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Antioxidant Activity of Ghanaian Underutilized Crops

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Antioxidant Activity of Ghanaian Underutilized Crops independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 22. 4. 2022

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Abstract

Oxidative stress is a significant contributor to major non-communicable chronic diseases such as cancer, cardiovascular diseases, diabetes and neurodegenerative diseases. These are on the rise in developing countries including Ghana, due to an increasing dependence on staple crops that are energy rich but nutrient poor. Fruit-based nutritional supplements contain antioxidants which can reduce the impact of oxidative stress. There is a great potential of finding new sources of antioxidants among NUS traditionally utilized by small-scale farmers which are often high in macro- and micronutrients. In frame of this thesis, ethnobotanical data of selected fruits and vegetables NUS species from Ghana were collected. Subsequently, their *in vitro* antioxidant activity was evaluated using the DPPH and ORAC assays. According to the collected ethnobotanical data, fruit of *Chrysophyllum albidum* is the most popular for consumption among local people. Furthermore, mesocarp of *C. albidum* showed the highest antioxidant activity with the IC_{50} value of 18.54 ± 0.27 µg/ml in the ORAC assay. The mesocarp of *Chrysophyllum subnudum* showed remarkably high activity in the DPPH assay with the IC_{50} value of 45.18 ± 8.61 µg/ which is the first report on its antioxidant activity. These findings may serve as a basis for further development of new food products for the prevention of some oxidative stress-associated diseases.

Key words: Antioxidants, Underutilized crops, Neglected crops, DPPH assay, ORAC assay

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List of the abbreviations used in the thesis

AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
CSIR	Council of Scientific & Industrial Research
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAO	Food and Agriculture Organization
HAT	Hydrogen atom transfer
K2PO4	Dipotassium phosphate
KH2PO4	Potassium dihydrogen phosphate
NUS	Neglected and underutilized species
ORAC	Oxygen radical absorbance capacity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Standard deviation
SET	Single electron transfer
SPF	Sun protection factor
UV	Ultraviolet radiation

1. Literature Review

1.1. Oxidative Stress and Free Radicals

The first mentions of oxidative stress and free radicals in literature can be found in the work of Denham Harman, M.D., Ph.D. from the year 1956. According to him the reactive molecules, which are freed within the normal chemical processes, catalyse various cellular damage, cancer and other diseases and aging by the cause of their destructive reactions in the cells and tissues (Harman 1956; Dröge 2002; Gutteridge & Halliwell 2010; Škrovánková et al. 2012). Later in the year 1969 McCord and Fridovich identified superoxide dismutases, enzymes which activate the dismutation of superoxide radicals to oxygen and hydrogen peroxide, thus ensuring the cellular defence against reactive oxygen species (Valko et al. 2007; Wang et al. 2015). Since then, the view of oxidative stress is constantly evolving over the years. Momentarily recognised mechanism is that oxygen free radicals may accumulate in the body because the body cannot effectively eliminate them beyond their scavenging capacity. This can result in tissue and organ damage, stimulate inflammation and negatively affect emotional wellbeing (Dröge 2002; Sharma 2014).

1.1.1. Biochemistry

Oxidative stress is closely associated with chronic psychological stress (Yegorov et al. 2020) and may be caused by the overproduction of oxygen free radicals, concretely reactive oxygen species (ROS) or reactive nitrogen species (RNS); or by inadequate activity of endogenous systems resisting the oxidative attack (Qi & Dong 2021). It can be defined as an imbalance between the ROS/RNS formation and the antioxidant defence mechanisms, including glutathione and ROSscavenging enzymes, such as peroxide dismutase, catalase and glutathione peroxidase, or non-enzymatic compounds such as bilirubin and albumin, which regulate the levels of ROS (Uttara et al. 2009; Škrovánková et al. 2012; Araújo et al. 2016; Francenia Santos-Sánchez et al. 2019; Gabr et al. 2019). This defence mechanism is called "redox regulation" and protects living organisms from various oxidative stresses by maintaining the "redox homeostasis" (Valko et al. 2007; Sharifi-Rad et al. 2020). When the steady state of the "redox balance" is disturbed either by an increase of free radicals or the decrease in the antioxidant systems activity, the redox signalling is used and the signal is delivered through redox reactions. The physiological demonstration of redox regulation involves a temporary shift of the intracellular redox state toward more oxidising conditions (Valko et al. 2007). When the response to oxidative stress in human body is insufficient it may trigger some physiological and physiopathological processes, inflammatory diseases and cell aging (Škrovánková et al. 2012; Irivibulkovit et al. 2018; Francenia Santos-Sánchez et al. 2019; Sharifi-Rad et al. 2020).

