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Afr. J. Biomed. Res. Vol. 24 (January, 2021); 99 - 108

Research Article

Pineapple Fruit Extract Prolonged Lifespan and Endogenous Antioxidant Response in *Drosophila melanogaster* Exposed to Stress.

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ABSTRACT

Free radical-induced cellular damage accelerates ageing. The WHO anticipated that 22% of the population will be over 60years by the year 2050 and 80% of those will live in low-and-middle-income countries (LMIC). It is, therefore, paramount to find local and readily available compounds that could reduce age-induced burden in LMIC. The methanol fruit extract of *Ananas sativa* and its fractions were evaluated for anti-ageing activity in the fruit fly, *Drosophila melanogaster* w^{1118} flies. Experimental flies were fed on food supplemented with 5, 10, and 20mg/ml methanolic fruit pulp-juice extract lifespan, reproduction, learning, memory and stress resistance assays were evaluated. Phytochemical composition and the percentage inhibition of the fruit extract on 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) were evaluated. Successive bioassay-guided fractionation was used to elucidate the fraction with higher bioactivity. There was a dose-dependent effect, significant at 20mg/ml of extract on lifespan, fertility and oxidative stress resistance ($p < 0.05$), but not on learning and memory (Tukey's post hoc test, n =50 flies/group). Male and female % mean survival time were 6.3±0.7, 4.5±1.4 and 13.6±0.5%, and 4.3±0.4, 12.5±0.4 and 20.7±0.4% respectively. The IC50 value of the extract on DPPH was only three times that of Vitamin C, a known pure antioxidant. The Ethyl Acetate fraction increased stress resistance ($p < 0.05$), but the sub-fractions obtained did not show any anti-ageing activity at the concentrations tested. The methanol fruit extract of *Ananas sativa* possesses anti-ageing bioactivity through oxidative stress resistance in *Drosophila melanogaster* w^{1118} flies.

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

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Received: November, 2020; Accepted: June, 2020

Abstracted by:

Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Ageing is the highest risk factor for non-communicable diseases and disabilities (Beard *et al*., 2015). Age-related disease burden is on the rise due to accelerated ageing in Sub-Saharan Africa (Gyasi and Phillips, 2019), as a result, 799 deaths per 100,000 population were reported between 1990-2017 (Bcheraoui *et al*., 2020). Ageing is a cumulative loss in biological function due to free radical-induced damage to cellular macromolecules (Lopez-Otin *et al*., 2013). The ability to live longer with minimal physiological decline has remained a challenge, therefore, new approaches into healthy ageing research should be adopted (WHO Report, 2014; Beard *et al*., 2015).

Africa is endowed with a variety of natural products majority of which are yet to be screened for anti-ageing bioactivity. In the quest to identify new anti-ageing bioactive compounds from natural fruits, the action of certain botanical products have been studied (Oladele *et al*., 2012; Balasubramani *et al*., 2014). Most of these fruits are rich in antioxidants, which are health-promoting compounds. The mode of action of these antioxidants is to delay the onset of oxidative damage to target molecule caused by reactive oxygen species which are inevitable side products of metabolism (Kregel and Zhang, 2007; Ziegler, Wiley and Velarde, 2015).

Environmental stress factors such as toxins, smoke, heat, contaminants from air and water, contribute to the generation of reactive oxygen species within biological systems (Abolaji *et al*., 2017). Antioxidants such as phenolic compounds, Vitamin C and Vitamin E play crucial roles in mediating oxidative stress effect in the cell. Apart from the other biological importance of Vitamin C in cell division and protein modification, it is an active antioxidant which plays a key role in healthy ageing (Salehi *et al*., 2020). *Ananas sativa* (Pineapple) is the third most abundant fruit in the tropics which is not only rich in antioxidants (Lu *et al*., 2014) but also minerals and vitamins which acts as cofactors in free radical scavenging pathways (Nimse and Pal, 2015). Some of the nutritional composition of the pineapple fruit include manganese, copper, magnesium, iron, selenium, zinc and ascorbic acid (Kongsuwan *et al*., 2009; Shittu, 2013).

Majority of the supporting evidence for ageing theories are 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 make them suitable for anti-ageing research (Pandey and Nichols, 2011). The fruit fly was used in the present study because it is a whole organism, compared to using human cell lines which is not representative of a whole biological system. Moreover, oxidative stress and nutrient-sensing pathways are well conserved in the model organism (Kim, 2007; Lee *et al*., 2015). Despite the nutritional importance of *Ananas sativa*, its anti-ageing effect is yet to be fully clarified. Therefore, the aim of this study was to evaluate the anti-ageing bioactivity of the crude methanol fruit extract of *Ananas sativa* (MEAS) and its fractions, in *Drosophila melanogaster*.

MATERIALS AND METHODS

Plant Collection, Authentication, and Extraction: This research was carried out at the Institute of Biomedical Research of the Kampala International University-Western Campus. Freshly harvested ripe fruits of *Ananas sativa* were obtained from a private plot in Bushenyi district of Western Uganda. The fruit was identified as *Ananas sativa* by a Botanist, Madam Olive Wanyanna of Makerere University in Uganda. Accession number 43344 was assigned to the sample voucher which was deposited at the University herbarium. A 100g of pineapple fruit pulp were dry blended and extracted in 1000 ml of 80% methanol. The resulting extract was filtered and concentrated at 40^{o}C using a rotatory evaporator and the resulting extract was kept in the cold for subsequent use (Lu *et al.*, 2014). The obtained fruit extract was 118.92 g (equivalent to a yield of 8.2% w/w; Percentage (%) Yield = (Final weight of crude extract / original weight) x 100).

Toxicity analysis of the crude fruit extract was conducted in a preliminary investigation in which flies were fed with food supplemented with the crude *Ananas sativa* fruit extract at the following concentrations; 1, 2, 5, 10, 20, 40, 100, 500, and 1000 mg/ml. The number of live and dead flies were counted and recorded after every 24hrs. The concentrations used in this study was based on the outcome of the preliminary investigation.

