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ANANAS SATIVA INCREASES LONGEVITY THROUGH OXIDATIVE STRESS RESISTANCE IN *DROSOPHILA MELANOGASTER*

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Abstract

Background: Ageing is the major risk factor for most non-communicable diseases. It occurs as a result of free radical damage to macromolecules in the cell, coupled with environmental stress assaults. In the quest to identify new anti-ageing bioactive compounds from natural products, and despite the therapeutic values of *Ananas sativa*, its anti-ageing activity has not been fully elucidated. Therefore, the aim of this study was to screen the crude, methanol fruit extract of *Ananas sativa* (MEAS) and its fractions, for anti-ageing bioactivity in the fruit fly, *Drosophila melanogaster* w^{1118} wild type flies.
Materials and Methods: Flies reared on food supplemented with 5, 10, and 20mg/ml fruit pulp and juice extract were assayed for longevity, fertility and stress resistance according to established protocols. Phytochemical composition and scavenging activity of extract on 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) were equally evaluated. The crude extract anti-ageing activity was assayed through a successive bioassay-guided fractionation approach, with different extraction solvents; n-Hexane, Chloroform, Ethyl acetate and Acetone.
Results: There was a dose-dependent effect of the fruit extract on lifespan, fertility and oxidative stress resistance, with a unique information on the anti-ageing bioactivity of *Ananas sativa*. The IC_{50} values of the fruit extract and Ascorbic acid positive control in the free radical scavenging activity were 248.15µg/ml and 81.51µg/ml respectively. However, the bioactive fraction obtained from the Ethylacetate fraction F1, did not exhibit oxidative stress resistant effect in the model organism.
Conclusion: The methanol fruit extract of *Ananas sativa* possesses anti-ageing bioactivity through oxidative stress resistance.

Keywords: Longevity; *Ananas sativa* fractions; antioxidants; free radical scavenging; oxidative stress resistance; *Drosophila melanogaster*.

Abbreviations: ROS; Reactive Oxygen Species, **DPPH**; 2, 2-diphenyl-1-picrylhydrazyl free radical, **PQ**; Paraquat (1, 1-dimethyl-4-4-bipyridynium dichloride), **MEAS**; Methanol Fruit Extract of *Ananas sativa*, **FRTA;** Free Radical Theory of Ageing, **MDA**; Malondialdehyde.

Introduction

Ageing is a gradual loss in biological function at the cellular, tissue and organ levels (Lopez-Otın *et al.,* 2013). As a result of its complex nature, it remains one of the most deliberated concepts. There are many factors that affect the ageing process, these factors include genetic, environmental, nutrition, cellular redox status, among others. Experimental aggravation of any of these factors could accelerate the process, while an experimental amelioration of any of these factors could slow down the on-set of ageing (Lopez-Otın *et al.,* 2013). Ageing is the major risk factor for diversity of non-communicable diseases such as diabetes, cancer, cardiovascular disorders, and neurodegenerative disorders. The role of nutrition in ageing is still an emerging area of research interest.

Nutrition and exogenous antioxidants from fruits and vegetables play very important role in ageing. However, despite the well documented nutritional and medicinal value of *Ananas sativa* (Pineapple pulp and juice), its possible role in anti-ageing is yet to be fully elucidated. *Ananas* which belong to the Bromeliaceae family, is the third most abundant fruit crop in the tropical region of the world (Lu *et al.,* 2014). It is highly valued for its sweet taste and aroma. It is a very good source of antioxidants, vitamins and minerals which are essential for normal metabolic cellular processes. It consists of a proteolytic enzyme complex called Bromelain. Bromelain is a potent bioactive compound in pineapple fruit and stem, which has been reported to have variety of medicinal and therapeutic importance (Taussig and Stanley, 1988).

Furthermore, the synergistic effect of the phytochemicals found in pineapple endorses the plant to be effective in cancer and cardiovascular diseases prevention (Liu, 2003; Rui Hai Lu, 2004). Antioxidants are molecules which enhance the scavenging of free radicals produced inevitably within the cells, these are essential components of the fruit. The activity of endogenous antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidases have been shown to be increased with regular consumption of exogenous antioxidants, and this may play a very crucial role in the ageing process (Guerra-Araiza *et al.,* 2013). The mechanism of action of these endogenous antioxidant enzymes, are highly enhanced by coenzymes and cofactors, most of which are obtained from the minerals and vitamins in fruits and vegetables. Some of these minerals and vitamins such an manganese, copper, magnesium, iron, selenium, zinc and ascorbic acid are nutritional components of *Ananas* (Ancos *et al.,* 2017).

Other parts of the fruit, such as the pineapple peel, has also been shown to possess medicinal and pharmacological activities, such as antibacterial activities (Lawal *et al.*,2013). The peel is equally rich in phytochemicals such as flavonoids, tannins, terpenoids, alkaloids, saponin and phytosterols (Lawal, 2013), the phytoconstituents in the fruit of Pineapple could ameliorate oxidative stress induced damage, such as cancer (Kalaiselvi *et al.,* 2013). Pineapple bromelain has been reported to be part of the active ingredients in topical formulations in cosmetics and creams of therapeutic importance (Lourenc *et al.,* 2016). However, the central role of the fruit in ameliorating the process of ageing is still an emerging area of interest.

