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IN VITRO PROTECTIVE EFFECT OF *TELFAIRAI OCCIDENTALIS* (FLUTED PUMPKIN) LEAVES AGAINST OXIDATIVE DNA DAMAGE INDUCED BY REACTIVE OXYGEN SPECIES

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ABSTRACT
Oxidative DNA damage is an inevitable consequence of cellular metabolism leading to reactive products that cause cellular damage. *Telfairai occidentalis* is a tropical vine grown in West Africa as a vegetable and for its edible seeds. This study aimed to evaluate the in vitro protective effect of *T. occidentalis* leaves extract against DNA damage induced by Fenton's system. *T. occidentalis* leaves were extracted using three solvents; water, n-butanol (n-BuOH) and ethyl acetate (EtOAc). pVax plasmid DNA (476 ng) was incubated with Fenton's system ($FeSO_4/H_2O_2$) in the presence/varying concentrations of the extract (10 mg/mL, 5 mg/mL and 1 mg/mL) at 37°C for 30 minutes while the control was devoid of extract. The incubate were analysed in 0.8 % agarose gel. The ethyl acetate and the butanolic extract were subjected to GC-MS analysis. The protective effect of *T. occidentalis* leaves extracts demonstrated an effective protective activity against oxidative stress-induced DNA damage and was dose-dependent with higher dose being most protective. Oleic acid and 3-hydroxy benzoic acid were found to be most likely the bioactive components of the butanolic and the ethyl acetate extract respectively.

Keywords: Reactive Oxygen Species, DNA damage, Telfairai occidentalis

INTRODUCTION

Reactive Oxygen Species (ROS) produced during normal physiological events is a significant contributor to cellular and molecular damage and interacts with biomolecules like lipids, proteins, and nucleic acids, leading to oxidative damage. One of the most concerning effects of ROS is oxidative damage to DNA, which can result in carcinogenesis, mutagenesis and other degenerative diseases such as cancer and neurodegenerative conditions. Mitigating the effects of ROS for biological systems and protecting the DNA from oxidative damage has become an important focus in preventing related diseases (Chaudhary et al 2023). ROS is eliminated by antioxidant defense mechanisms, protecting the cells of its toxic effects have of recent aroused considerable interest due to their broad range of effects in biological systems. Overproduction of ROS in pathological conditions are associated with several chronic disease prevention and development (Kumar and Pandey, 2013, Shen *et al*. 2022). Naturally occurring antioxidant compounds from plant sources have generated increased attention as potential therapeutic agents for combating ROS and are increasingly investigated for use in foods to replace synthetic antioxidants due to their side effects (Hassanpour and Doroudi, 2023). They are favored for their low toxicity, availability and wide variety of bioactive compounds. *T. occidentalis* is a tropical vine from West Africa for culinary utilization. It is known for its antioxidant capabilities, but the potential protective effects of the leaves against oxidative damage induced by ROS is yet to be extensively studied. Understanding its protective effect against oxidative DNA damage could provide insight into its potential as a natural antioxidant (Zhuang et al. 2023) and may lead to novel approaches in preventing oxidative stress-related diseases (Gomaa et al. 2022). We investigate the *in vitro* antioxidant and protective effect of *T. occidentalis* leaves against DPPH induced oxidative stress DNA damage.

METHOD

T. occidentalis plant leaf samples were obtained and confirmed at the Herbarium of the department of biological Science, ABU Zaria. Aqueous extract of shade dried, pulverized leaves was fractionated with water, *n*-butanol and ethyl acetate. The IC_{50} and free radical scavenging activities of *T. occidentalis* on DPPH free radicals was determined according to Oktay *et al*. (2003) with absorbance measured spectrophotometrically at 517nm. The protective effect of *T. occidentalis* on DNA damage was determined using Fenton's system according to Li *et al*. (2013) on *pVax* plasmid DNA (476ng), the outcome was analyzed on 0.8% agarose gel electrophoresis.