Fractionation of the methanol fruit extract of *Ananas sativa*: The dried methanol fruit extract (200g) was further subjected to a successive extraction, using different extraction solvents in their order of polarity, (50%); n-Hexane, Chloroform, Ethyl acetate, and Acetone (Krishnamurthy *et al*, 2015). Five resulting *Ananas* fractions were obtained in the following order; n-Hexane extract, Chloroform extract, Ethyl acetate extract, Acetone extract and the water residue. The ethyl acetate fraction gave a significant longevity effect and was further fractionated. Thirteen (F_{13}) fractions were obtained and subjected to DPPH free radical scavenging activity.

Fly stock husbandry: In the present study, *Drosophila melanogaster* w^{1118} (white) strain of flies were used. Virgin males were separated from the virgin females and they were maintained at 25^{o}C under a 12/12hour light and dark cycle in a digital fly incubator (SPX-150A), on a standard culture cornmeal medium. Flies were raised at a standard density of 10 flies per fresh food medium and changed twice a week into another fresh food medium (Kinghorn *et al*., 2015). A day-old virgin flies were treated with food supplemented with 0, 5, 10, and 20 mg/ml methanol fruit extract of *Ananas sativa* (MEAS) throughout their life, these doses are based on preliminary findings.

Oxidative stress induction: After fourteen (14) days of pre-treatment with the extract, flies were starved for 3 hours in an empty vial, after which they were introduced into vials containing either hydrogen peroxide or Paraquat. As for the H_2O_2 oxidative stress-induced flies, after 3hrs of starving, they were made to feed on food supplemented with 5% H_2O_2 (Slack *et al.*, 2010). As for the Paraquat oxidative stress-induced flies, a previously described method was adopted (Jahromi *et al.*, 2013) in which starved flies were introduced into vials containing filter papers impregnated with 50µl each of 15mM Paraquat and 5% Sucrose solution. The flies were then monitored for lifespan.

Dietary stress induction: Inactive yeast was used as a source of protein (w/v) in the present study. Briefly, a stainless-steel tray was placed in a pre-heated oven at 30^{o}C for 1hr, 60 grams of dry yeast grains was measured into the tray and sterilized for another 1hr to obtain inactive yeast. (Burger *et al*., 2010). In a preliminary investigation, the effect of protein on longevity was analyzed in 1%, 2%, 5%, 10%, 12% and 15% concentrations. The outcome of this finding was the basis for

choosing 1% protein as a poor protein diet, a form of dietary stress.

Heat stress induction: Flies were subjected to a range of temperatures from 29-39^{o}C. The outcome of the preliminary finding was the basis for choosing 29^{o}C as the optimum temperature for heat stress. Flies were exposed to heat stress for 8hrs every day by keeping them in an oven at 29^{o}C. The control group were kept at 25^{o}C. The number of live and dead flies was recorded daily for the lifespan analysis.

Lifespan assay: Longevity assay was carried out according to established procedures (Kinghorn *et al.*, 2015). The rate of survival was evaluated by a daily counting of live flies until all the flies have died. 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 the control group.

Climbing assay: Climbing performance of flies was carried out with the aid of a negative geotaxis assay in which 10 flies were introduced into a 25ml pipette with a funnel. The pipette was gently tapped to get all the flies to the base. The percentage of flies that reached the 25cm mark of the pipette in 45seconds was calculated (Kinghorn *et al.*, 2015). Paraquat-treated flies were also subjected to climbing activity in the locomotor deficit assay (Jahromi *et al.*, 2013).

Reproductive performance assay: Fertility assay was carried out by putting ten (10) females with ten (10) males in the same experimental food vial. This was done for each of the treatment groups. They were allowed to mate for ten days. Thereafter, all the parent flies were removed completely from the food vials, and the number of offspring that appeared from the eggs laid by the female flies were counted and recorded every 24 hours for ten days.

Fly whole-body homogenization for biochemical assays: The whole body of forty (40) flies were homogenized in ice-cold phosphate buffer saline 2 mL (0.1 M, pH 7.4), this was centrifuged for ten minutes at 2,500g and the resulting homogenate was used for all biochemical test.

Catalase activity: *In-vivo* catalase activity was measured as previously described (Iwase *et al.*, 2013). The calculation of the number of micromoles of H_2O_2 that decomposed per unit-time is the catalase activity.

Lipid peroxidation assay: Lipid peroxidation reaction was assayed using the previously established protocol (Ohkawa, Ohishi and Yagi, 1979), the 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, bovine serum albumin was used as a standard.

Learning and Memory Experiment: Adults that emerged from parents groomed in food medium supplemented with 5, 10 and 20mg/ml *Ananas sativa* fruit extract at different age of young (5days old), mid-age (25days old) and aged flies (50days old) were used in this study. The model organisms were exposed to olfactory-aversive learning and memory tests. In the learning phase, they were taught to associate an odour to mechanical shock and then tested for the choice between this odour and another odour in a T-maze later in the absence of the shock. The odourants used were methylcyclohexanol, MCH and octanol OCT. As for the memory test, different memory stages were assayed. Short term memory-STM which was conducted 1hr after training, midterm memory-MTM was conducted 3hr after training, and long-term memory-LTM was conducted 24hr after training respectively. The organisms were introduced into the T-maze again after the learning stage and kept in the middle of the T-maze for about 1min 3s. They were made to choose between the two odours. Flies at the right or left arm of the T-maze were counted and recorded. Memory scores are calculated and used as the performance index (Burger, Buchel and Tadeusz, 2010; Malik and Hodge, 2014).