The Free Radical Theory of Ageing (FRTA) which was proposed by Denham Harman in 1956 is currently the most widely accepted theory of Ageing. Harman proposed that accumulation of free radical damage to cellular macromolecules is the major underlying factor of ageing and the major determinant of lifespan (Harman, 1956). The cumulative damage by free radicals, over time, increases the susceptibility of organisms to diseases and death, which is accompanied by age advancement (Harman, 1981). Majority of the supporting evidence for the theories of ageing comes from investigations using model organisms such as *Drosophila melanogaster;* fruit fly, *Caenorhabditis elegans;* worms, and *Saccharomyces cerevisiae;* yeast. These model organisms have common ageing pathways which makes them suitable for ageing researches, basically, due to their relatively short lifespan (Pandey *and* Nichols, 2011). *Drosophila melanogaster* has been a wonderful tool in the genetics of ageing and in the discovery of some therapeutic agents. The present study made use of this simple but powerful model organism to reveal the ability of *Ananas sativa* fruit extract to increase resistance to oxidative stress.

In the W.H.O report on ageing and health (2015), recommendations were made on feasible future prospect into healthy ageing, by focusing on the concept of functional ability, through various approaches of ageing research (Beard *et al.,* 2015). Therefore, the central aim of this study was to evaluate the anti-ageing bioactivity of the crude methanol fruit extract of *Ananas sativa* and its fractions, and the possible process by which they could enhance longevity in a simple model organism; *Drosophila melanogaster*.

Materials and methods

Materials

Experimental procedure and animal handling were in compliance with standard operating measures. The model organism used in this study does not have the capability of transmitting infections and diseases whatsoever. They are safe model organisms for biomedical research. All chemicals and reagents used in this study were of analytical grade from Sigma-Aldrich and Sigma Zanyo: Methanol, Triton X-100, Catalase from bovine liver (2000-5000 units/mg protein, the product number C9322-5G, Nipagin (methyl-*p*-hydroxybenzoate), n-Hexane, Chloroform, Ethyl acetate, Acetone, Propionic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), L-Ascorbic acid and Paraquat (PQ) (1, 1-dimethyl-4-4-bipyridynium dichloride), Thiobarbituric acid, Silica gel powder for column chromatography (0.040-0.063mm) M = 60.08g/mol (Germany).

Method: Plant Collection, Authentication, Extraction, and Fractionation

Fresh fruits of *Ananas sativa* were obtained from the western part of Uganda at Bushenyi district. The fruit was identified as *Ananas sativa* by a Botanist at Makerere University in Uganda. Accession number of 43344 was assigned to the fruit which was deposited at the University herbarium. The fresh fruit were extracted according to previously established protocol (Lu *et al.,* 2014). Briefly, 100g of pineapple fruit pulp was dry blended and extracted in 1000ml of 80% methanol, after sieving using a muslin cloth, extract was concentrated at 40^{o}C using a rotatory evaporator. The crude extract, a light golden brown concentrate, was stored in an air tight container, wrapped in a foil paper, at 4^{o}C for further experimental use. The crude extract obtained was 118.92 g (equivalent to a yield of 8.2% w/w; % Yield = (Final weight of crude extract / original weight) x 100). Methanol was used for extraction due to its wide solubility properties.

After establishing the anti-ageing effect of the crude methanol fruit extract of *Ananas* in the flies using the following assays; longevity, oxidative stress resistance, climbing activity, and reproductive performance, the dried methanol fruit extract (200g) was further subjected to a successive extraction, using different extraction solvents (50%); n-Hexane, Chloroform, Ethyl acetate, and Acetone. Five resulting fractions were obtained in the following order; n-Hexane extract, Chloroform extract, Ethyl acetate extract, Acetone extract and the water residue. Extracts were kept at 4^{o}C and their longevity effect were evaluated. Thereafter, with the aid of a column chromatographic technique, the ethyl acetate fraction, which gave the highest bioactivity, was further fractionated according to previously described method (Krishnamurthy *et al.,* 2015). Thirteen (F_{13}) fractions were obtained and subjected to free radical scavenging activity. The most active fraction from the F_1-$F_{13}$was tested for anti-ageing activity through oxidative stress resistance.

Fly stock husbandry and Oxidative stress Induction

In the present study, *Drosophila melanogaster* w^{1118} (white) strain was used. Virgin males were separated from the virgin females. Flies were maintained at 25^{o}C under a 12/12 hour light and dark cycle in a digital fly incubator (SPX-150A), on a standard culture cornmeal medium and raised at a standard density of 10 flies per fresh food medium changed twice a week. Each experiment included five replicas and was repeated on three independent occasions.
A day-old synchronized w^{1118} virgin flies were reared and treated with food supplemented with 0, 5, 10, and 20 mg/ml methanol fruit extract of *Ananas sativa* (MEAS) throughout their life. These concentrations were obtained based on preliminary toxicity studies. Flies were subjected to lifespan, climbing and reproductive performance assays. Heat stress assay was carried out by subjecting flies to a high temperature of 29^{o}C for 8hrs daily. As for the oxidative stress assay, after starving flies for 3hrs, oxidative stress was induced according to previously described method (Slack *et al.,* 2010). Briefly, 15mM Paraquat on filter paper impregnated with 5% Sucrose, after fourteen (14) days of pre-treatment with the *Ananas sativa* fruit extract. Paraquat (PQ) oxidative stress was induced within a period of 3hrs for 24 to 96hrs (Jahromi *et al.,* 2013). Dietary Stress was induced by rearing flies in a poor protein diet; 1% yeast w/v, food containing protein at a concentration of 1%, the inactive yeast used as a source of protein was prepared according to previously described method (Burger *et al*., 2010).