1.1.2. Free Radicals

Free radicals can be produced by exogenous factors such as solar radiation and its ultraviolet rays, which cause the homolytic breakdown of bonds in molecules, or chemical intoxication in form of drugs, contaminants, pesticides, additives, etc. The conversion of toxic substances to less dangerous ones promotes the release of free radicals (Russo et al. 2018; Francenia Santos-Sánchez et al. 2019). Furthermore, tobacco smoke, burning of fossil fuels and certain pollutants such as ozone are also exogenous sources of free radicals (Škrovánková et al. 2012; Russo et al. 2018). Other factors giving rise to free radicals are endogenous. Many of chemical reactions involved in metabolic processes require free radicals and their main source is a mitochondrial respiratory chain (Poljsak 2011; Araújo et al. 2016; Ndhlala et al. 2018; Russo et al. 2018; Sharifi-Rad et al. 2020). Other endogenous sources are inflammatory processes; free radicals which occur in the cleansing cells of the immune system participate in the destroying of pathogenic microorganisms. When the production of free radicals during this process is excessive it may cause tissue damage (Uttara et al. 2009; Francenia Santos-Sánchez et al. 2019; Sharifi-Rad et al. 2020). Free radicals such as hydroxyl, superoxide, alkoxyl, and peroxyl radicals, are molecules which have an unpaired electron in the outer orbit and thus are generally unstable and very reactive. Hydrogen peroxide and singlet oxygen are not free radicals but may lead to free radical reactions (Škrovánková et al. 2012). It is complicated to identify which agent is accountable for a certain biological effect because various reactive species coexist in the reactive environment (Dröge 2002). The most important free radicals are ROS and RNS. One of the greatest sources of ROS production is the mitochondria (Benaroudj et al. 2001; Poljsak 2011; Yegorov et al. 2020; Qi & Dong 2021). It is an undesirable side product of the activity of the respiratory chain and oxidoreductases (Dröge 2002; Qi & Dong 2021). Despite the fact that mitochondria are involved in its formation, they can also be harmed by ROS (Qi & Dong 2021). Furthermore, the aging-specific pro-inflammatory changes are intensified because of the cells inability to maintain the optimal state of mitochondria (Yegorov et al. 2020). Other sources where ROS can be generated are peroxisomes, endoplasmic reticulum, plasma membrane, cytosol, lysosomes, microsomes and nuclear envelope (Qi & Dong 2021). ROS play a dual role since they can be either harmful or beneficial to living systems. When their concentration is high, they can react with different macromolecules and thus damage cellular lipids, proteins or DNA by inhibiting their normal function which leads to a number of diseases and aging processes (Benaroudj et al. 2001; Valko et al. 2007; Uttara et al. 2009; Gabr et al. 2019). When the concentration of ROS is low or moderate, the beneficial effects involve physiological roles in cellular responses in defence against infectious agents and in the function of a number of cellular signalling systems (Valko et al. 2007). Moderate concentration of ROS plays an important role as regulatory mediator in signalling processes. Actually, many of the ROS-mediated responses protect the cells against oxidative stress and help to re-establish „redox homeostasis“ (Valko et al. 2007; Qi & Dong 2021). RNS as well are generated in small amounts during normal cellular processes such as cell signalling, muscle relaxation, peristalsis, neurotransmission, blood pressure modulation, immune system control, phagocytosis, production of energy in the cell and regulation of cell growth (Uttara et al. 2009; Francenia Santos-Sánchez et al. 2019). Nitric oxide is a reactive radical generated in biological tissues by specific nitric oxide synthases. It is an important oxidative biological signalling molecule in various physiological processes, including neurotransmission, blood pressure regulation, defence mechanisms, muscle relaxation and immune regulation (Pacher et al. 2007; Valko et al. 2007; Sharifi-Rad et al. 2020). Overproduction of RNS which exceeds the ability to neutralise and eliminate them in the system leads to a nitrosative stress. That can cause inhibition of normal functions of proteins by structural alterations. During inflammatory processes, cells of the immune system produce the superoxide anion and nitric oxide. Mutual reaction of these two may produce high amounts of a very oxidative active molecule peroxinitrite anion, which can cause DNA fragmentation and lipid oxidation (Valko et al. 2007). Thus, oxidative stress creates favourable conditions for the onset of various non-communicable diseases (Dröge

2002; Wood et al. 2006; Valko et al. 2007; Uttara et al. 2009; Sharma 2014; Araújo et al. 2016; Russo et al. 2018).