Head homogenization for brain chemistry analysis: The head of flies were homogenized in ice cold sodium-phosphate buffer (1 ml, 0.1 M, pH 8.0) and centrifuged at 4^{o}C (2,500g, 10 min), solution was filtered, and the sample was kept at 4^{o}C and later used for the biochemical tests.

Acetylcholinesterase activity and Lipid peroxidation test in fly heads: The reaction mixture was 20µl of 10mM DTNB; 5, 5-dithiobis 2-nitrobenzoic acid, 50µl sample, a milliliter of phosphate buffer, 0.1 M, at pH 8.0 and 20µl of 8mM acetylthiocholine iodide. Absorbance was quantified at 412 nm. The enzyme activity was expressed as µmoles of substrate hydrolyzed/min/mg protein (Ellman *et al.*, 1961) . The lipid peroxidation in samples was carried out by Thiobarbituric acid reaction. Reaction mixture was 1.5 ml of 20 % Acetic acid, pH 3.5, 500µL of sample, 0.8 % w/v of 1.5ml of TBA, and sodium lauryl sulphate (SDS) (8 % w/v). Absorbance of malondialdehyde (MDA) produced was measured at 532 nm and quantified as malondialdehyde equivalents (Ohkawa, Ohishi and Yagi, 1979). Protein concentrations in the fly homogenates were measured via the method of Lowry (Lowry *et al.*, 1951).

Catalase and SOD measures in fly heads: Catalase activity was measured as previously described by monitoring the clearance of H_2O_2 25^{o}C at 240nm in a reaction mixture containing 1800µL of 50mM Phosphate buffer (pH 7.0), 180µL of 300mM H_2O_2, 20µL sample . Reaction was allowed to take place for 2m and calculated values were obtained by taking absorbance at 240nm using a UV-visible spectrophotometer. Catalase activity was expressed as mM of H_2O_2 consumed per min/mg of protein) = (ΔA) / (ε.d.t.c), where ε = 39.4 M-1 cm-1 (H_2O_2 extinction coefficient), d = 1 cm path length, t = 2 min (incubation time), c = protein content in the test sample (mg). The Superoxide dismutase activity was measured method of Marklund and Marklund (1974), about 0.5 ml of 2 mM pyrogallol was added into a total volume of 3 ml reaction mixture containing 200 µl sample and 0.1 M Tris HCl-buffer, pH 8.2 in a test tube, changes in absorbance was measured at 412 nm using a spectrophotometer. The specific activity of the enzyme was expressed as units (Marklund and Marklund, 1974).

Qualitative phytochemical screening and *in vitro* DPPH free radical scavenging activity: The phytochemical analysis

of the fruit extract and its five fractions were carried out according to standard methods (Kasolo *et al*., 2010). As for the DPPH radical scavenging assay, crude methanol fruit extract was prepared in concentrations of 10, 20, 50 100, and 200µg/ml (Kanti, Chatterjee and Ghosh, 2015) and the absorbance was measured at 517 nm with a UV-VIS spectrophotometer using ascorbic acid as a standard. The percentage inhibition exerted by the extract on the DPPH free radical was calculated thus; Percentage % DPPH free radical scavenging = $(A_0 – A_1) / A_0$ x 100.

Statistical Analysis: All experimental data were expressed as mean ± standard deviation (SD), with the aid of Graph Pad Prism 6 Statistical software. Survival analysis was evaluated with a log-rank test. Climbing, reproductive performance and biochemical assay analyses were graphically represented, significant differences between the treatment and control groups were analyzed using one-way or two-way ANOVA with Dunnet's or Tukey's multiple comparison tests as appropriate. The DPPH free radical scavenging activity was analyzed using linear regression. $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Pineapple fruit extract increases lifespan and reproduction: Toxicity analysis of the methanolic extract of *Ananas sativa* fruit showed that the toxic lethal doses are the 500 and 1000 mg/ml respectively, and lower concentrations of 1 and 2 mg/ml did not show an extension in lifespan with respect to the control group. The concentrations which showed an increase in lifespan were 5, 10, and 20 mg/ml, therefore these are the concentrations used in the present study based on the outcome of the preliminary findings.

There was a dose-dependent effect of the fruit extract on longevity and reproductive performance (Fig. 1), with the most effective concentration being the 20mg/ml, $p < 0.05$. The percentage increase in the mean survival times of the male and female flies in the three treatment concentrations were 6.3 ± 0.7, 4.5 ± 1.4 and 13.6 ± 0.5%, and 4.3 ± 0.4, 12.5 ± 0.4 and 20.7 ± 0.4% respectively (Table 1). The lifespan-extending effect of *Ananas* may be due to its antioxidant value, and its ability to increase oxidative stress resistance through certain nutritional pathways as previously shown with other plant products, as in the mechanism of lifespan extension of Rapamycin in fruit flies (Bjedov *et al.*, 2010).

Oxidative stress resistance is peculiar to most anti-ageing compounds (Kregel and Zhang, 2007). Minerals and vitamins play significant roles in longevity (Lee *et al.*, 2015). The role of diet and nutrition in longevity has been shown in different model organisms including *Drosophila melanogaster* (Fontana and Partridge, 2015). Resveratrol, the plant polyphenol found in grape berry skin was shown to increase lifespan (Bass *et al.*, 2007) Plant phytoconstituents was reported to regulate cellular oxidative stress in *Drosophila* (Balasubramani *et al.*, 2014).