Lifespan, Climbing and Fertility assays

Longevity, climbing, and reproductive performance assays were carried out with advancement in age according to established procedures (Kinghorn *et al.,* 2015). Rate of survival was evaluated by a daily counting of live flies until all the flies have died. Climbing performance of flies was carried out with the aid of a negative geotaxis assay. The climbing activity of flies exposed to PQ was carried out after 24hr of exposure (Jahromi *et al.,* 2013). Survival analysis data were presented as cumulative survival curves and analyzed using log rank tests. Percentage (%) Increase in mean survival time = (T/C-1) X 100, where T is the mean survival time of treatment group and C is the mean survival time of control group. As for the reproductive performance assay or fertility assay, briefly, ten (10) females were housed with ten (10) males for each of the treatment groups and they were allowed to mate for ten days. After day ten, all the adults (parents) were removed from the vials. Thereafter, the number of offsprings that emerged from the eggs laid by the adult females were counted and recorded every 24hrs for ten days. The mean number that emerged for the ten days gave a measure of the reproductive performance.

Biochemical assays: Catalase activity and Lipid peroxidation assay

The whole body of forty (40) flies per group were rendered motionless by chilling them on ice, they were manually grounded and homogenized in ice cold phosphate buffer saline 2 mL (0.1 M, pH 7.4), centrifuged at 2,500g for 10 min at 4^{o}C, supernatant was filtered, resulting homogenate was kept at 4^{o}C for the biochemical assay. *In-vivo* catalase activity was measured as previously described by Iwase *et al* (2013). Catalase activity unit is defined as the number of micromoles of hydrogen peroxide decomposed per unit time. Lipid peroxidation reaction was assayed using the previously established protocol (Ohkawa *et al.,* 1979), absorbance of lipid peroxidation product; malondialdehyde (MDA) produced

was measured at 532 nm. The concentration of protein in the homogenates of flies were measured using the standard protocol of Lowry *et al* (Lowry *et al.,* 1951) and bovine serum albumin was used as a standard.

Qualitative Phytochemical screening and *in vitro* Free radical scavenging activity

Phytochemical screening of MEAS and its five fractions were carried out according to standard established procedures. Extract was prepared in concentrations of 10, 20, 50 100, and 200µg/ml, and the free radical scavenging activity of the varying concentrations was measured using 2, 2-diphenyl-1-picrylhydrazyl DPPH (Kanti *et al.,* 2015).

Absorbance was measured at 517nm with a UV-VIS spectrophotometer (Spectronic 21D, Milton Roy). Ascorbic acid was used as a positive control. The percentage inhibition exerted by the extract on the DPPH free radical was calculated thus; % DPPH free radical scavenging = $(A_0 - A_1) / A_0 \times 100$.

Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD), with the aid of Graph Pad Prism version 6 Statistical software. Survival analysis was carried out with log rank tests. Significant differences were analyzed using one-way or two-way ANOVA with Dunnet's and Turkey's post hoc or multiple comparison tests. The DPPH free radical scavenging activity was analyzed using a linear regression, and the results obtained were considered as statistically significant if $p < 0.05$.

Results and Discussion

***Ananas sativa* supplementation increases lifespan and fertility.**

In order to test the anti-ageing effect of MEAS, *Drosophila melanogaster* Wild type virgin flies were exposed to different concentrations of the fruit extract. There was a concentration dependent effect of the extract on longevity and fertility (Fig. 1), with the most effective concentration being the 20mg/ml. The percentage increase in the mean survival time of the male and female flies were 5, 3, and 11% and 3, 11, and 20% respectively. That of the offspring was 8, 8 and 15% relative to control (Table 1). Climbing activity with age, which is a marker of health span, was also enhanced by the extract, (Suppl. Fig1). The lifespan extending effect of *Ananas* could be due to its antioxidant value, and its ability to increase oxidative stress resistance through various nutritional pathways as previously shown with other plant products, as in the mechanism of lifespan extension by Rapamycin in fruit flies (Bjedov *et al.,* 2010). Pineapple could have enhanced longevity in the model organism, due to its nutritive constituents, certain nutrients such as carbohydrates, proteins, minerals and vitamins have been shown to play significant roles in longevity of organisms (Lee *et al.,* 2015). The role of diet and nutrition in longevity has equally been shown in variety of model organisms including *Drosophila melanogaster* (Fontana *and* Partridge, 2015). Furthermore, Resveratrol, the plant polyphenol found in grape berry skin has been shown to increase lifespan in the same model organism (Bass *et al.,* 2007), the same phenomenon same goes for Pomegranate juice and *Bacopa monnieri*, whose phytoconstituents have been shown to regulate cellular oxidative stress in *Drosophila* (Balasubramani *et al.,* 2014; Subramanian *et al.,* 2014). The mechanism by which the reported plant products enhances longevity is in their modulation of oxidative stress and their role in acting as dietary restriction mimetic in the nutrient sensing pathways (Wang *et al.,* 2012).

