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A Biochemical Evaluation of the Anti-Diabetic and Antioxidant Activities of *Tetrapleura tetraptera*

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Abstract

The anti-diabetic and antioxidant activities of the methanolic extract of *Tetrapleura tetraptera* fruits and seeds were investigated. The dried fruits and seeds of *T. tetraptera* were separately collected, grounded and extracted in methanol and water. Alpha–amylase inhibitors are used to achieve greater control over hyperglycemia in type-II diabetes mellitus. The present study tends to screen alpha amylase inhibitors from natural sources like plants (*T. tetraptera*) in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycemia. Similarly, the retinol, ascorbic acid, tocopherol, terpinoids, phenols, flavonoids, beta-carotene and lycopene contents of *T. tetraptera* were also studied using standard methods. Inhibition of Fe^{2+}-induced lipid peroxidation in brain homogenate was evaluated on the seed, as free radical scavenging activity was determined on the fruit using DPPH (2,2-diphenyl-1-picryhydrazyl) method. Results for alpha-amylase inhibition show that the %inhibition (IC_{50}) of *T. tetraptera* fruit as compared to the standard acarbose were 4.276ug/ml and 4.087ug/ml respectively. Whereas that of *T. tetraptera* seed as compared to standard acarbose were 15110.00ug/ml and 521.89ug/ml respectively. *T. tetraptera* fruit showed high free radical scavenging activity as against the standard Butylhydroxyanisol (BHA) with effective concentrations (EC_{50}) of 423.467ug/ml and 307.50ug/ml for extract and BHA respectively. Similarly, *T. tetraptera* seed had a good inhibition on lipid peroxidation as compared to standard BHA with %inhibition (IC_{50}) of 575.75ug/ml and 728.75ug/ml for sample and standard respectively. In addition, results also showed that *T. tetraptera* contained 7.92±65mg/100mg and 7 ascorbic acid, 6.34±0.65mg/100g retinol, 4.86±0.57mg/100g tocopherol, 11.19±4.72GAE/100g phenols, 0.48±0.22CE/100g flavonoids, 18.67mg/g β-carotene and 6.825mg/100g terpenoids. These results suggest that *T. tetraptera* fruits and seeds can be used in the management of diabetes as well as oxidative stress reduction.

Keywords

Tetrapleura tetraptera, Antidiabetes, Antioxidant, Alpha-Amylase, Hyperglycermia

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1. Introduction

Oxygen is, no doubt, an indispensable part of aerobic life. However, under certain circumstances, it can seriously affect our well-being through the formation of reactive oxygen species (ROS) representing both free radical and non-free radical species, and their potential delirious effects such as arteriosclerosis, ischemic heart disease, ageing, inflammation, diabetes, immune suppression,

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neurodegenerative diseases, cancers owing to lipid peroxidation and others [1]. The most encountered free radicals are the hydroxyl radical (OH^-), the superoxide radical (O_2^-), the nitric oxide radical (NO^-) and the lipid peroxyl radical (LOO^-), while non-free species are principally hydrogen peroxide (H_2O_2) and singled oxygen (O_2) [2].

Natural antioxidants from plants have the ability to protect the body against oxidative damage [3], by scavenging the free radicals and inhibiting peroxidations and other radical mediated processes. Many phytochemicals have antioxidant activity and reduce the risk of many diseases, for example, alkyl sulphide (found in onions and garlic), carotenoids (from carrots), and flavonoids (present in fruits and vegetables). Phenolic compounds in plants function as antioxidants due to their redox properties. They are therefore useful as reducing agents, hydrogen donors, free radical quenchers, and metal chelators. In recent years, significant attention has been directed towards exploring plant-based natural antioxidants, especially the phenolics and tocopherols [4-5]. A lot of antioxidants have already been extracted and isolated from different parts of plants and plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs [6]. In fact, more than 80% of people in developing countries are estimated to depend on traditional herbal medicine (WHO) while more than 60% of patients are reported to use herbs in management and treatment of cancer and diabetes [7].