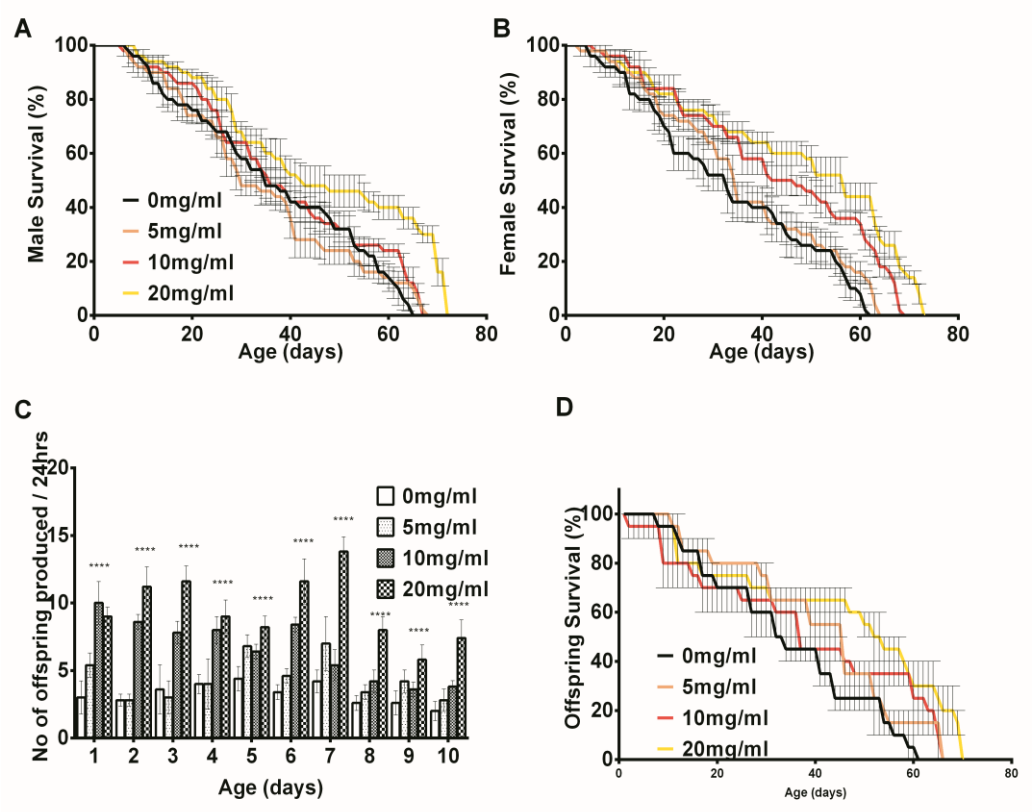

Figure 1.
Effect of *Ananas sativa* on longevity and reproductive performance.
(A) There was a significant longevity effect in 20mg/ml male group ($P < 0.0384$) and **(B)** female flies showed significant effect in the 10 and 20mg/ml group relative to control ($P < 0.0229$ and 0.0006). **(C)**The health 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). **(D)**The effect of the extract on the lifespan of the offspring produced, 20mg/ml group ($P < 0.005$). n=50 flies per treatment group, ANOVA, Dunnet's test. **** means $p < 0.0001$.

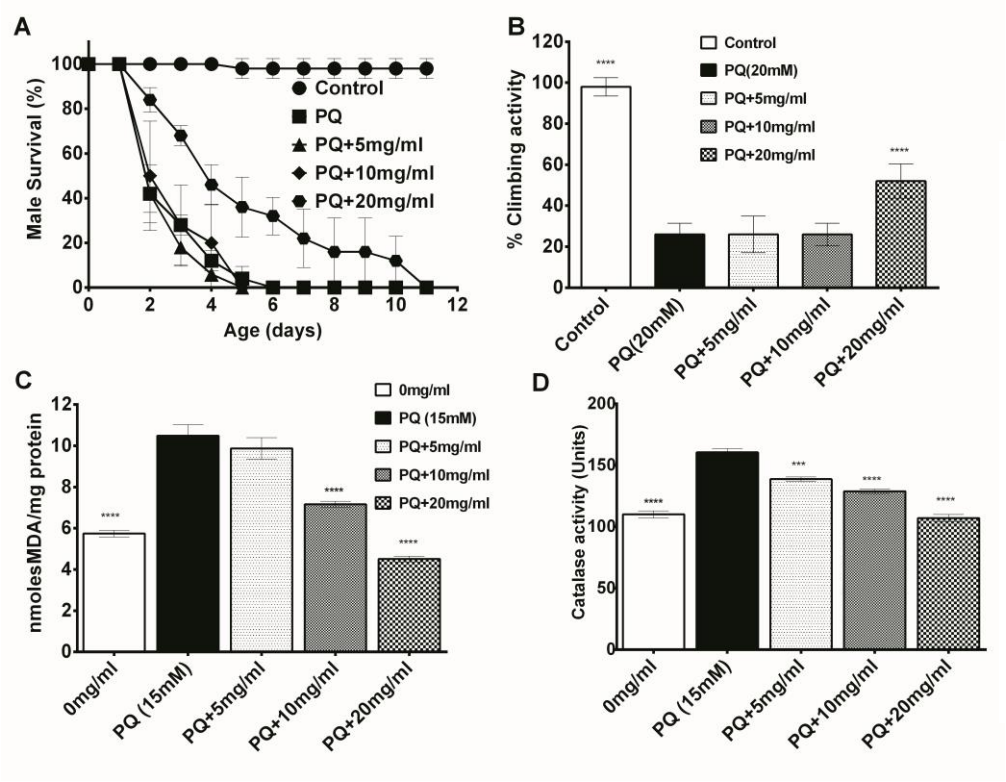

Figure 2.
Effect of *Ananas sativa* on Oxidative stress resistance.
(A) There was a significant effect of the 20mg/ml extract on oxidative stress resistance after exposure of flies to 15mM Paraquat ($P < 0.0001$) **(B)** and *Ananas sativa* alleviates Paraquat induced locomotor deficit in the 20mg/ml 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 ($P < 0.05$) **(D)** Catalase activity in fly revealed a dose-dependent significant oxidative stress resistance in flies ($P < 0.05$). *** means $P < 0.001$ **** $P < 0.0001$