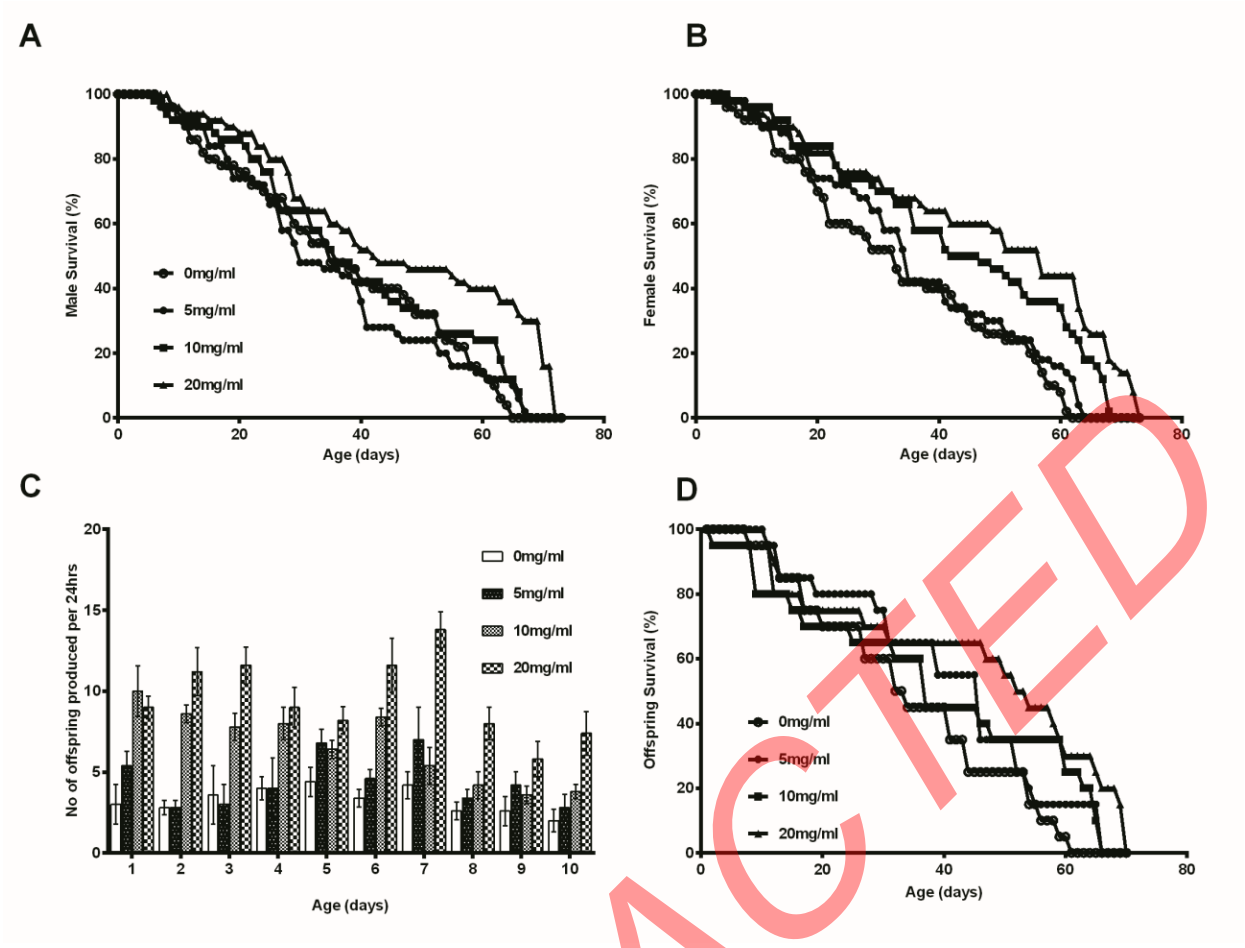


Figure 1: Effects of *Ananas sativa* on longevity and fertility

(A) The Survival of male flies showed a significant effect only in the 20mg/ml group ($P < 0.0384$) and **(B)** female fies showed significant effect in the 10 and 20mg/ml group relative to control ($P < 0.0229$ and 0.0006). **(C)** The health span promoting effect of *Ananas sativa* fruit extract was evident by a significant increase in the number of offspring produced per 24hr, only the 10 and 20mg/ml gave a significant high effect ($P < 0.001$ and 0.009).as evaluated by ANOVA, Dunnet's test. **(D)** The effect of the extract on the lifespan of the offspring produced, equally showed a significant increase with respect to the control in the 20mg/ml group ($P < 0.005$).

Table 1: Percentage increase in mean survival time of *Ananas* treated flies. There was an increase in mean survival time of the *Ananas* treated male flies by 5, 13 and 11%, and of the females by 3, 11 and 20% respectively. In the dietary stress assay, the lifespan of the offspring increased by 8, 8, and 15% respectively. The lifespan of the mothers subjected to reproductive performance assay increased by -9, 9 and 18% all with respect to the concentration of extract at 5, 10 and 20mg/ml respectively. The F1 fraction did not cause an increase in oxidative stress resistance in the flies MST = 0%.

Treatment group	% Increase in Mean Survival time		
	5 mg/ml	10 mg/ml	20 mg/ml
Males	5	3	11
Females	3	11	20
Offspring	8	8	15
Mothers	-9	9	18
F1 (2mg/ml)= 0%			

***Ananas sativa* supplementation increases resistance to oxidative stress**

Environmental factors such as pollutants, contaminants, UV radiation, chemical exposure, and neurotoxin like Paraquat could lead to generation of free radical induced damage, which is the front liner in the etiology of human diseases,

phytoconstituents from fruits and vegetables has been shown to ameliorate this effect (Menezes *et al.*, 2014) as well as ageing (Ziegler *et al.*,2015). In order to test the protective effect of MEAS on oxidative stress, flies were exposed to Paraquat-induced oxidative stress, heat stress and dietary stress (1% Protein diet). However, treatment with different concentrations of MEAS resulted in an increase in lifespan through resistance to oxidative stress (Fig. 2). Also shown in the present study was the significant ameliorative effect of the extract on climbing performance of flies as age progressed, (Suppl Fig. 1) and an increase in resistance to heat and dietary stress (Fig. 2). Therefore, the present study suggests a protective antioxidative and nutritive effect of MEAS in ameliorating accelerated ageing induced by stress and its ability to increase oxidative stress resistance. The characteristics of most anti-ageing compounds is stress resistance enhancement, they usually have the ability to increase longevity through oxidative stress resistant pathways (Kregel *and* Zhang, 2007). In addition, phytochemicals from fruits and vegetables, primarily activate a mild to moderate oxidative stress in cells so as to stimulate a beneficial effect within the cell, however, this is still an area of scientific deliberation (Bjørklund *and* Chirumbolo, 2017).