Diabetes mellitus is a pathological condition characterized by hyperglycemia due to partial or total loss of insulin secretion [8]. It is a metabolic disorder as it causes disturbances of carbohydrate, fat and protein metabolism, leading to several complications such as nephropathy, neuropathy, and retinopathy. Current statistics suggest that about 382 million people are living with diabetes worldwide and this number is projected to increase to 552 million by 2035, while in Africa, 19.8 million people are diagnosed with the disease [9]. In patients with diabetes, acarbose, which potently inhibits alpha amylase and alpha glucosidase, is expected to show a beneficial effect on their pathological conditions by inhibiting digestion and absorption of carbohydrates when ingested with meals.

Thus, the search for the discovery of antidiabetic drugs that can perform the function of acarbose, from medicinal plants is an important strategy required to combat the widespread nature of diabetes mellitus in the world. This is because present synthetic drugs have many disadvantages ranging from limited efficacy and several side effects such as hypoglycemia, weight gain and chronic tissue damage [10]. One such plant in the management of these metabolic disorders is *T. tetraptera*.

Tetrapleura tetraptera belongs to the mimosaceae family. It is referred locally to as Aridan in Yoruba and oshosho in Igbo. It is generally found in the lowland forest of tropical Africa. The fruit consist of a fleshy pulp with small, brownish-black seeds. The dry fruit has a pleasant aroma [11]. It is used as a popular seasoning spice in Southern and Eastern Nigeria. The fruit is used to prepare soup for mothers from the first day of birth to prevent post-partum contraction. The fruits are also used for the management of convulsions, leprosy, inflammation, rheumatism, flatulence, jaundice and fevers [12]. The anticonvulsant activity of the volatile oil from fresh fruits of *T. tetraptera* in mice has been reported. Its leaves are essential for the treatment of epilepsy and present strong molluscicidal activity. The root extract has also been proven to be used for the treatment of gastrointestinal related clinical problems.

Consequently, the objective of the present study is to investigate the antidiabetic and antioxidant potentials of *Tetrapleura tetraptera.*

2. Materials and Methods

2.1. Collection, Identification, and Preparation of Sample

Fruits of *T. tetraptera* were purchased from a market in Awka, Anambra state. The fruits were cut open and the seeds separated from it. The non-infected fruits and seeds were separately washed, dried, ground into a fine powder using a corona manual grinder. The fine powders of both fruits and seeds were separately macerated in methanol and distilled water (4:1) for 48 hours and concentrated to obtain the different extracts for both fruits and seeds using rotary evaporator.

2.2. Estimation of Alpha-Amylase Inhibition

This was done using the methods of M. A. BHUTKAR and S. B. BHISE Government College of Pharmacy, KARAD, INDIA. This was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay condition. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. 1ml of the aqueous extract of *Tetrapleura tetraptera* was pre-incubated with α-Amylase for 30 minutes. Afterwards 1ml of 1%w/v starch solution was added. Then incubate at 37°C for 10 minutes. The reaction was stopped by adding 1ml of DNS reagent and the contents were heated in boiling water bath for 5 minutes. A blank was prepared without having the *Tetrapleura tetraptera* extract, and another without amylase enzyme, replaced by equal quantities of buffer (20mM Sodium phosphate buffer with 6.7mM Sodium chloride, pH 6.9 at 20°C). The absorbance was measured at 540nm for both

fruits and seed separately, using a UV spectrophometer. The reducing sugar released from starch was estimated as maltose equivalent from the standard graph. Acarbose was used as positive control for both fruits and seeds.

2.3. DPPH Radical Scavenging Assay

The capacity to scavenge the stable free radical DPPH was monitored. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the non-radical form of DPPH-H, various concentration of fruit extract was mixed with methanolic solution containing DPPH radicals. The mixture was shaken vigorously and left to stand for 60 minutes in the dark. The reduction of DPPH radical was determined by measuring the absorption at 517nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration

$$\%RSA = [(ADPPH - AS) / ADPPH] \times 100$$

Where ADPPH is the absorbance of the DPPH solution, and AS is the absorbance of the solution when *T. tetraptera* fruit extract has been added at a particular level. The extract concentration providing 50% of radical scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. BHA was used as a standard control.