Anti-ageing plant products enhanced longevity through modulation of oxidative stress response, and ability to act as dietary restriction mimetic in nutrient-sensing pathways (Wang *et al.*, 2012). The free radical scavenging effect of *Ananas* (Pineapple) could delay the onset of age-long free radical-induced damage to cellular macromolecules (Vrianty *et al.*, 2019). However, the anti-ageing activity of the fruit extract is yet to be reported. For the first time, we hereby present the anti-ageing bio-activity of the fruit extract and its fractions in *Drosophila melanogaster.*

Endogenous antioxidant activity of Pineapple fruit extract: To understand the process by which *Ananas* ameliorates oxidative stress, the effect of the extract on endogenous catalase activity and lipid peroxidation were measured, using lipid peroxidation product, malondialdehyde, MDA a biomarker of oxidative stress. Treated flies exposed to Paraquat exhibited a significant reduction in malondialdehyde production in their homogenates which is a modulation of lipid peroxidation (Fig. 2). This modulatory effect observed could be as a result of the presence of polyphenolic compounds and flavonoids in the fruit. A recent study reported that pineapple can prevent free radical-induced diseases due to the synergistic action of the fruit's antioxidants (Lu *et al.*, 2014; Vrianty *et al.*, 2019). Inflammation, a peculiar oxidative stress response was ameliorated by *Ananas* in another study due to its bioactive constituent (Maurer, 2001; Manzoor *et al.*, 2016)

DPPH free radical scavenging activity of the fruit extract fractions: The Ethylacetate fraction gave the most significant highest free radical scavenging activity effect (Table 1) (Fig.3). There was an increase in resistance to oxidative stress by the fraction (Fig. 3C). Ananas Ethylacetate fraction, F1 was tested for oxidative stress resistance. There was no significant increase in mean lifespan of flies treated with this fraction (1 and 2mg/ml) compared to the Paraquat control group. DPPH free radical scavenging effect of *Ananas* may be associated with its antioxidant components, e.g. flavonoids, phenolic compounds, and tannins as shown in this study. It was previously reported that *Ananas* exhibits a significant antioxidant and free radical scavenging activity, due to its phytoconstituents (Agnes Jenitha and Anusuya, 2016). Previous studies have shown the role of Pineapple in aiding digestion (Subandiyono, Hastuti and Nugroho, 2018), and antiplatelet activity due to its bioactive composition (Rahmawati, Setiasih and Hudiyono, 2020).

Pineapple fruit extract enhanced longevity during exposure to heat and dietary stress: There was a significant increase in heat stress resistance of treated flies by the increase in lifespan of treated flies relative to control in the 20mg/ml group of male and female flies ($p < 0.002$) (Fig 4A and B). Effect of the different concentration of the extract was also tested on flies exposed to poor protein diet. The rate of egg-laying during exposure to poor protein diet (1% protein) was ameliorated significantly in the 20mg/ml group ($p = 0.0001$) relative to the control (Fig. 4C). The longevity of the mothers that produced the eggs in the same poor protein diet was also evaluated, the lifespan of the 20mg/ml treated flies significantly improved as age progressed relative to the control group, Fig. 4D, ANOVA, Tukey's post hoc test ($P < 0.0001$). Heat stress and dietary stress have been reported to induce cellular oxidative stress in organisms, this process can be influenced through dietary means (Akbarian *et al.*, 2016).

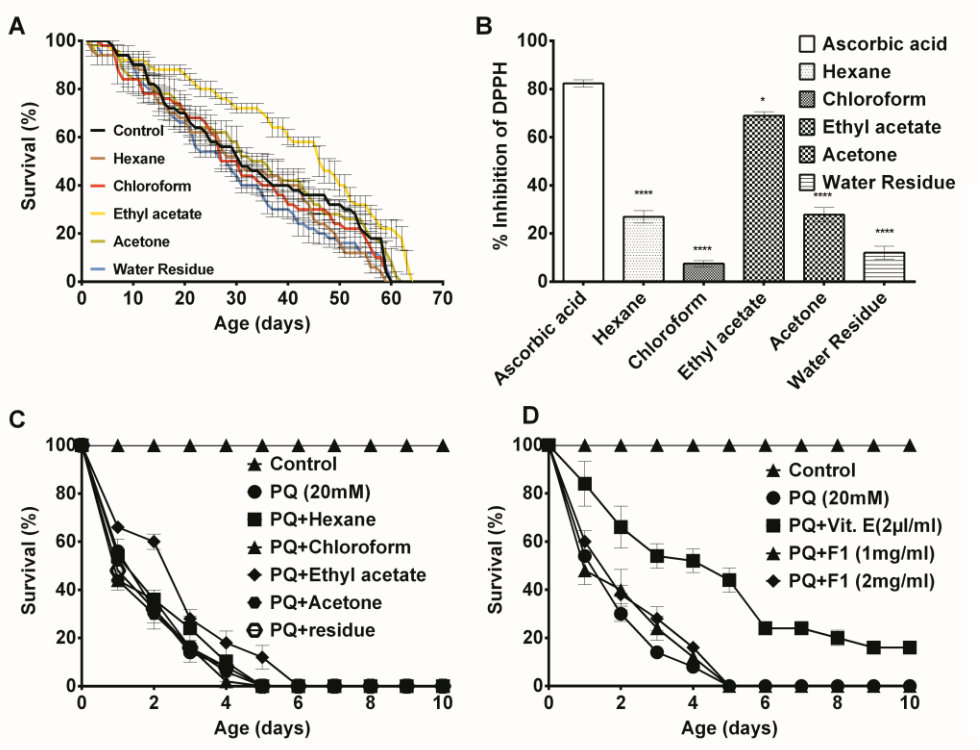

Figure 3.
Effect of different fractions of *Ananas sativa* on longevity and oxidative stress resistance.
(A) The Ethylacetate fraction of *Ananas* increased longevity ($P < 0.05$) and **(B)** gave the highest free radical scavenging activity ($P < 0.05$) and **(C)** the highest oxidative stress resistance ($P < 0.0001$) relative to the PQ control **(D)** The 1 and 2mg/ml F1 of the Ethylacetate fraction did not show a significant change in oxidative stress resistance with respect to Paraquat control group (P: 0.705, 0.072), Tukey's multile comarison test , and Vitamin E is a positive control. * means $p < 0.05$; **** means $p < 0.0001$