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RESULTS

Free radical scavenging activities of *T. occidentalis* on DPPH free radicals

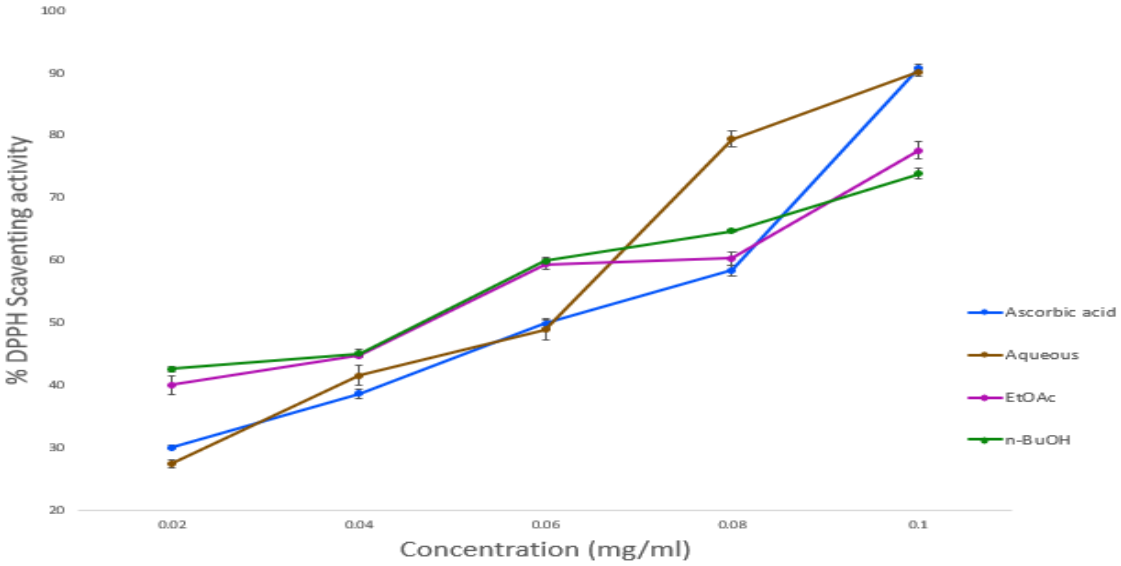

Figure 1: DPPH scavenging activity of the different extracts of *T. occidentalis*

Figure 1 demonstrated the reduction capability indicating its potential antioxidant activity. IC_{50} for aqueous, ascorbic acid, EtOAc and *n*-BuOH was 50.78, 54.93, 45.77 and 42.38 µg/mL respectively.
The potential of *n*-BuOH extract of *T. occidentalis* showed free radical scavenging activity using DPPH test exhibited significant protection against free radical (DPPH) scavenging activity in a dose dependent pattern, this enhanced the levels of an antioxidant defense system

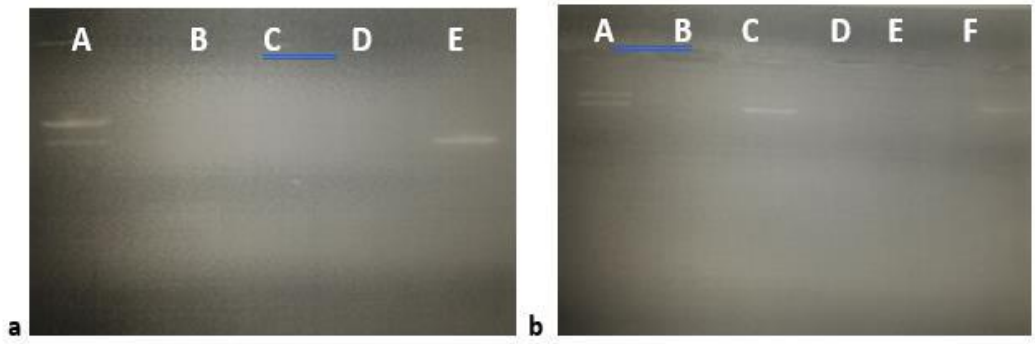

Plate 1: 0.8% Agarose gel electrophoregram of (a) aqueous and (b) *n*-BuOH extract of *T. occidentalis* leaves

(c) A = Plasmid + 10.0 mg/ml extract + Fenton's, B = Plasmid + 5.0 mg/ml extract + Fenton's, C = Plasmid + 1.0 mg/ml extract D = Plasmid + Fenton's, E = Plasmid + water
(d) A = Plasmid + extract, B = Plasmid + Fenton's, C = Plasmid + DMSO/PBS (1:1), D = Plasmid + 10.0 mg/ml extract + Fenton's, E = Plasmid + 5.0 mg/ml extract + Fenton's, F = Plasmid + 1.0 mg/ml extract + Fenton's