2.4. Inhibition of Lipid Peroxidation

This was determined by the method of Barros [13]. Determination of the extent of inhibition of lipid peroxidation was carried out using homogenate of brain of a goat 130kg as the source of poly unsaturated fatty acids. The brain was dissected and homogenized with pestle and mortar in an ice-cold Tris-HCL buffer (pH 7.4, 20mM) to produce 10% w/v brain homogenate which was centrifuged at 3000rpm for 10mins. An aliquot (0.1ml) of the supernatant was incubated with 0.2ml of the *Tetrapleura tetraptera* seed extract at various concentrations (100-1000µg/ml) in the presence of 0.1ml of 10µM ferrous sulphate and 0.1ml of 0.1mM ascorbic acid at 37°C for 1 hour. The reaction was stopped by the addition of 0.5ml of 28% TCA followed by the addition of 0.38ml of 2% TBA. The mixture was then heated at 80°C for 20 minutes. After centrifugation at 3000rpm for 10mins to remove the precipitated protein, the colour of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532nm using spectrophotometer. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio } (\%) = [(A-B)/A] \times 100\%$$

Where A and B are the absorbance of the control and the compound solutions respectively. The seed extract concentration providing 50% lipid peroxidation inhibition (EC 50) was calculated from the graph of antioxidant activity percentage against the extract concentration. BHA was used as the standard.

2.5. Determination of Total Phytochemicals

Total phenols and flavonoids were determined by methods described by Barros [13]. Beta-carotene, Lycopene, Retinol, Tocopherol, and Ascorbic acid were determined by methods described by Rutkowski [14]. Whereas, terpene was determined by method described by Narayan [15].

3. Results and Discussion

The evaluation of the antidiabetic and the antioxidant activities of *T. tetraptera* fruits and seeds revealed that the methanolic extract of *T. tetraptera* fruit has a maximum alpha-amylase inhibitory activity (IC_{50}) of 4.276ug/ml as against IC_{50} 4.087ug/ml for standard acarbose (figure 1), while the seed had a maximum inhibitory activity (IC_{50}) of 15110.00ug/ml as against IC_{50} 521.89ug/ml for standard acarbose (figure 2). Likewise, figure 3 shows the free radical scavenging activities of *T. tetraptera* fruits with effective concentration (EC_{50}) of 423.467ug/ml as against standard BHA EC_{50} of 307.50ug/ml. Figure 4 shows the inhibition of lipid peroxidation in brain homogenate by *T. tetraptera* seed with IC_{50} of 575.09ug/ml against 728.75ug/ml for standard BHA. Figure 5 and Table 1 contain the results for the bioactive components contained in both fruits and seeds respectively.

Table 1. Bioactive components in *T. tetraptera* seed.

Parameter	Result
Phenol	11.19 ± 4.72GAE/100g
Flavonoid	0.48 ± 0.22CE/100g
B-carotene	18.67 ± 2.52mg/g
Lycopene	Not detected