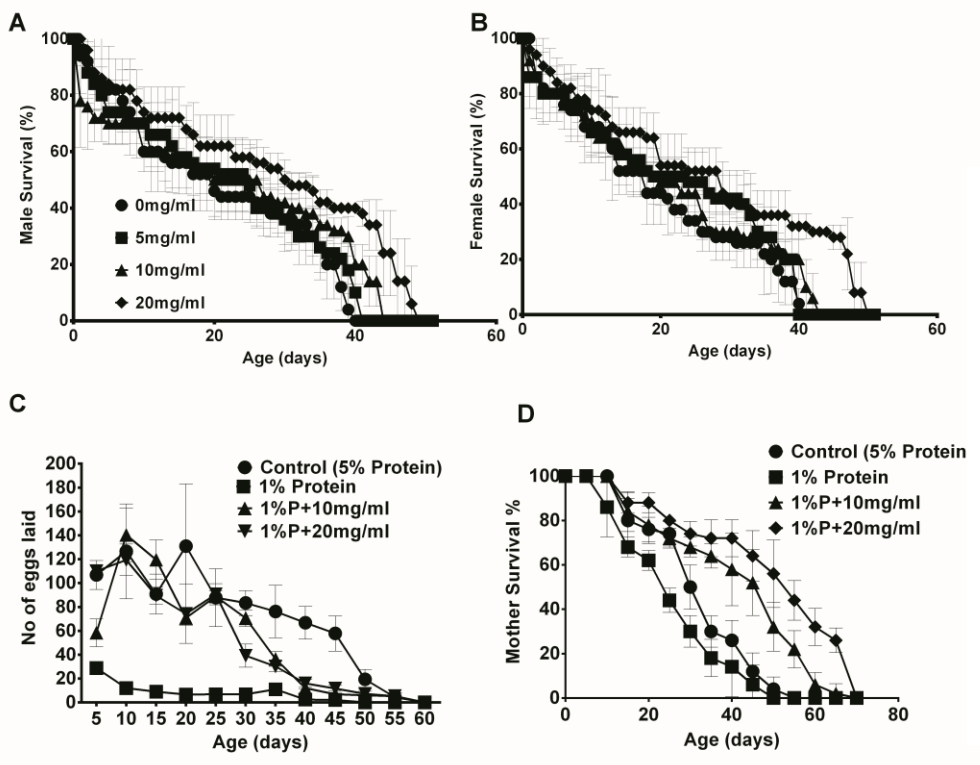

Figure 4
Effect of *Ananas sativa* fruit extract on longevity during exposure to heat and dietary stress.
The 20 mg/ml group of treated flies showed significant increase in heat stress resistance in male and female flies ($p < 0.002$) **(A-B)**. The rate of egg-laying during exposure to poor protein diet (1% protein) was improved significantly in the 20mg/ml group, ANOVA, Tukey's post hoc test ($p = 0.0001$) **(C)**. The longevity of the mothers that produced the eggs in the same poor protein diet was significantly improved as age progressed relative to the control group (1% protein group) ($P < 0.0001$) **(D)** ANOVA, Tukey's post hoc test.

Effect of Pineapple fruit extract on learning and memory
To ascertain the impact of the fruit extract on age-related memory decline, treated flies were made to undergo the learning and memory test at a young , middle and old age respectively. The flies treated with fruit extract of *Ananas sativa* showed no significant difference relative to the control group in learning and memory ($n = 50$ flies/group). The memory scores of flies in the Short Term Memory (STM), 1 hour test, did not show significant changes, the memory scores in the Medium Term Memory (MTM), 3 hour test, revealed a decrease in performance, $P = 0.0256$ in 10 and 20 mg/ml treated groups. The memory scores in the Long Term Memory (LTM), 24 hour test, revealed no significant changes ($P < 0.4041$) relative to the control (Figure 5).

