *in vitro* and *in vivo* antioxidant potency of *B. pinnatum*. Similar results were also reported in a recent study which has demonstrated paraquat induced oxidative stress in *C. elegans* and its mitigation by phycocyanin [22].

Hence, present investigation indicates antioxidant, anti-ageing and stress tolerance efficacy of *B. pinnatum* using *C. elegans*.

CONCLUSION

Natural herbs are widely used for various medication due to their efficacy, safe mode of action and least side effects. This study confirms *in vitro* and *in vivo* antioxidant activities of *B. pinnatum* in *C. elegans*. Results of the present study reveal a hitherto unknown role of *B. pinnatum* in prolonging the lifespan and health condition in *C. elegans*. Moreover, the present investigation also indicates that treatment with *B. pinnatum* not only enhances the lifespan but also causes delay in the ageing mechanism. Our findings provide quite strong evidence for *B. pinnatum* as a potent anti-ageing and stress

tolerance agent. It is also suggested that the protective and lifespan extending action of the crude extract is not only due to its antioxidant capacity but may also be mediated by modulation of certain related signalling pathways. In addition, further research needs to be carried out using mammalian systems to evaluate potential efficacy of *B. pinnatum* as a wonder drug to enhance lifespan and reduce oxidative stress.

ACKNOWLEDGMENTS: None.

CONFLICT OF INTEREST: None.

FINANCIAL SUPPORT: None.

ETHICS STATEMENT: None.

REFERENCES

- [1] Buonocore D, Rucci S, Vandoni M, Negro M, Marzatico F. Oxidative system in aged skeletal muscle. *Muscles Ligaments Tendons J.* 2011; 1(3): 85–90.
- [2] Finkel T. Signal transduction by reactive oxygen species. *J Cell Biol.* 2011; 194(1): 7–15.
- [3] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature.* 2000; 408(6809): 239–47.
- [4] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature.* 2006; 440(7086): 944–8.
- [5] Beckman KB, Ames BN. The Free Radical Theory of Ageing. *Physiol Rev.* 1998; 78(2): 547–81.
- [6] Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and ageing. *Science.* 1996; 273(5271): 59–63.
- [7] Kenyon C, Jean C, Gensch E, Adam R, Ramon T. A *C. elegans* mutant that twice as long as wild type. *Nature.* 1993; 366(6454): 461–4.
- [8] Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. Daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science.* 1997; 277(5328): 942–6.
- [9] Arya U, Das CK, Subramaniam JR. *Caenorhabditis elegans* for preclinical drug discovery. *Curr Sci.* 2010; 99(12): 1669–80.
- [10] Hercus MJ, Loeschke V, Rattan SIS. Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology,* 2003; 4(3): 149–56.
- [11] Beese K, Baur B. Expandable spermatheca influences sperm storage in the simultaneously hermaphroditic snail *Arianta arbustorum*. *Invertebr Reprod Dev.* 2006; 49(1–2): 93–01.
- [12] Longo VD, Antebi A, Bartke A, Barzilai N, Brown-Borg H, et al. Interventions to slow ageing in humans: Are we ready? *Ageing Cell.* 2015; 14(4): 497–10.
- [13] Van Raamsdonk JM, Hekimi S. Reactive oxygen species and ageing in *Caenorhabditis elegans*: Causal or casual relationship? *Antioxidants Redox Signal.* 2010; 13(12): 1911–53.
- [14] Klass MR. Ageing in the nematode *Caenorhabditis elegans*: Major biological