1.1.3. Diseases

Some of the most serious chronic and degenerative diseases in whose emergence oxidative stress is involved are cancer, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, Parkinson's and Alzheimer's disease and aging processes (Dröge 2002; Wood et al. 2006; Valko et al. 2007; Uttara et al. 2009; Sharma 2014; Araújo et al. 2016; Russo et al. 2018). These effect populations around the world and are recently on the rise in developing countries including Ghana; primarily because of the significant change in food habits which has caused high dependence on staple crops that are energy rich but nutrient poor. That leads to worse malnutrition and food insecurity issues together with the increased incidence of obesity and related non-communicable chronic diseases (Aboagye et al. 2007; Nti et al. 2012; Nyadanu & Lowor 2015; JLI 2018; Laar et al. 2020). Cancer belongs to leading causes of death worldwide, with almost 10 million deaths every year (Cancer Research UK 2022). 65% of these cases occur in developing countries (Singh 2015). Several factors are involved in the development of cancer and oxidative stress is one of them. When some of the cells in the body grow and spread uncontrollably they may form tumours, those can be benign or malignant. Benign tumours usually do not invade nearby tissue and therefore are not so harmful. On the other hand, malignant tumours are more fatal, they spread into other tissues, are able to travel through the bloodstream and form so-called metastasis. Carcinogenesis may be triggered both by genetic predisposition and by environmental factors. There are many mechanisms through which can reactive species induce mutagenesis. The main process is the permanent modification of genetic material (Valko et al. 2007; Araújo et al. 2016; Russo et al. 2018; Sharifi-Rad et al. 2020). Oxidative stress is also involved in the emergence of chronic inflammation which has a direct impact on the carcinogenesis (Russo et al. 2018). Well known carcinogenic sources are for example tobacco smoke, high alcohol consumption and ultraviolet rays (Valko et al. 2007; Sharifi-Rad et al. 2020). Furthermore, free radicals play an important role in the genesis of several cardiovascular diseases (Sharma 2014; Sharifi-Rad et al. 2020). One of those is atherosclerosis; causing thickening of the vessel wall and accumulation of fibrous and cholesterol rich plagues, which can lead to thrombosis formation and blood flow obstruction. This often results in strokes and myocardial infarctions (Araújo et al. 2016). In the past years, cardiovascular diseases are the leading cause of death worldwide (Russo et al. 2018; WHO 2020a), affecting especially elderly people, whose heart tolerance to oxidative stress is lower due to a reduction in the concentration of antioxidant enzymes (Russo et al. 2018; Sharifi-Rad et al. 2020). Some of the risk factors for atherosclerosis are high blood pressure, a high fat diet, obesity and diabetes. Diabetes mellitus is a metabolic disease that belongs to major global health problems, accounting for 1.6 million deaths annually (Oyetayo et al. 2021). Once the intracellular glucose level is increasing, it promotes the formation of free radicals and decreases the antioxidant potential of cells to neutralize them. That leads to macro- and microvascular complications. There are two types of diabetes. Type 1 is caused by insufficient production of insulin by pancreas and mostly affects adults who follow unhealthy diet and have a lack of exercise. Type 2 is caused by incorrect response of target cells to insulin and appears in childhood (Maritim et al. 2003; Sharma 2014; Russo et al. 2018; Sharifi-Rad et al. 2020). Another one of major causes of morbidity in the world is a chronic obstructive pulmonary disease. Free radicals affecting this condition come from cellular and environmental sources, such as tobacco smoke. Those induce the immune response which may lead to the development of chronic bronchitis, emphysema and other lung conditions as well as higher susceptibility to bacterial and viral infections (Russo et al. 2018). Oxidative stress also has a significant impact on the pathogenesis of large number of neurodegenerative diseases, mainly Parkinson's and Alzheimer's disease (Sharma 2014; Oboh et al. 2018; Sharifi-Rad et al. 2020). Increased lipid peroxidation, damage of DNA and proteins and degeneration of dopaminergic neurons play major role in the development of Parkinson's disease. This leads to decreased glutathione levels, thus higher susceptibility to oxidative damage. The main symptoms include rhythmic tremor in limbs, problems in movement control and muscle rigidity (Sharma 2014). In developed countries, approximately 40% of people over 80 years are being afflicted by Alzheimer's disease. However, due to the improvement of the quality of lifestyle and related increase of life expectancy in developing countries including Ghana, these populations could be more at risk in the upcoming years (Go et al. 2021). One of the causes of Alzheimer's disease is mitochondrial dysfunction due to oxidative stress, leading to the damage of neuronal synapses. It affects cognitive functions, such as memory and communication ability (Sharma 2014; Oboh et al. 2018; Russo et al. 2018). Oxidative stress is the basic principle of most theories of aging, which is linked with a progressive loss of tissue and decreased organ function. It is caused mainly by the accumulation of damage in cells and connective tissues (Russo et al. 2018; Sharifi-Rad et al. 2020). This is only a brief summary of examples of diseases connected to oxidative stress which is a major contributor to much larger number of different complaints (Valko et al. 2007; Sharma 2014).

