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Research Article

PHYTOCHEMICAL ANALYSIS OF TETRAPLEURA TETRAPTERA FRUIT AND ANTIOXIDANT ACTIVITIES OF ITS DIETARY INCLUSION IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Tetrapleura tetraptera (TT) known as Aridan in Yoruba (Nigeria) has been used in folklore for ages. This study examined the phytochemical composition of TT and antioxidant effects of its dietary inclusion in *Drosophilia melanogaster*. Experiments with *D. melanogaster as* an animal model were conducted. Dried TT fruit was ground into powder. The antioxidant effects of dietary inclusion of TT on *D. melanogaster* and mRNA expression of antioxidant genes by real time-PCR assay were also determined. Phytochemical screening showed the presence of important secondary metabolites. The results indicate that TT fruit exhibited antioxidant property by reducing production of Malondialdehyde (MDA) and up-regulating superoxide dismutase (SOD) and catalase (CAT) expression significantly ($p < 0.05$) in D. melanogaster groups fed with 0.1% and 1% concentrations of TT compared to the negative control. These findings further established the antioxidant potential of TT in folklore.

Keywords: *D. melanogaster*, *T. tetraptera*, Malondialdehyde, Superoxide dismutase, Catalase

INTRODUCTION

Drosophila melanogaster, an arthropod fruit fly is a member of the Drosophillidae family. It has been explored in biological sciences researches for over 100 year [1], and now considered as a fitting model to assess biological activities of therapeutic against divers' human ailments [2, 3]. Its distinctive physiological characteristics are alike to that of vertebrates [4], and have been accepted scientifically to replace the usage of higher animals in biomedical researches [5]. Neurodegeneration and neural dysfunction is mainly caused by oxidative stress in the central nervous system [6]. It appears that the major contributor to aging, diabetes and cardiovascular diseases is cell damage caused by reactive oxygen species [7]. Reactive oxygen species (ROS) are often produced as a result of exogenous factors or biological reactions [8], playing numerous positive functions within the body [9].

The balance between the production and neutralization of ROS by antioxidants is very delicate, and if this inclines to the overproduction of ROS, the cells begin to suffer the consequences of oxidative stress [10]. ROS attacks cell membranes lipid and destroy DNA, promote oxidation causing membrane lipid peroxidation and reduce membrane fluidity [9]. The human system combats oxidative stress and oxidative mediated damage with the endogenous antioxidants which performs a key role to maintain optimal cellular functions and well-being [11, 12]. Superoxide dismutase converts superoxide anions into hydrogen peroxide which is subsequently converted into water and molecular oxygen by catalase [13]. Several diseases had been treated and managed using substances from therapeutic plants and herbs [14].

Tetrapleura tetraptera, called "aridan" by the Yoruba's' in Nigeria is a deciduous tree, belonging to the family of Mimosaceae and commonly found in the coastal Africa tropical forest [15]. The dry fruit has a pleasant aroma, used as spice in Central and West Africa [16]. The fruits are made up of a fleshy pulp with small brownish seeds and have high nutritional value [17]. Currently, studies have it that the pod, fruit and bark of *T. tetraptera* exhibited some antioxidant activities and anti-diabetic properties [17, 18]. These aforesaid properties posit *T. tetraptera* as a great functional food. It is exciting to note that functional plant foods have recently received great consideration due to their significance nutritionally and therapeutically [16, 19]. Therefore, advanced investigation of *T. tetraptera* health benefits is vital. This study examined some phytochemical constituents and the antioxidant potentials of *T. tetrapleura* dietary inclusion in *Drosophilia melanogaster*. Gene expression of SOD and CAT were also examined.

Materials and methods

Sample Collection and Preparation

Tetrapleura tetraptera (TT) fruits were purchased from Okusa market at Akungba-Akoko, Ondo State. The plant was identified and authenticated in the Department of Plant Science and Biotechnology, Adekunle Ajasin University. The fruits were washed, sliced, air dried, blender and kept in a dry container at room temperature before further analysis.

Chemicals and Equipment

Ethanol, Chloroform, Agar nutrient, Nipagin, Glucose, Iron Chloride, Chloroform, Isopentyl propanol, Nuclease frees water were procured from Sigma Scientific. All chemicals and reagents were of analytical grade.