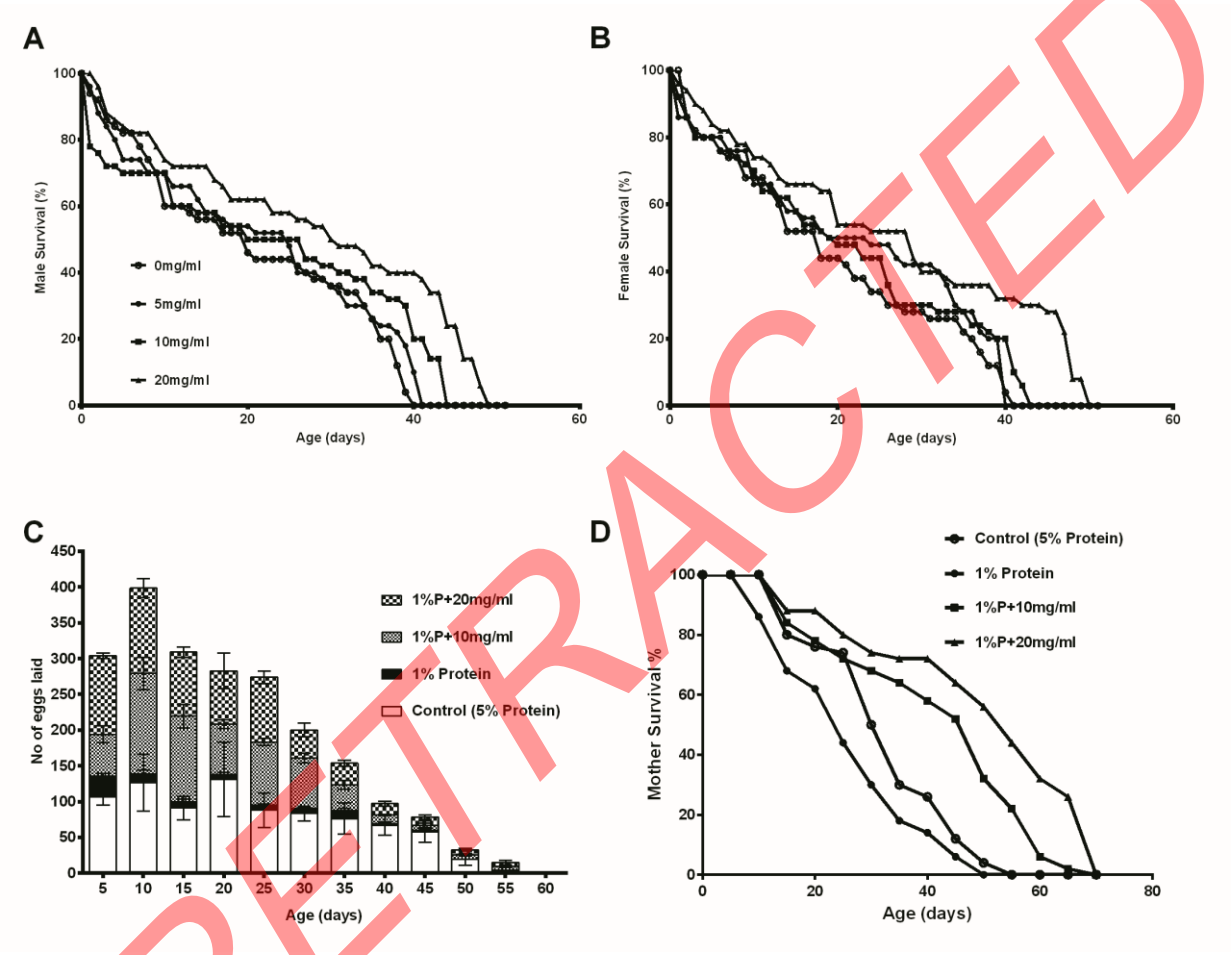


Figure 2: Effects of *Ananas sativa* on Heat stress and Dietary stress

(A and B) There was a significant increase in heat stress resistance as evident by the increase in lifespan of treated flies relative to control in the 20mg/ml group of male and female flies. **(C)** The rate of egg laying during exposure to poor protein diet (1% protein) was ameliorated significantly in the 20mg/ml group. **(D)** The longevity of the mothers who produced the eggs, in the same poor protein diet was equally improved with respect to the control group, as evaluated by two-way ANOVA, Tukey's post hoc test ($P < 0.0001$).

***Ananas sativa* modulates endogenous antioxidant defense mechanism**

In order to understand the mechanism by which *Ananas* ameliorates oxidative stress, the effect of the extract on endogenous lipid peroxidation and catalase activity were measured, as lipid peroxidation product, malondialdehyde, MDA is a biomarker of oxidative stress. Flies exposed to Paraquat and treated with the extract exhibited a significant reduction in malondialdehyde production in their homogenates which is a modulation of lipid peroxidation (Fig. 3). This modulatory effect observed could be as a result of the presence of vitamins and minerals which act as cofactors for antioxidant enzymes.

Polyphenolic compounds and flavonoids in the in the fruit could also have contributed to its modulation of oxidative stress and enhancement of endogenous antioxidant enzyme activities, as previously reported, that pineapple is able to prevent free radical induced diseases due to the synergistic action of the fruit antioxidants (Liu, 2004), inflammation, which is peculiar to oxidative stress response, have also been shown to be ameliorated due to the bioactive constituent of pineapple; bromelain. Bromelain has anti-inflammatory properties (Maurer 2001; Manzoor *et al.*, 2016).