1.1.4. Prevention and Treatment

It is generally more efficient to prevent the formation of excess free radicals than attempting to neutralize them once they have been produced. There are many ways how to decrease oxidative stress by either keeping the respiratory chain in cells working optimal without an increase of free radicals, or by increasing of the defence and repair systems of the body (Poljsak 2011; Araújo et al. 2016). Paradoxically, when an organism is exposed to a low-intensity stressor, it results in specific responses that lead to an increase in cellular resistance. One of the examples is regular physical activity. Whereas long exhausting exercise may cause oxidative modification of proteins, moderate low intensity training is connected with higher antioxidant activity in the body, protection against oxidation of DNA and proteins, improved insulin sensitivity and overall reduction of oxidative stress and inflammation (Poljsak 2011; Sharifi-Rad et al. 2020). Another example of beneficial mild stress is caloric restriction. This energetic stress activates a defence response of an organism, helps to maintain the optimal state of intracellular environment and regulates free radicals (Poljsak 2011). One of the most important sources of free radicals is tobacco smoke. Induced oxidation may damage lipids, proteins, nucleic acids; and strongly contributes to inflammation and carcinogenesis. Therefore, it is crucial to avoid smoking in order to prevent further health risks, such as cancer, chronic obstructive pulmonary disease and cardiovascular diseases (Caliri et al. 2021). Also excessive sun exposure leads to cell damage caused by oxidative stress. Some of the consequences are inflammation, premature skin aging and cancer. It is recommended to avoid the sun exposure in the peak hours and using a sunscreen with at least SPF 30 to protect the skin from ultraviolet radiation (Albrecht et al. 2019). Another significant contributor to oxidative damage is psychological stress, which is on the rise in the modern society. Excessive adrenalin, released into the bloodstream during a stressful situation, oxidizes and increases the production of free radicals. Immoderate psychological stress should be therefore reduced. At the same time, adequate recovery sleep is recommended to be implemented into the lifestyle because of the production of melatonin (Poljsak 2011). Last but not least, a healthy diet is necessary in order to prevent oxidative stress. It is highly recommended to limit the consumption of processed food, refined sugar and saturated fats. According to the World Health Organization, daily intake of fruits and vegetables should be at least 400g in an effort to prevent chronic diseases and micronutrient deficiencies (WHO 2020b). Not only that fruits and vegetables contain essential nutrients such as dietary fibre, protein and sugar but also vitamins, minerals and other health-promoting phytochemicals (Poljsak 2011; Oloyede & Oloyede 2014; Achaglinkame et al. 2019). A healthy diet can be supported by several fruit-based nutritional supplements and functional foods which carry biologically active compounds with health benefits for the prevention and treatment of chronic diseases. These supplements contain wide range of nutrients, vitamins, minerals and various plant and herbal extracts (Alfonso Valenzuela et al. 2003; Škrovánková et al. 2012; Wilson et al. 2017; Achaglinkame et al. 2019).