Drosophila Melanogaster Stock Culture

Wild type *D. melanogaster* (Harwich strain) stock culture was obtained from Drosophila research laboratory, Functional Food and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology Akure, Nigeria. The flies were maintained and reared on normal diet made up of corn meal medium containing 1% w/v brewer's yeast and 0.08% v/w nipagin at constant temperature and humidity (25 ± 1°C; 60% relative humidity respectively) under 12 h dark/light cycle conditions. All the experiments were carried out with the same *D. melanogaster* strain [20].

Diet formulation

The basal diet was based on the traditional corn meal medium containing 1% w/v brewer's yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar and 0.08% v/w nipagin. The diet was prepared once a week. The *T. tetraptera* supplemented diet was prepared by adding 0.1 and 1.0 mg/g basal diet respectively. The *T. tetraptera* was dissolved in 1% ethanol (vehicle) and added to the basal diet at 0.1 and 1.0 mg/g diet, and the same volume of

ethanol, but no *T. tetraptera*, was added to the basal diet. The media were then mixed and distributed into vials.

Phytochemical Screening of Plant Extracts

Chemical tests were conducted on the powdered plant samples using the standard methods described by Edeoga *et al.* [21]. This was conducted for the determination of the presence of saponins, tannins, flavonoids and alkaloids.

Test for Tannins

0.5g of powered sample of each plant was boiled in 20.0ml of distilled water in a test tube and filtered. The filtration method used here was a normal method, which include a conical flask and filter paper. 0.1% $FeCl_3$ was added to the filtrate samples and observed for brownish green or a blue – black coloration, which showed the presence of tannins.

Test for Saponins

2.0g of powered sample of each plant was boiled in 20.0ml of distilled water in a water bath and then filtered. To obtain a stable persistent front, the 10.0ml of the filtrate was mixed with 5.0ml distilled water in a test tube and shaken vigorously. 3 drops of olive oil was mixed with the frothing, observed for emulsion formation, signifying the presence of saponins.

Test for Flavonoids

To the aqueous TT extract in a test tube, a few drops of 1% NH_3 solution was added, followed by the addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappears on standing.

Test for Alkaloids

200.0ml of 10% CH_3COOH in C_2H_5OH was added to 5.0g of the plant sample was prepared in a beaker. The mixture was covered, allowed to stand for 4 hours, and then filtered. The extract was concentrated in a water bath until the initial volume was quartered. The precipitate was realized by continuous addition of concentrated NH_4OH until completion. The precipitate was collected after the settling of the whole solution and washed with diluted NH_4OH and then filtered. The residue is alkaloid.

***In vivo* experiment**

Preparation of sample for biochemical assays

The flies were anesthetized in ice and homogenized in 0.1M phosphate buffer, pH 7.4, using a Teflon homogenizer. The resulting homogenates were centrifuged at 10,000g, 4°C for 10 minutes in a Kenxin refrigerated centrifuge Model KX3400C (KENXIN Intl. Co., Hong Kong). Subsequently, the supernatant was separated from the pellet into Eppendorf tubes and used for anti-lipid peroxidation assay.

Anti-Lipid Peroxidation Assay

A modified Ohkawa *et al*. [22] method was employed for the anti-lipid peroxidation assay. A reaction mixture containing 30µL of 0.1M pH 7.4 Tris-HCl buffer, plant extract (0-100µL) and 30µL of 250µM freshly prepared $FeSO_4$ were mixed with 100µL S1 fraction briefly. The volume was top up to 300µL with distilled water and incubated at 37℃ for 1hour. 300µL 8.1% Sodium dodecyl sulphate (SDS) was added to the reaction mixture for colour reaction development, afterwards followed by the addition of 500µL of acetic acid/HCl (pH 3.4) and 500µL 0.8% thiobarbituric acid (TBA) mixture. This mixture was incubated at 100℃ for 1hour. The produced thiobarbituric acid reactive species (TBARS) from the reactions were measured at 532nm using a JENWAY UV-Visible spectrophotometer. The absorbance was compared against malondialdehyde (MDA) standard curve.