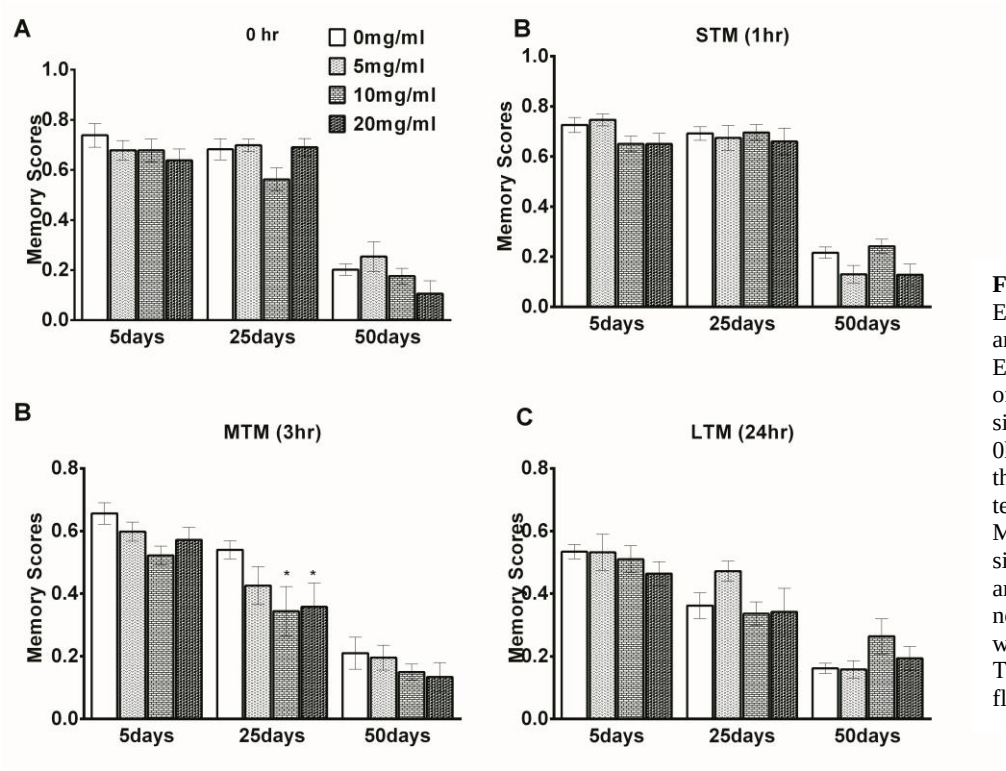

Figure 5.
Effect of *Ananas sativa* on learning and memory with age increase
Effect of extract on different phases of memory. **(A)** There was no significant change in learning phase 0hr, p = 0.1591 and **(B)** memory of the flies with age in the STM; Short term memory, p = 0.3071, **(C)** MTM; Mid-term memory, p = 0.0256, a significant decrease in performance and **(D)** LTM; Long term memory, no significant change, p = 0.4041 with respect to the control group. Tukey's post hoc test (n = 50 flies/group). * means p < 0.05

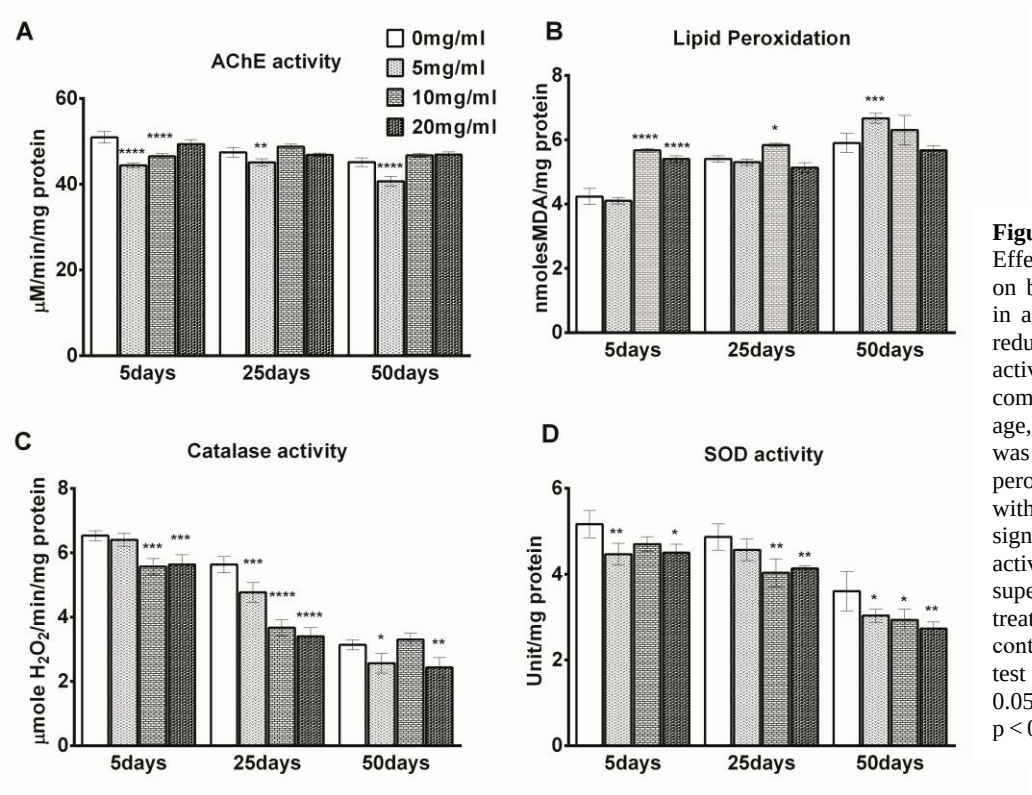

Figure 6
Effect of *Ananas sativa* fruit extract on brain antioxidants with increase in age (A) There was a significant reduction in the acetylcholinesterase activity of the treatment group compared to the control group with age, p < 0.0001, (B) however, there was a significant increase in lipid peroxidation in the treatment groups with age, p = 0.0001, (C) there was a significant decrease in catalase activity, p = 0.0003 and (D) superoxide dismutase activity in the treatment groups compared to the control, p = 0.0095. Tukey's post hoc test (n = 50 flies/group). * means p < 0.05, ** means p < 0.01, *** means p < 0.001 and **** means p < 0.0001

Effect of Pineapple fruit extract on brain AChE activity and Lipid peroxidation: To test the effect of fruit extract on brain acetylcholinesterase activity and lipid peroxidation as age progressed, fly-brain homogenates were analyzed for the bio-markers involved. The enzyme acetylcholinesterase (AChE) splits the neurotransmitter acetylcholine to produce choline and acetate. There was a significant decrease in acetylcholinesterase activity of the 5mg/ml group as age progressed, 5days; P < 0.0001, 25days; P = 0.0080, and 50days; P < 0.0001, however, other concentrations of the extract gave no significant changes in the enzyme activity as age progressed (Fig. 6A). There was a significant increase in malondialdehyde, MDA in the treated groups compared to the control 5days; P < 0.0001, 50days; P = 0.0003 (Fig. 6B).

Effect of Pineapple fruit extract on brain-antioxidant enzymes: To examine the effect of the fruit extract on the activity of brain antioxidant enzymes, brain samples of treated flies were tested for the enzymes. There was a significant decrease in the activity of Catalase enzyme as age progressed, 5days; P = 0.0006, 25days; P < 0.0001, 50days; P = 0.0070 in all the 20mg/ml groups (Fig. 6C). There was equally a decrease in the superoxide dismutase, SOD activity in the treatment groups as age increased, 5days; P = 0.0139, 25days; P = 0.0065, and 50days; P = 0.0014 in the 20mg/ml groups (Fig. 6D).