DNA nicking induced by hydroxyl radical
The pVax plansmid from plate 1a, in Lane A: Plasmid + 10.0 mg/mL + Fenton's reagent showed the pVax plasmid was protected, in lane B: Plasmid + 5.0 mg/mL extract + Fenton's reagent was degraded, in lane C: Plasmid + 1.0 mg/mL + Fenton's reagent was also degraded, in lane D: Plansmid + Fenton's (negative control) was degraded and in lane E: Plasmid + water being the positive control with the reference standard was protected.
While the pVax plasmid in plate 1b, in Lane A: Plasmid + extract was protected, lane B: Plasmid + Fenton's reagent was degraded,

lane C: Plasmid + DMSO/PBS (1:1) was protected, lane D: Plansmid + 10.0 mg/mL extract + Fenton's reagent was partially protected, lane E: Plasmid + 5.0 mg/mL extract + Fenton's reagent was slightly protected, and lane F: Plasmid + 1.0 mg/mL extract + Fenton's reagent was protected.

The electrophoregram in Plate 1 showed the protection ability of *T occidentalis* (a and b) and reference standard (E) when compared with (D) the negative control was found to be effective in protecting *pVax* plasmid DNA against the strand breakage induced by hydroxyl radicals in a Fenton's reaction mixture.

DISCUSSION

The current study investigated the protective effect of *T. occidentalis* leaf extract. The *in vitro* antiplasmodial activities of *T. occidentalis* extract demonstrated the leaves to possess significant protective activity, suggesting that *T. occidentalis* contain biologically active substances. This supports the traditional use of this plant for various medicinal and culinary purposes. *T. occidentalis* and the reference compound (ascorbic acid) the free radical (DPPH) scavenging activity increased steadily with the increase in concentration. The absorbances at 517 nm of *T. occidentalis* and ascorbic acid showed that *T occidentalis* can act as electron donor and can react with free-radicals to convert them to more stable products and thereby terminate radical chain reactions (Ding *et al.* 2020). The IC_{50} value of *T occidentalis* was observed to be at 50.78, 54.93, 45.77 and 42.38 µg/mL for aqueous, ascorbic acid, EtOAc and *n*-BuOH was respectively, suggesting a moderately active antplasmodial activity relative to ascorbic acid. According to the norm the extract is active when IC_{50} < 5 µg/mL, moderately active when 5 µg/mL< IC_{50} <50 µg/mL and weak when IC50 >50 µg/mL (Berre *et al*. 2022, Garcia-Molina *et al*. 2022). The reducing power of plant compounds might be due to the di- and mono-hydroxyl substitution in the aromatic ring which possesses potential hydrogen donating abilities (Ding *et al.* 2020). The reducing properties are generally associated with the presence of reductones, known to exert antioxidant activity by breaking the free-radical chain by donating a hydrogen atom (Konecny *et al.* 2018, Savel *et al.* 2012).

Hydroxyl radical is the most reactive among reactive oxygen species (Behnaz *et al*. 2022), and considered to be responsible for much of the biological damage in free-radical pathology (Mittal et al. 2023), has the capacity to cause strand breakage in DNA, and contributes to carcinogenesis, mutagenesis and cytotoxicity (Chaudhary et al 2023). The DNA protective effect of aqueous, n-BuOH and EtACc extract of *T. occidentalis* leaf was checked against Fenton's induced DNA damage on *pVax* plasmid DNA. The protection offered against DNA damage by *T. occidentalis* was concentration dependent at concentration 0.02-0.1mg/mL, protection exhibited was more effective and slightly close to that of ascorbic acid. ROS however play a two-fold role as both toxic (generating oxidative stress) and beneficial (indulge in cellular signaling and immune function) compounds to the organism's system (Chaudhary et al 2023). The result showed a correlation between the effect and concentration, and attributed to the presence of high amounts of polyphenols, a potent antioxidants

Conclusion

T. occidentalis extracts exhibited an effective ability to scavenge DPPH radicals in a dose-dependent manner. It was found to be effective in protecting plasmid DNA against damage induced by hydroxide radicals in Fenton's reagent and can be a rich source of antioxidants that mitigate oxidative stresses on susceptible biomolecules.

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