1.2. Antioxidants

Exposure to free radicals from different sources has led organisms to develop a series of defence mechanisms. Those involve preventative mechanisms, repair mechanisms, physical defences and antioxidant defences (Valko et al. 2007). Antioxidants can be defined as substances that neutralize free radicals and the main characteristic is the prevention or detection of a chain of oxidative propagation and stabilization of the generated radical (Škrovánková et al. 2012; Francenia Santos-Sánchez et al. 2019; Yuniarti et al. 2020), thus preventing oxidative stress-related damage (Sharifi-Rad et al. 2020). They use several mechanisms to inhibit free-radical reactivity, such as donation of hydrogen, radical scavenging and singlet oxygen quenching (Irivibulkovit et al. 2018). Some antioxidants have the ability to eliminate excess free radicals in the body and reduce the damage of free radicals to carbohydrates, lipids and proteins in cells and also to protect the body tissues from further damage. Certain antioxidants enhance the activity of antioxidant enzymes and reduce the ROS production by regulating the endogenous antioxidant system (Serafini 2006; Wood et al. 2006; Qi & Dong 2021).

1.2.1. Classification

One of the classifications may be made between endogenous and exogenous antioxidants. The first category includes antioxidants which can be synthetized by cells, whilst exogenous antioxidants have to be taken through the diet, because their synthesis in cells is not possible. Another classification divides antioxidants into enzymatic and non-enzymatic. Enzymatic antioxidants can be further divided into primary and secondary enzymes. Primary ones can directly scavenge free radicals, whereas secondary enzymes support other endogenous antioxidants (Wood et al. 2006; Sharifi-Rad et al. 2020). Enzymatic antioxidant defences include superoxide dismutase, glutathione peroxidase and catalase. Non-enzymatic antioxidants include ascorbic acid (Vitamin C), tocopherol (Vitamin E), glutathione, carotenoids, flavonoids and other. Glutathione is a cofactor of several detoxifying enzymes against oxidative stress and also scavenges hydroxyl radical and singlet oxygen directly. It is also able to regenerate vitamins C and E back to their active forms (Alfonso Valenzuela et al. 2003; Wood et al. 2006; Valko et al. 2007; Sharifi-Rad et al. 2020). Glutathione represents the main cellular redox buffer that helps to maintain the intracellular “redox homeostasis“ (Dröge 2002; Valko et al. 2007).

1.2.2. Methodology for Assessing Antioxidant Activity

It is important to distinguish between the antioxidant activity and capacity in the study of antioxidant compounds and mechanisms. The antioxidant activity refers to the rate constant of a reaction between an antioxidant and an oxidant, whereas the antioxidant capacity is a measure of the amount of a certain free radical captured by an antioxidant sample (McDonald-Wicks et al. 2006; Francenia Santos-Sánchez et al. 2019). The antioxidant capacity can be tested using both *in vivo* and *in vitro* techniques. However, the *in vivo* methods, which mostly involve animal testing (Alam et al. 2013), are of higher cost and therefore *in vitro* methods are more commonly used (McDonald-Wicks et al. 2006). The extraction technique, conditions, solvent used and particular assay methodology are important to appropriately determine the antioxidant capacity