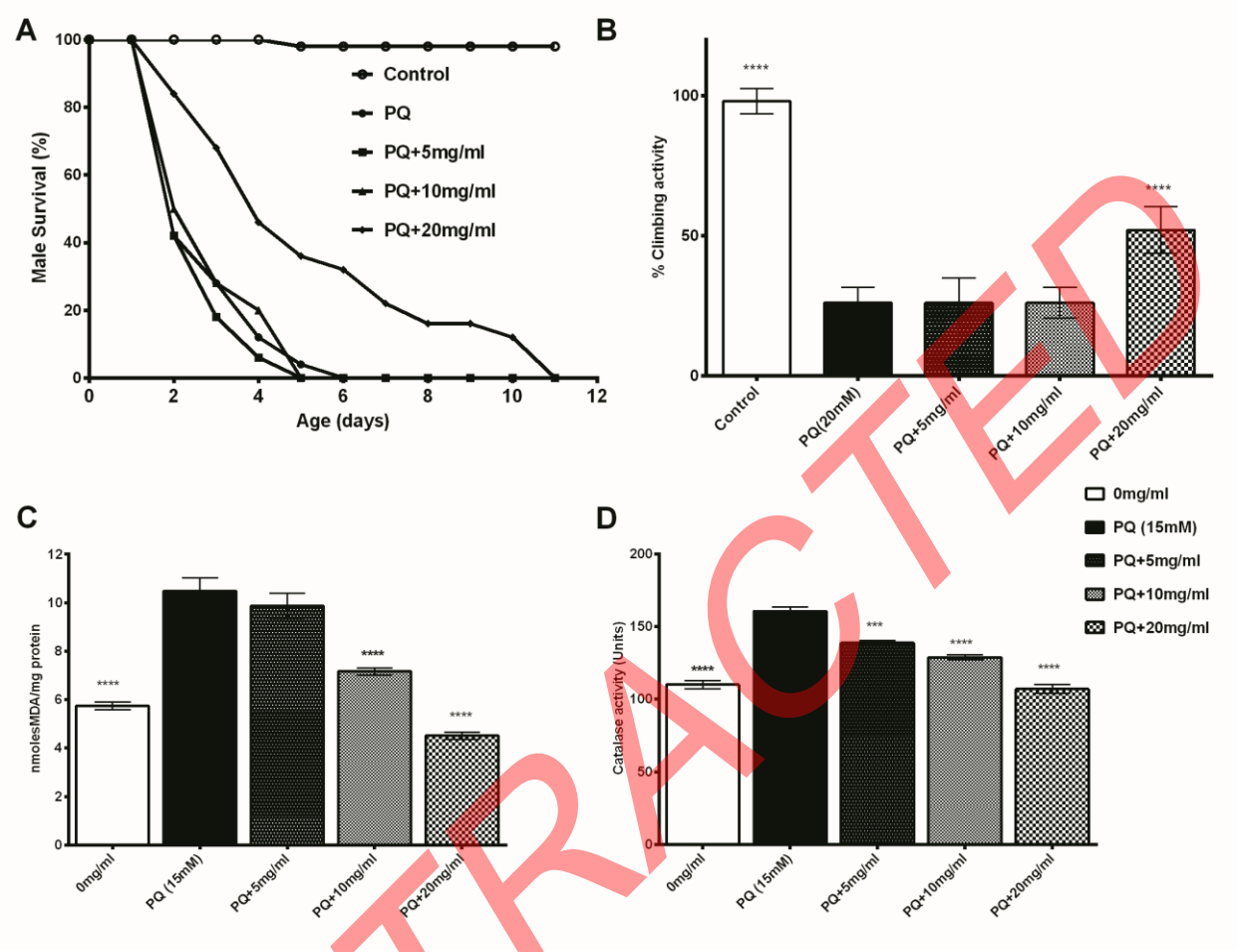


Figure 3: Effects of *Ananas sativa* on Oxidative stress resistance

(A) There was a significant effect of the 20mg/ml extract on oxidative stress resistance after exposure of flies to 15mM Paraquat ($P < 0.0001$) **(B)** and *Ananas sativa* alleviates Paraquat induced locomotor deficit. Pre-treatment of flies with the extract exerted a significant improvement in the locomotor activity of the 20mg/ml group compared to the Paraquat oxidative stress group ($p < 0.0001$). **(C)** There was a significant decrease in MDA levels of treated flies when compared to the Paraquat group **(D)** Catalase activity in fly homogenates equally revealed the ability of extracts to alleviate the effect of Paraquat induced oxidative stress and its modulatory effect showed a significant increase in oxidative stress resistance in fly homogenate samples.

Ananas sativa Ethylacetate fraction F_1 did not increase resistance to oxidative stress

The bioassay guided fractionation was adopted at each time point to identify the fraction of *Ananas sativa* which may be responsible for its longevity effect, The Ethylacetate fraction gave the most significant mean survival time of flies and also gave the highest free radical scavenging activity effect (Table 1) (Fig. 4A and B), and there was equally an increase in resistance to oxidative stress by the fraction (Fig. 4C). On testing the free radical scavenging activity of the ten Ethylacetate fractions, the F1 gave the highest free radical scavenging activity against DPPH radical (Suppl. Fig. 1), therefore the F1 fraction was tested for oxidative stress resistance. There was no significant increase in mean lifespan of flies treated with the F1 (1 and 2mg/ml) with respect to the Paraquat control group of flies, vitamin E was used as a positive control (Fig. 4D). Therefore, the protection exhibited by *Ananas* in scavenging the DPPH free radical may be associated with its antioxidant components, e.g. flavonoids, phenolic compounds, and tannins as previously suggested that *Ananas* exhibits a significant antioxidant and free radical scavenging activity (Jenitha *and* Anusuya, 2016). However, the present study has been able to show the anti-ageing bio-activity of the different fractions of the fruit extract.