RNA Isolation and mRNA Analysis of SOD and CAT by real-time PCR

In brief, the total RNA was collected from the tissue of *D. melanogaster* and extracted using the commercial extraction agent TRIzol. Fruit flies were homogenized in 1 mL of TRIzol solution and then centrifuge at 12,000 g at 4 °C for 5 min. The supernatant was transferred to another new tube containing 160 µL Chloroform. The mixture was then subjected to centrifugation at 12,000g 4 °C for 5 min. The upper layer was mixed with 400 µL 100 %v iso-amyl propanol to precipitate the RNA. After 1 hour of incubation at -20 °C, the samples were centrifuged at 12, 000 g at 4 °C for 20 min. The pellet was washed with cold 70 % ethanol followed by vortexing and then re-centrifugation. Finally, the RNA pellet was re-suspended in an appropriate amount of nuclease-free water. The concentration and purity of RNA obtained were determined by measuring their absorbance at 260 nm and 280 nm. cDNA was obtained by reverse transcription and then stored at room temperature for 24 hours before further use. Real time-PCR assay was applied to determine and amplify SOD and CAT gene mRNA expression. All reactions were done with a CFX96 real-time PCR detection system. The thermal cycle conditions were: initial denaturation step (4 °C, 5 min), denaturation (25 cycles, 94 °C, and 30 s), annealing (50 °C, 30 s) and elongation (72 °C, 30 s). Relevant information on the gene primers are listed in Table 1. Agarose gel electrophoresis was used for purification and visualization of the amplified cDNA fragment. The expressed gene was visualized on the photophoresis machine [23].

Table 1: Real time PCR primers used to measure *Drosophila melanogaster* antioxidant mRNA gene expression

Gene	Forward primer 5' -3'	Reverse primer 5'- 3'
SOD1	GACGAGAATCGTCACCTGGG	TTGACTTGCTCAGCTCGTGT
CAT	TGCATGGTCGTCTGTTCTCC	CATTCCTGGGGACCGTTGAA
GaPdH	GTCTCACCCCATTCTACCGC	AGTTTGGCTACTCCAACCGC

Data Analysis

Data were expressed as mean ± SD. Significant difference between samples was assessed using a Student t-test. Differences were considered significant when $P < 0.05$. Statistical Package for the Social Sciences (SPSS) version 17.0 software was used for the analysis [24].

Results and discussion

Phytochemical composition

The results of the phytochemical analyses on *Tetrapleura tetraptera* (fruit) is shown in Table 2. The result revealed the presence of tannins, saponins, flavonoids and alkaloids in the sample. Bioactive compounds in plants have been demonstrated to have good health benefits and prevent the risk of chronic diseases such as diabetes, cancer, obesity, neurodegenerative and cardiovascular diseases [25]. Plant phytochemicals have been demonstrated to possess higher antioxidant potential [26].

Table 2: Phytochemical screening on aqueous extract of *Tetrapleura tetraptera* fruit

Test	*Tetrapleura tetraptera*
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+

Key: +: Present

The protective effect of *T. tetraptera* extract against Fe^{2+} induced lipid peroxidation is presented in Figure 1. This result shows a significantly ($P < 0.05$) higher MDA level in the negative control (6.73 µMol. MDA /mg prot) compared to the normal control (2.99 µMol. MDA /mg prot).While, pretreatment with *T. tetraptera* extract (0.1% and 1%) reduced MDA level (4.28 µMol. MDA /mg prot and 5.38 µMol. MDA /mg prot respectively) significantly ($p < 0.05$) when compared to the negative control (6.73 µMol. MDA /mg prot), with 0.1% TT showing the highest MDA inhibition. This finding agrees with a study earlier reported by Oboh *et al* [27]

where MDA production was significantly increased in the presence of Fe^{2+}. In this present study, *T. tetraptera* extract showed a higher inhibition of lipid peroxidation which is characterized by decrease in MDA level. This protective effect against lipid peroxidation may be due to the presences of some essential phytochemicals in the TT extract [28].