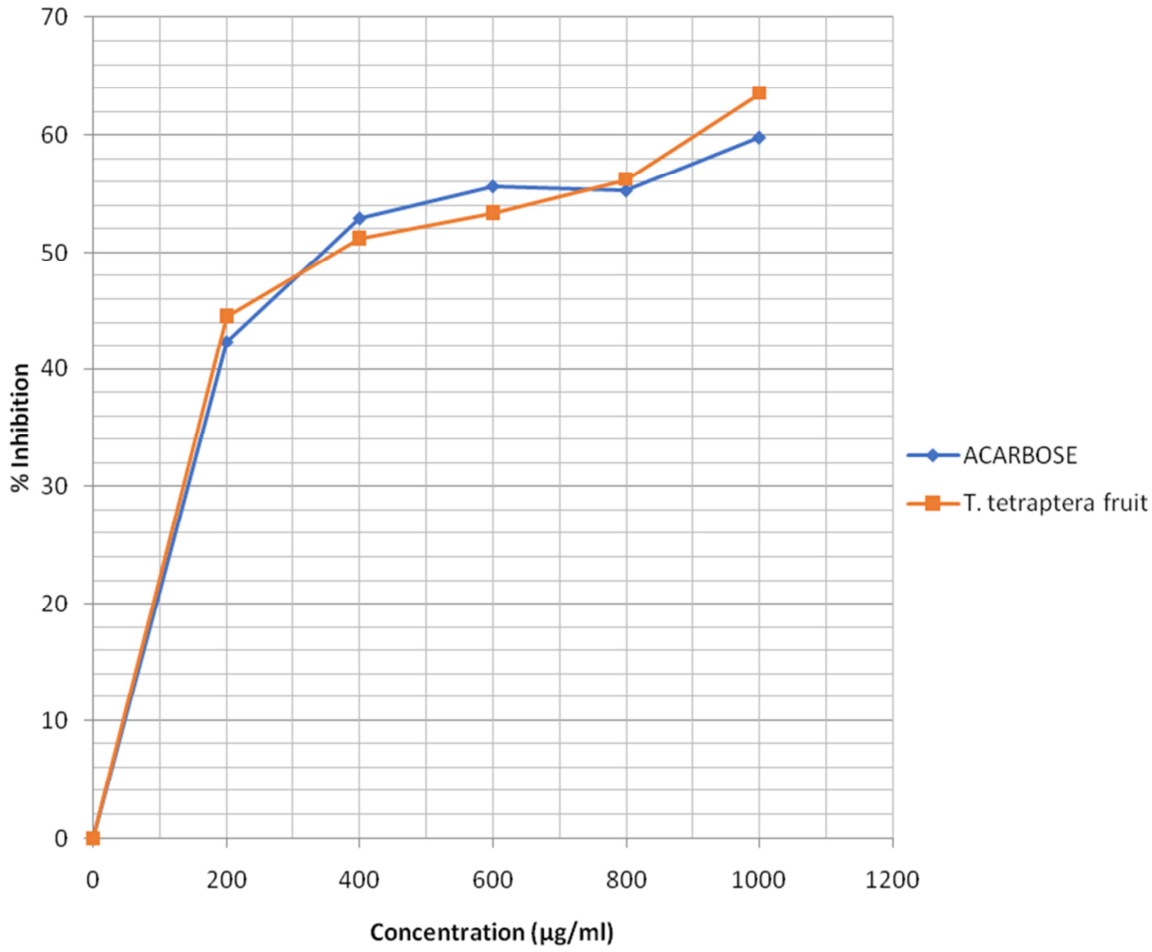

Figure 1. Alpha-amylase inhibitory activity of *T. teraptera* fruit.