Lifespan extending effects of phytochemicals have been reported in previous studies, for instance, flavonoids in blueberry and tea extracts have been shown to extend lifespan in *Drosophila melanogaster* (Peng *et al.*, 2012).

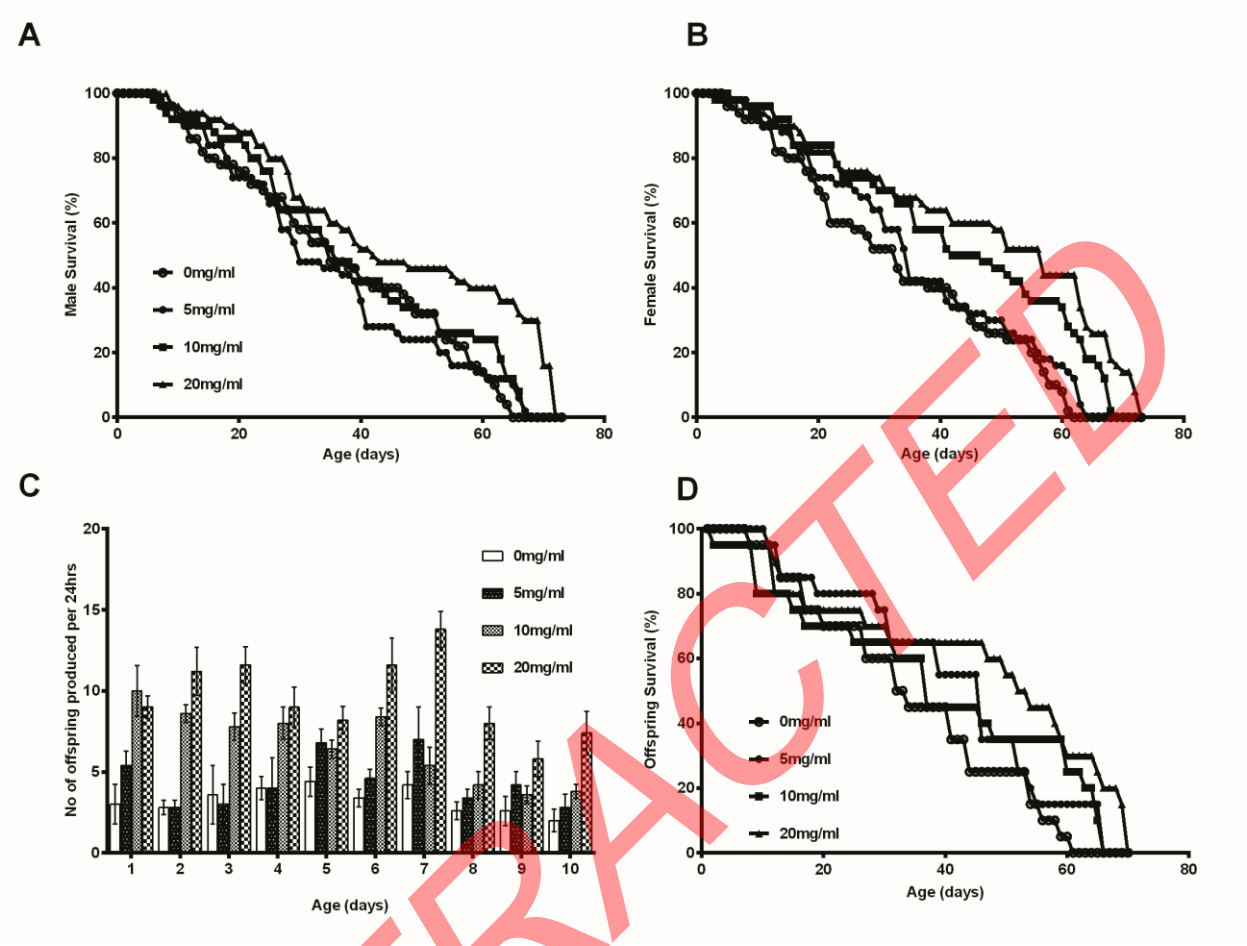
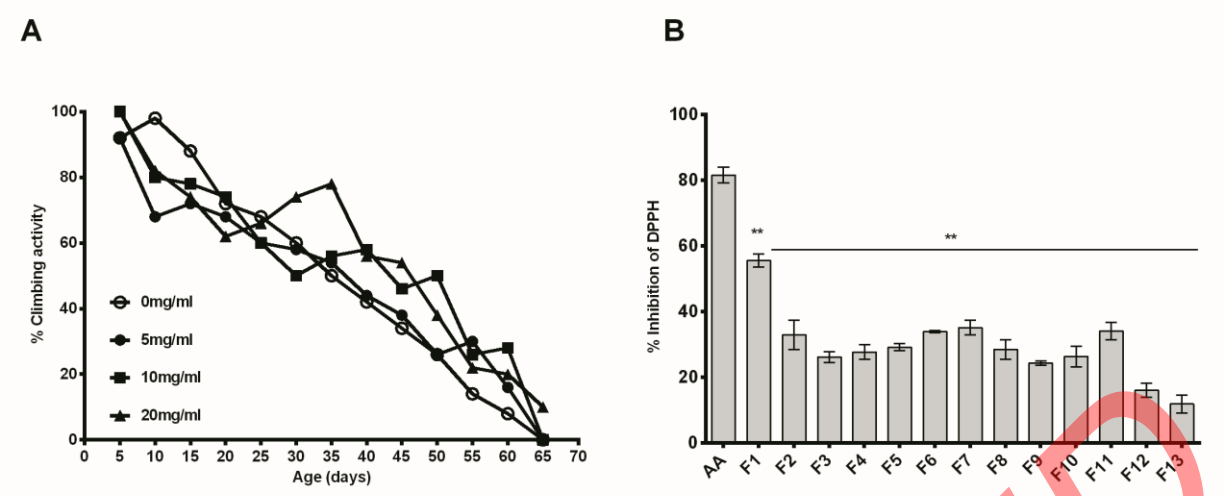