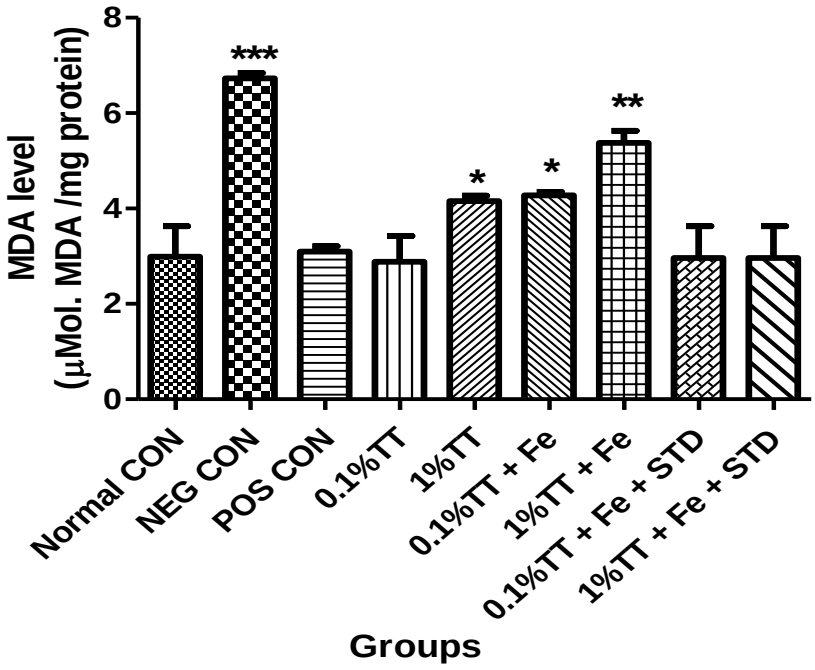

Fig. 1: Effect of dietary inclusion of *T. tetraptera* fruit on Fe^{2+} induced MDA in *D. melanogaster.*
Values represent mean ± SD. ***P<0.001, **P<0.01, *P<0.05 vs. normal con;
Keys:CON: Control; NEG: Negative; POS: Positive; TT: *Tetrapleura tetraptera*; Fe: $FeSO_4$; STD: Standard (0.1 % Gallic acid)

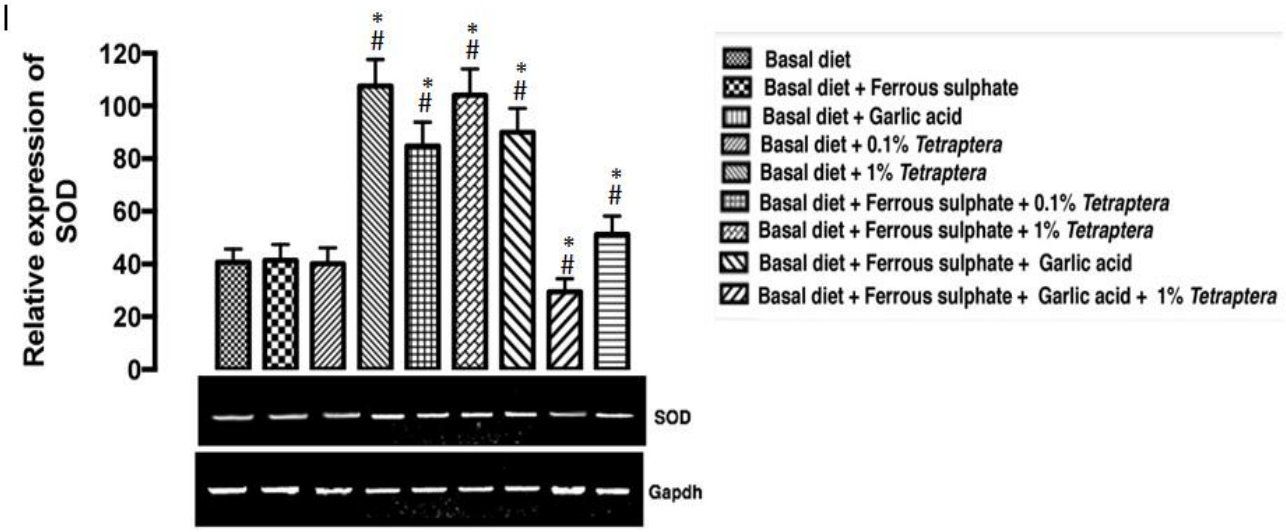

Fig. 2: The effects of *Tetrapleura tetraptera* on the expression of SOD gene in *D. melanogaster.*

Results are presented in mean ± SEM. Relative expression of Catalase gene vs. GAPDH in all groups. P < 0.05 (95% confidence level). # indicate significant difference when groups were compared with basal diet (normal control), while * indicate significant difference when groups were compared with basal diet + ferrous sulphate (negative control).