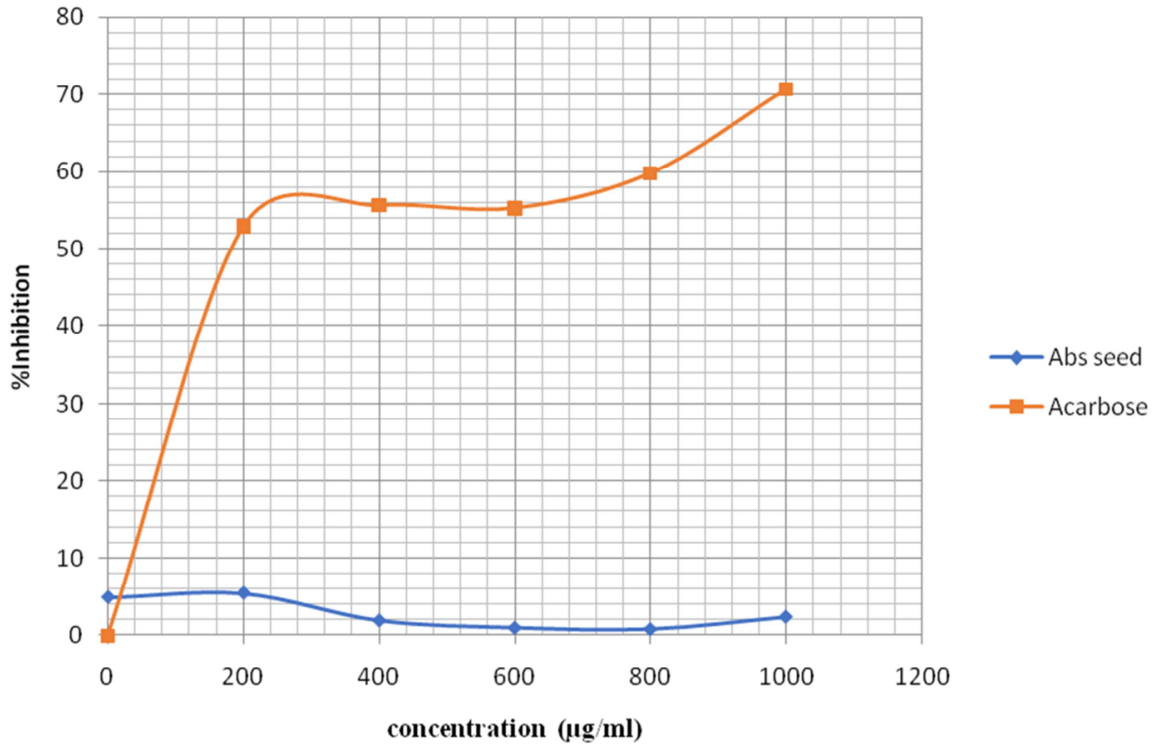

Figure 2. Alpha-amylase inhibitory activities of *T. tetraptera* seed.