(Škrovánková et al. 2012). Current *in vitro* methods for determining antioxidant activity are divided into three general groups; based either on ROS/RNS scavenging, single electron transfer mechanisms (SET) or spectrophotometric determination through hydrogen atom transfer (HAT) (McDonald-Wicks et al. 2006; Irivibulkovit et al. 2018; Francenia Santos-Sánchez et al. 2019). The result of both SET and HAT methods is the inactivation of free radicals, but the reaction mechanisms involved in the process are different. SET methods detect the capacity of a potential antioxidant for the transmission of a chemical species and are shown by a change of colour as the oxidant is reduced by antioxidant. HAT methods measure the ability of an antioxidant to inactivate free radicals via the donation of a hydrogen atom (Francenia Santos-Sánchez et al. 2019). Example of the most widely used SET-based method is DPPH assay and of the HAT-based method is the ORAC assay. The DPPH assay is fast, simple and inexpensive (Alam et al. 2013); it is associated only with the DPPH radical reagent and the antioxidant and requires mild experimental conditions. This colorimetric method is established on reduction of the violet DPPH radical by the antioxidant through a hydrogen atom transfer mechanism to induce a change in the colour to the stable pale-yellow DPPH molecules. To determine the antioxidant activity, the remaining violet DPPH radical is measured by a UV-Vis spectrophotometer at approximately 515 – 520 nm. The method uses percentage of DPPH remaining to obtain the necessary quantities that are required to reduce the initial concentration to 50% (IC_{50}). Low IC_{50} values show a high antioxidant strength. Due to the stability of DPPH radical, this method allows a reaction with almost any type of antioxidant and there is enough time even for weak antioxidants to react. The assay delivers useful information on the antioxidant capacity to donate hydrogen atoms, mechanisms between the free radical and the antioxidant, and the reducing capacity of the reaction (Sharma & Bhat 2009; Alam et al. 2013; Musa et al. 2013; Irivibulkovit et al. 2018; Francenia Santos-Sánchez et al. 2019; Yuniarti et al. 2020). ORAC method measures antioxidant scavenging activity against peroxyl radical induced by 2,2′-azobis(2- amidinopropane) dihydrochloride (AAPH) at 37 °C (Ou et al. 2001; Alam et al. 2013). The mechanism of the reaction competes between the fluorescence probe (fluorescein) and antioxidants for a radical. The source of free radical is AAPH which promotes the degradation of fluorescein. The examined antioxidant promotes the elimination of the peroxyl radicals, thus protecting the fluorescein from degradation. Trolox, a water-soluble derivative of vitamin E (Brewer 2011), is used as a standard in this assay (Alam et al. 2013; Francenia Santos-Sánchez et al. 2019).

1.2.3. Sources of Natural Antioxidants and Health Benefits

Plant materials such as fruits, vegetables and herbs are promising sources of effective antioxidants, including phenolic compounds, vitamins and terpenoids (Frankel & German 2006; Uttara et al. 2009; Borokini et al. 2014). Phenolic compounds are the most abundant secondary metabolites of plants and are involved in signalling and defence mechanisms; for example defence against UV radiation, pathogens, parasites and predators. They can also act as reducing agents and have an ideal structure for free radical scavenging activities as hydrogen-donating antioxidants. These characteristics are associated with the decrease of risk of some neurodegenerative diseases, such as various types of cancer and cardiovascular diseases. Phenolic compounds in plants include phenolic acids, flavonoids, terpenes, tannins, stilbenes and lignans (Škrovánková et al. 2012; Suhaila & Mohamed 2015; Francenia Santos-Sánchez et al. 2019). The most abundant polyphenols in human diet are flavonoids, which are divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones and anthocyanins. Flavonoids can interfere with the propagation reactions of the free radicals and also with their formation, either by chelating the transition metal or by inhibiting the enzymes involved in the initiation reaction. They have a synergic effect with other antioxidants and these combinations show as more efficient (Škrovánková et al. 2012). Plants also contain a wide range of vitamins that play essential role in human nutrition and health. Those are compounds which need to be taken up in the diet because human body cannot synthetize them. Several vitamins have a strong antioxidant potential or act as cofactors. To those belong both water-soluble (vitamins B, C) and lipid-soluble (vitamins A, D, E, and K) compounds. Most attention among these is given to tocopherols (vitamin E), ascorbate (vitamin C) and carotenoids (pro-vitamin A) (Asensi-Fabado & Munné-Bosch 2010; Brewer 2011). Tocopherols play an important role in controlling singlet oxygen levels by regulating ROS accumulation in plastids. Higher levels of tocopherols are present mainly in seeds of some medicinal plants. One of the most powerful synergists of tocopherols and other phenolic antioxidants is ascorbic acid (Asensi-Fabado & Munné-Bosch 2010; Brewer 2011; Škrovánková et al. 2012). Vitamin C acts as hydrosoluble antioxidant, reacts with most of the physiologically important ROS and plays a major role in cellular signalling and in preventing oxidative damage to macromolecules (Asensi-Fabado & Munné-Bosch 2010; Francenia Santos-Sánchez et al. 2019). Carotenoids, particularly b-carotene, lutein and zeaxanthin are important in controlling singlet oxygen levels in plastids (Škrovánková et al. 2012). Antioxidant properties of carotenoids are related to their high capacity for electron donation and are characterized as excellent peroxyl radical scavengers. Therefore, carotenoids have an important role in protecting the cell membranes and lipoproteins against peroxyl radicals (Francenia Santos-Sánchez et al. 2019). Recent evidence shows that vitamin B compounds could also play a significant antioxidant role in plants, mainly as a cofactor in scavenging of the singlet oxygen (Asensi-Fabado & Munné-Bosch 2010). All these compounds can be found in well-known fruits and vegetables such as blueberries, strawberries, raspberries, goji, figs, citruses, tomatoes, garlic and many more. Berries contain high levels of flavonoids and lower the risk of heart attack and infections. Tomatoes are high in vitamin C and lycopene which helps to reduce the risk of cardiovascular diseases and cancer. Garlic has beneficial effects on brain functions and can be used as pharmacotherapy for Alzheimer’s disease (Wilson et al. 2017). These ingredients are usually a common part of the human diet. However, there are many lesser-known fruits and vegetables which are even higher in antioxidant levels. For example acerola and camu camu are remarkably rich in vitamin C, phenolic compounds and carotenoids. They are effective in reducing inflammation, blood pressure and improving blood sugar levels. Both are highly perishable and therefore mainly taken in dietary supplement forms as powders, pills or as a juice (Garcia et al. 2020). Another example are acai berries, which contain more antioxidants (especially anthocyanin) than other berries and their consumption lowers the risk of age related brain diseases, cardiovascular diseases and cancer development (Sadowska-Krępa et al. 2015). Many previously neglected plants are becoming popular and commercialized as food supplements and are expected to make a contribution as future crops mainly under water limited conditions. The potential to find interesting new species is high, especially in West Africa, due to its large biodiversity. That could lead to development of new products and improving the nutrition of local communities (Chivenge et al. 2015; Joshi et al. 2020).