- and environmental factors influencing lifespan. *Mech Ageing Dev.*1977; 6(C): 413–29.
- [15] Bolanowski MA, Russell RL, Jacobson LA. Quantitative measures of ageing in the nematode *Caenorhabditis elegans*, Population and longitudinal studies of two behavioral parameters. *Mech Ageing Dev.*1981; 15(3): 279–95.
- [16] Harrington LA, Harley CB. Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. *Mech Ageing Dev.*1988; 43(1): 71–78.
- [17] Wolkow CA, Kimura KD, Lee MS, Ruvkun G. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science.* 2000; 290(5489): 147–50.
- [18] Antebi A. Genetics of Ageing in *Caenorhabditis elegans*. *PLoS Genet.* 2007; 3(9): 1565–71.
- [19] Artal-Sanz M, de Jong L, Tavernarakis N. *Caenorhabditis elegans*: A versatile platform for drug discovery. *Biotechnol J.* 2006; 1(12): 1405–18.
- [20] Noroozi M, Angerson WJ, Lean MEJ. Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. *Am J Clin Nutr.*1998; 67(6): 1210–18.
- [21] Kamboj A, Saluja A. *Bryophyllum pinnatum* (Lam.) Kurz.: phytochemical and pharmacological profile: a review. *Pharmacognosy Reviews*, 2009; 3(6): 364.
- [22] Singh NK, Sonani RR, Awasthi A, Prasad B, Patel AR et al. Phycocyanin moderates ageing and proteotoxicity in *Caenorhabditis elegans*. *J Appl Phycol.*2016; 28(4): 2407–17.
- [23] Sonani RR, Singh NK. Phycoerythrin extends lifespan and health span of *Caenorhabditis elegans*. 2014; 9717: 1-14.
- [24] Pandey R, Gupta S, Shukla V, Tandon S, Shukla V. Antiageing, antistress and ROS scavenging activity of crude extract of *Ocimum sanctum* (L.) in *Caenorhabditis elegans*. *Indian J Exp Biol.* 2013; 51(7): 515–21.
- [25] Abbas S, Wink M. Green tea extract induces the resistance of *Caenorhabditis elegans* against oxidative stress. *Antioxidants.* 2014; 3(1): 129–43.
- [26] Vining Smith J, Luo Y. Elevation of oxidative free radicals in Alzheimer's disease models can be attenuated by *Ginkgo biloba* extract EGb 761. *J Alzheimer's Dis.* 2003; 5(4): 287–300.
- [27] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Internationale pharmaceutica scientia.* 2011; 1(1): 98-06.
- [28] Azwanida NN, A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants.* 2015; 4(196): 2167-0412.
- [29] Herald TJ, Gadgil P, Tilley M. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *J Sci Food Agric.* 2012; 92(11): 2326–31.
- [30] Brenner S. The genetics of *Caenorhabditis*

- elegans*. Genetics. 1974; 77(1): 71–94.
- [31] Porta-de-la-Riva M, Fontrodona L, Villanueva A, Cerón J. Basic *Caenorhabditis elegans* Methods: Synchronization and Observation. JoVE J. Vis. Exp. 2012; 64: e4019–e4019.
- [32] Solis GM, Petrascheck M. Measuring *Caenorhabditis elegans* life span in 96 well microtiter plates. JoVE (Journal of Visualized Experiments). 2011Mar; 18(49): e2496.
- [33] Cai WJ, Huang JH, Zhang SQ, Wu B, Kapahi P et al. Icaritin and its derivative icaritin II extend healthspan via insulin/IGF-1 pathway in *C. elegans*. PLoS One. 2011; 6(12): 1–11.
- [34] Phulara SC, Shukla V, Tiwari S, Pandey R. *Bacopa monnieri* promotes longevity in *Caenorhabditis elegans* under stress conditions. Pharmacogn Mag. 2015; 11(42): 410–16.
- [35] Tepe B, Eminagaoglu O, Akpulat HA, Aydin E. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and *S. verticillata* (L.) subsp. *amasiaca* (Freyn & Bornm.) Bornm. Food Chem. 2007; 100(3): 985–989.
- [36] Rathee JS, Hassarajani SA, Chattopadhyay S. Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract. Food Chem. 2007; 103(4): 1350–7.
- [37] Sandhya Kumari TD, Sudha Madhuri TD, Singara Charya MA, Subba Rao K. Antioxidant and anticancer activities of *Nyctanthes arbor-tristis*. Int J Pharm Pharm Sci. 2012; 4(4): 452–454.
- [38] Anand T, Naika M, MSL S, Khanum F. Antioxidant and DNA damage preventive properties of *Bacopa monniera* (L.) Wettst. Free radicals antioxidants. 2011; 1(1): 84–90.
- [39] Yoon D, Lee MH, Cha D. Measurement of Intracellular ROS in *Caenorhabditis elegans* Using 2',7'-Dichlorodihydrofluorescein Diacetate. Bio-Protocol. 2018; 8(6): 1–8.