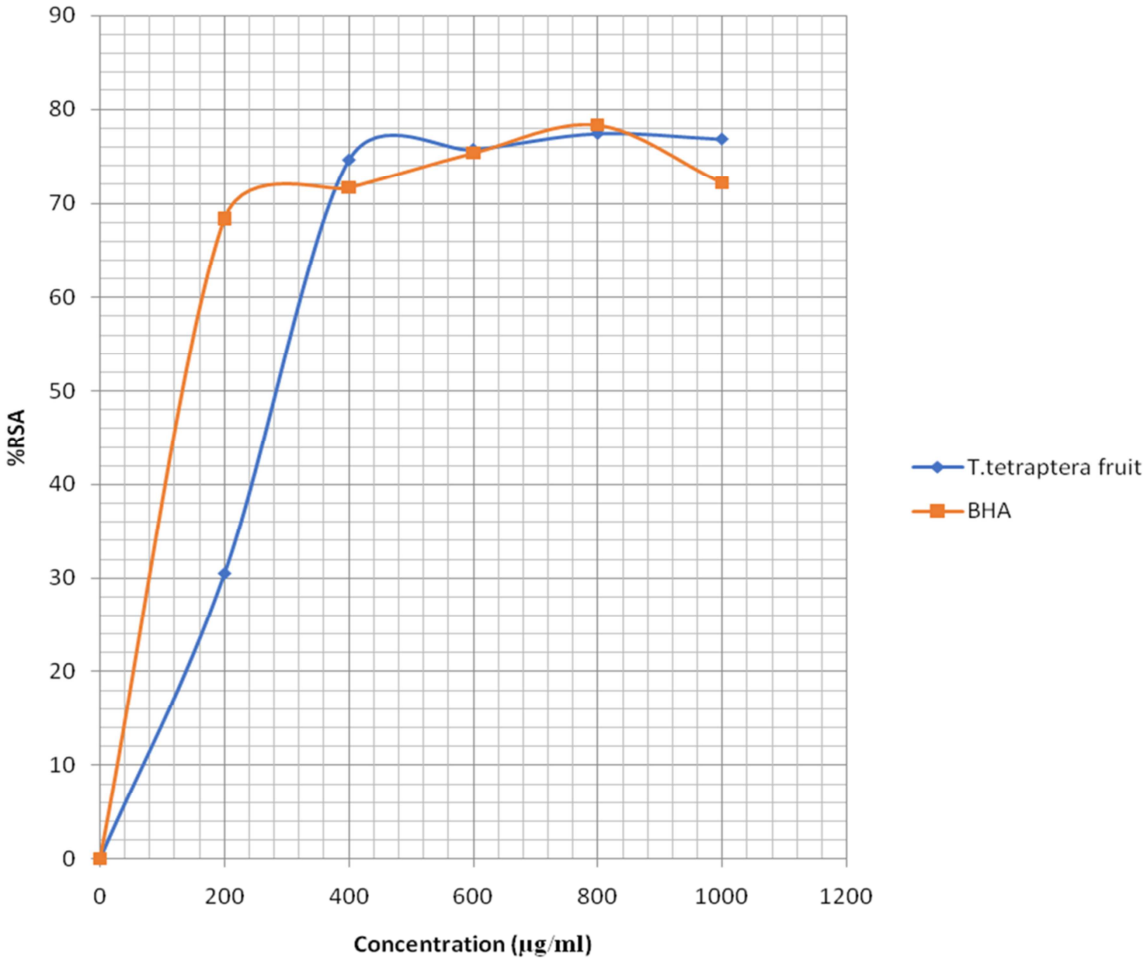

Figure 3. Free radical scavenging activity of *T. teraptera* fruit.

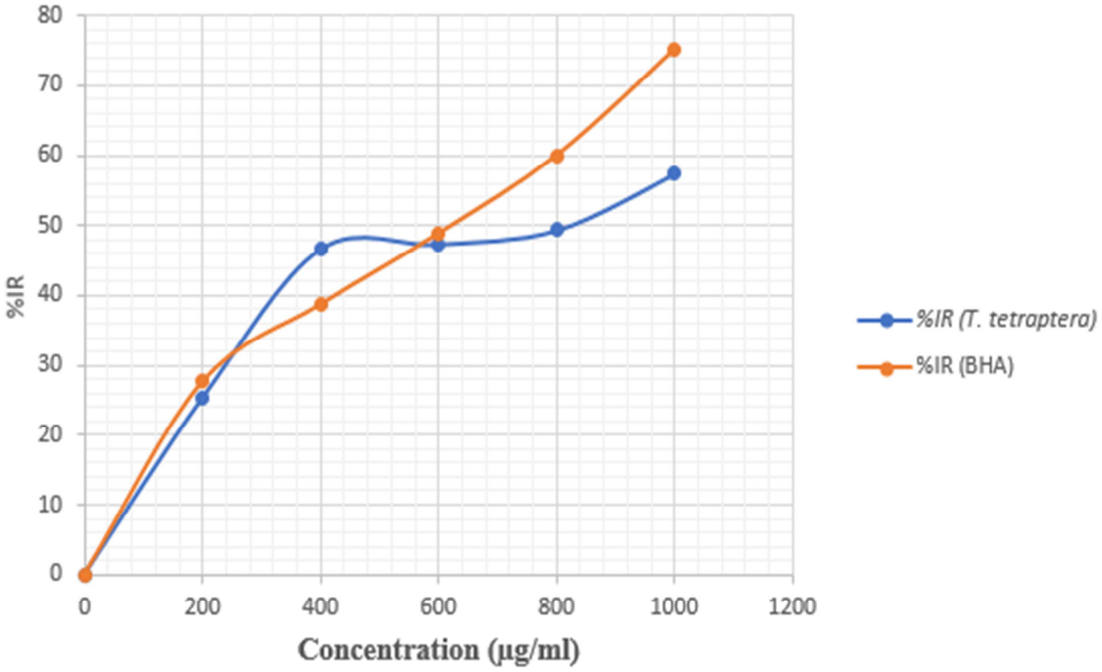

Figure 4. Inhibition of lipid peroxidation in brain homogenate by *T. tetraptera* seed.