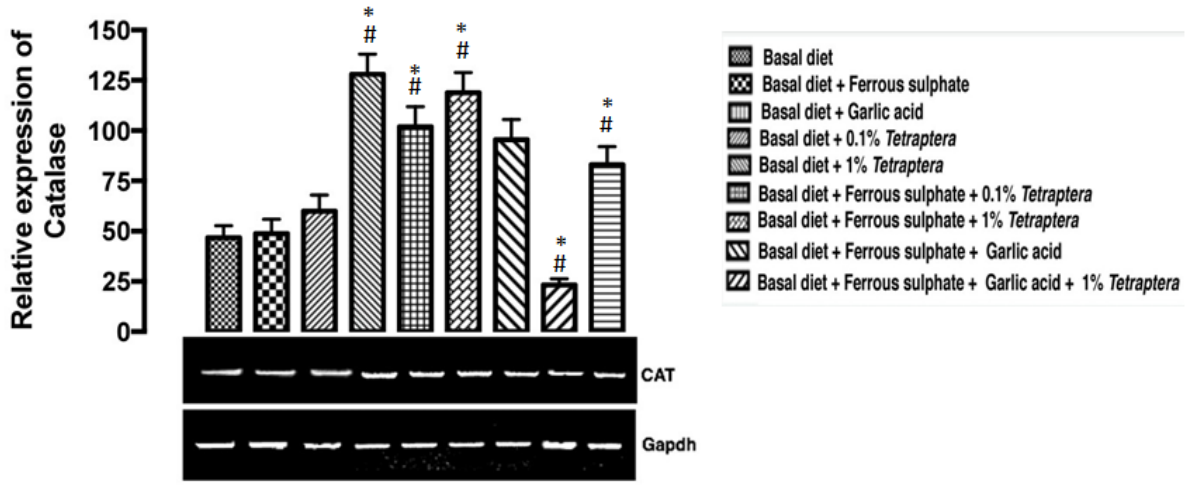

Fig. 3: The effects of *Tetraptera tetrapleura* on the expression of Catalase gene in *D. melanogaster.*

Results are presented in mean ± SEM. Relative expression of Catalase gene vs. GAPDH in all groups. P < 0.05 (95% confidence level). # indicate significant difference when groups were compared with basal diet (normal control), while * indicate significant difference when groups were compared with basal diet + ferrous sulphate (negative control).

Effect of *T. tetraptera*-supplemented diet on SOD and CAT gene expression level in *D. melanogaster*

Superoxide dismutase (SOD) is the first detoxification enzyme and most potent cellular antioxidant. It is an important endogenous antioxidant enzyme that acts as a component of first-line defense system against ROS [29]. The effect of *T. tetraptera*-supplemented diet on SOD and CAT genes expression level in *D. melanogaster* were examined and the result presented in Fig. ii and iii' respectively. As presented in Figure 2 and 3, *T. tetraptera* (0.1 % and 1 %) caused a significant increase in mRNA expression level when compared with control. *D. melanogaster* is a typical anti-oxidation and anti-aging animal model and the experimental result revealed that expression of SOD and CAT genes in all the groups fed with the *T. tetraptera* was significant (P < 0.05) compare to the normal control group and with the negative control group. This signifies that *T. tetraptera* increases the expression of SOD and CAT, thus the antioxidant system is optimized to ameliorate oxidative stress.

T. tetraptera was able to slightly up-regulate the expression of SOD and CAT across board in the groups that received treatment with the plant extract, although as expected, the levels of expression were different based on concentration. The down-regulation of SOD and CAT genes expressed in basal + ferrous sulphate + garlic group against both positive and negative control groups was notable. From the graph, the group that received ferrous sulphate + garlic has these genes repressed, suggesting that the antioxidants level of garlic was insufficient to mitigate the effects of free radicals generated by ferrous sulphate. The result showed that 0.1% *T. Tetraptera* was most effective at up regulating the mRNA expression of SOD and CAT scavenging free radicals that could cause oxidative stress in *Drosophila melanogaster*. This result is consistent with the results of Shanthi that hawthorn could prevent antioxidant levels such as glutathione and vitamin E from dropping, and maintaining the activities of antioxidant enzymes in the liver, aorta and heart [30].

In conclusion, *T. tetraptera* extract possesses various phytochemicals and antioxidant ability against oxidative stress mediated ion toxicity. Also, the study demonstrated that dietary supplementation with *T. tetraptera* upregulated SOD and CAT mRNA expression in *D. melanogaster* conferring its protective potential and its use in folklore.

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Conflict of interest

The authors declare no conflict of interests.

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