Figure 4: Effects of different fractions of *Ananas sativa* on longevity and oxidative stress resistance

(A) The Ethylacetate fraction of *Ananas* increased longevity and **(B)** gave the highest free radical scavenging activity and **(C)** the highest oxidative stress resistance ($P < 0.0001$) relative to the PQ control **(D)**The 1 and 2mg/ml F1 of the Ethylacetate fraction did not increase resistance to Paraquat induced oxidative stress and longevity with respect to Paraquat control group as evaluated by Dunnet's multiple comparison test ($p = 0.79$ and 0.26 respectively).



Supplementary figure 1: Effect of *Ananas sativa* on climbing activity as age progressed and the free radical scavenging activity of fractions

(A) The health span promoting effect of *Ananas sativa* was apparent by a significant increase in climbing performance with age when compared to the control in the 20mg/ml group ($P < 0.02$), other groups had no significant effect, as evaluated by two-way ANOVA, Dunnet's test ($n = 50$ flies per treatment group, $p< 0.05$). Effects of *Ananas sativa* ethyl acetate fraction on DPPH free radical. **(B)**The Ethyl acetate fraction gave the highest free radical scavenging activity with respect to the Ascorbic acid control group as evaluated by Tukey's post hoc test. ($P<0.0001$).

DPPH free radical scavenging activity and Phytochemicals present in methanol fruit extract of *Ananas sativa* and its fractions

The free radical scavenging activity of the fruit extract gave a concentration dependent activity. The concentration at which 50% of the DPPH free radical was inhibited was deduced, as evaluated from a linear regression analysis. The IC50 value of the extract and Ascorbic acid are 81.51 and 248.15 µg/ml. (Table 2).The qualitative phytochemical screening of the crude methanol fruit extract of *Ananas sativa* and its fractions, confirmed the presence of an appreciable amount of phytochemicals in the extract, the following compounds were present in mild and moderate levels; flavonoid, phenolic compounds, steroid, tannin, alkaloid, reducing sugars, saponin, terpinoid, coumarin, free amino acids and proteins, however, there were no traces of cardiac glycosides and anthraquinones (Table 3). These finding are in line with previous studies (Jenitha *and* Anusuya, 2016).

Table 2: Percentage inhibition IC50 values of the DPPH free radical scavenging activity of the Crude *Ananas sativa* fruit extract and Ascorbic acid. Result revealed a concentration dependent effect. Data are expressed as mean ± SD, n=5. The IC_{50} values of the extract and of Ascorbic acid are 248.15µg/ml and 81.51µg/ml respectively.

Sample	Concentration (µg/ml), mean ± SD					IC50
	20	50	100	200	500	
Ascorbic acid	41.20±1.1	65.77±0.7	74.47±0.5	81.40±0.7	86.06±0.4	81.51µg/ml
Ananas sativa	10.84± 0.4	35.17±0.9	50.87±0.5	60.46±0.5	70.36±0.4	248.15µg/ml

Table 3: Phytochemical screening of the Crude Methanol Fruit Extract of *Ananas sativa* and its 5 fractions. The experiment was performed in triplicate and result shows the presence of high concentrations of phenolic compounds, flavonoids, and moderate amount of steroids which have been reported to possess a substantial potent antioxidant and cytotoxic effects. Keys: - (Absent), + (Mild), ++ (Moderate), +++ (High).

S/N	Phytochemicals	Crude MetOH	Hexane	Chloroform	Ethylacetate	Acetone	Water residue
1	**Flavonoids**	+++	-	-	+	-	-
2	**Poly phenolic compounds**	+++	-	-	+	-	+
3	**Steroids**	+	-	-	-	-	-
4	**Tannins**	++	-	+	+	-	-
5	**Alkaloids**	+++	-	+	+	-	-
6	**Reducing sugars**	+++	+	+	+	+	+
7	**Saponin**	+++	+	+	-	-	-
8	**Cardiac glycosides**	-	-	-	-	-	-
9	**Terpenoids**	++	-	-	+	-	-
10	**Coumarins**	+	-	-	-	-	-
11	**Free Amino acids**	+	-	-	-	-	-
12	**Proteins**	+	-	-	-	-	-
13	**Anthraquinone**	-	-	-	-	-	-

Conclusion

In summary, the methanol fruit extract of *Ananas sativa*, Pineapple fruit and pulp, exhibited anti-ageing bio-activity in the model organism; *Drosophila melanogaster*, by increasing longevity through resistance to stress, by enhancing the activities of cellular endogenous antioxidant enzyme activity, by enhancing fertility and by increasing cellular oxidative stress resistance. The fractions equally exhibited appreciable free radical scavenging activities. However, the supposed anti-ageing bioactive fraction obtained from the Ethylacetate fraction F1, did not exhibit oxidative stress resistant effect in the model organism. Hence, it could be suggested that *Ananas sativa* possesses anti-ageing bioactivity. Further work should be carried out by testing the anti-ageing bioactivity of each of the obtained fraction, isolation and identification of possible anti-ageing compounds from the fruit extract with other phenotypic features. Other molecular mechanisms or nutritional pathways, by which the extract exhibits anti-ageing bio-activity should be equally be explored.

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Conflict of interest: There is no conflict of interest associated with this study.

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