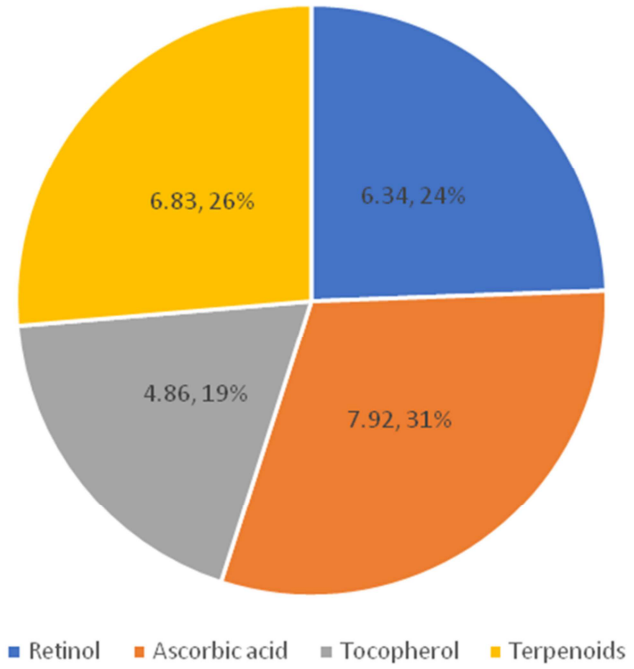

Figure 5. Bioactive components in *T. tetraptera* fruit (mg/100g).

It has been suggested that, the management of diabetes and oxidative stress should include therapeutic strategies to reduce hyperglycemia and decrease or control activities of free radical species. Natural alpha-amylase inhibitors from plant sources offer an attractive therapeutic approach to the treatment of hyperglycemia by decreasing glucose release from starch, which may be potentially useful in the treatment of diabetes mellitus, as well as the control of oxidative damage [16]. To support the quest for alternative therapies for diabetes as well as oxidative stress, we investigated the in vitro potential of *T. tetraptera* seeds and fruits to reduce oxidative stress as well as high carbohydrate digestion via the enzymes alpha amylase. The anti-amylase activity observed in the result for both seeds and fruits may be due to the presence of phenolic compounds and vitamins, which have been shown to interact with and/or inhibit the enzyme [17-18]. The reaction mechanism involved in the inhibition of alpha amylase may be due to the presence of flavonoids which have been reported to inhibit alpha amylase [19]. Similarly, phenols are prominently known to exhibit antioxidant activity through a variety of mechanisms, including free radical scavenging, inhibition of lipid peroxidation and chelating of metal ions [20].

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers, it was reasonable to determine their total amount in the seed and fruit extracts of *T. tetraptera*. Phenols and polyphenols can provide relief from certain physical ailments and degenerative diseases in humans, including the reduction of cardiovascular diseases and certain cancers [21]. The effects of dietary phenolics are of great current interest, due to their antioxidative and possible antidiabetic activities. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation [22]. The many pharmacological effects of phenols and flavonoids are linked to their ability to act as strong antioxidants and free radical scavengers, to chelate metals, and to interact with enzymes, adenosine receptors, and bio membranes.

Similarly, ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine [23]. Thus, the presence of ascorbic acid and other vitamins, with powerful antioxidant properties, indirectly contribute to the several key oxidative and reductive enzyme systems. Ascorbic acid has the ability to regenerate other biologically important antioxidant activities, such as glutathione and tocopherol, into their reduced states [24].

4. Conclusion

The findings of this study suggest that the use of these plant extracts will be greatly beneficial to reduce oxidative stress and the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes. Future studies will provide more insight for the molecular mechanisms by which these plant and its active compounds regulate oxidative stress as well as glucose homeostasis.

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