Table 1.
Percentage increase in mean survival time of *Ananas* treated flies. There was a percentage increase in mean survival time of the *Ananas* treated flies.

Group	Mean Survival Time			
	Control	5mg	10mg	20mg
Male MST	62.8 ± 1.8	66.8 ± 0.7	65.6 ± 1.4	71.4 ± 0.5
Female MST	60.6 ± 1.9	63.2 ± 0.4	68.2 ± 0.4	73.2 ± 0.4
% increase in MST (M)		6.3 ± 0.7	4.5 ± 1.4	13.6 ± 0.5
% increase in MST (F)		4.3 ± 0.4	12.5 ± 0.4	20.7 ± 0.4

*Percentage increase in mean survival time of treated flies = (T/C-1)*100. T= Mean survival time of treatment group. C= Mean survival time of control group*

Research has shown that the ageing brain can be ameliorated by certain nutrients from plant-based products (Ayers and Verghese, 2014). Potent antioxidants from plant-based products exhibit protective effects on the brain during ageing (Guerra-Araiza *et al.*, 2013). *Ananas* extract reduced cellular free radical induced damage (Vrianty *et al.*, 2019), despite the reported increased lipid peroxidation in aged cells (Andziak and Buffenstein, 2006).

Pineapple fruit extract possesses antioxidant, anti-inflammatory and anti-cancer properties (Taussig and Stanley, 1988; Agnes Jenitha and Anusuya, 2016; Vrianty *et al.*, 2019), however, its role on age-induced memory decline is not fully understood, this was presented in this study. The activity of brain endogenous antioxidants: catalase and superoxide dismutase tend to diminish with age (Sohal, Arnold and Orr, 1990), this is in line with our finding. Certain plant products have been shown to inhibit the activity of the acetylcholinesterase enzyme, which increases the availability of the neurotransmitter acetylcholine, a neurotransmitter that is highly implicated in memory (Khan, Khan and Sahreen, 2012; Colovic *et al.*, 2013). Brain lipid peroxidation-induced inflammation tend to increase with age (Bishop, Lu and Yankner, 2010). A previous study revealed that Ananas bioactive compound, a combination of sulfhydryl proteolytic enzymes, possess a broad spectrum effect, including anti-inflammatory effects (Manzoor *et al.*, 2016).

DPPH free radical scavenging activity and phytochemical analysis of the fruit extract and its fractions: The percentage inhibition of the free radical by the extract gave a concentration-dependent activity. The IC_{50} value of the extract and Ascorbic acid standard are 81.51 and 248.15 µg/ml (Table 2).The qualitative phytochemical screening of the fruit extract its fractions confirmed the presence of the following compounds; saponin, terpenoids, coumarin, free amino acids, flavonoid, phenolic compounds, steroid, alkaloid, reducing sugars, proteins and tannin (Table 3). These findings are in line with previous studies which showed the dose-dependent free radical scavenging effect of the fruit extract (Agnes Jenitha and Anusuya, 2016; Vrianty *et al.*, 2019).

Table 2.
DPPH free radical scavenging activity of *Ananas sativa* fruit extract. There was a dose-dependent effect. The IC_{50} values of the extract and Ascorbic acid are 248.15µg/ml and 81.51µg/ml respectively. Data = mean ± SD, n=5.

Sample	Concentration (µg/ml), mean ± SD					IC_{50} µg/ml
	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	81.51
A. sativa	10.84 ± 0.4	35.17 ± 0.9	50.87 ± 0.5	60.46 ± 0.5	70.36 ± 0.4	248.15

Table 3.
Phytochemical analysis of the fruit extract of *Ananas sativa* and its fractions. The result showed high concentration of phenolic compounds.

Phytochemicals	MEAS	MEAS Fractions				
	Crude MEAS	Hexane	Chloroform	Ethylacetate	Acetone	Water residue
Flavonoids	+++	-	-	+	-	-
Poly phenolic compounds	+++	-	-	+	-	+
Steroids	+	-	-	-	-	-
Tannins	++	-	+	+	-	-
Alkaloids	+++	-	+	+	-	-
Reducing sugars	+++	+	+	+	+	+
Saponin	+++	+	+	-	-	-
Cardiac glycosides	-	-	-	-	-	-
Terpenoids	++	-	-	+	-	-
Coumarins	+	-	-	-	-	-
Free Amino acids	+	-	-	-	-	-
Proteins	+	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-

Keys: - (Absent), + (Mild), ++ (Moderate), +++ (High), (n = 3).

In conclusion, the methanol fruit extract of *Ananas sativa* (MEAS), Pineapple fruit and pulp, exhibited anti-ageing bio-activity in the model organism *Drosophila melanogaster*. *The extract* increased longevity, enhanced endogenous antioxidant enzyme activity, enhanced reproductive performance, increased cellular oxidative stress response, increased heat and dietary stress resistance as age progressed in the model organism.. Its fractions exhibited substantial free radical scavenging activities. However, the suspected anti-ageing bioactive fraction obtained from the *Ananas* Ethylacetate fraction, F1, did not show significant changes in oxidative stress resistance when compared to the control group. There was no significant ameliorative effect on normal age-related memory and brain antioxidant enzyme decline. Further investigations should be carried out on its neuroprotective effect, characterization of its anti-ageing bioactive compound and other molecular mechanisms by which the extract exhibits anti-ageing bio-activity.

Acknowledgements

Special thanks goes to the Postgraduate Studies and Research Directorate of the Kampala International University-Western Campus, Biochemistry Department, Institute of Biomedical Research (IBR; KIU-WC), Sayansi, Trend in Africa and DrosAfrica.

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