1.2.4. Neglected and Underutilized Crops

There are approximately 7000 plant species, which have been used in the history for the human consumption. Only 30 of them are considered to be main crops to feed the world. Almost 50% of our worldwide caloric need is supplied by 3 major species – rice, wheat and maize (Aboagye et al. 2007; FAO 2012; Chivenge et al. 2015; Nyadanu et al. 2016; Joshi et al. 2020; Li et al. 2020). However, there is a large number of cultivated species with local importance which are referred to as neglected and underutilized crop species (NUS), minor species or orphan crops. These constitute an essential part of agricultural biodiversity. Among them we can find not only food crops, but also species used for fibre, oil, fuel, fodder, stimulants and medicinal effects (Hammer et al. 2001; Padulosi et al. 2011; Joshi et al. 2020; Hossain & Maitra 2021).

1.2.5. Importance

Although their full potential is not entirely appreciated, they are of significant local importance; especially due to their high ability of adaptation to harsh environments and contribution to diversification and resilience of agroecosystems (Padulosi et al. 2011, 2013; Nyadanu et al. 2016; Baa-Poku & Asante 2020; Mugiyo et al. 2021). NUS are great resources for food security, nutrition, traditional medicine and economical income for rural communities. They complement staple crops in the human diet and belong to some strategies of farmers to alleviate climate change consequences and economic risks (Dansi et al. 2012; Chivenge et al. 2015; Li et al. 2020; Mugiyo et al. 2021); and offer a great alternative in the event of failure of the main staple crops (Baa-Poku & Asante 2020). These crops appear in the wild and farmers retain them during cultivation of their lands, or are maintained by traditional practices (Dansi et al. 2012; Hossain & Maitra 2021). Some of these small-scale farmers use them for home consumption, as medicines or for an additional income from local markets and road side sales (Nyadanu et al. 2016). NUS usually remain neglected by researchers, thus inadequately characterized and underestimated (Dansi et al. 2012; Nyadanu et al. 2016; Hossain & Maitra 2021). Nevertheless, vast majority of them is rich in micro and macro-nutrients, and could therefore be helpful in tackling malnutrition caused by their deficiency, and combating “hidden hunger” and with that related poor health conditions of many Africans (Johns & Eyzaguirre 2006; Dansi et al. 2012; Borokini et al. 2014; Nyadanu &

Lowor 2015; Nyadanu et al. 2016). Many of these neglected species are rapidly disappearing and according to FAO it would be more difficult to adapt to climate change and ensure food security if we lose these irreplaceable resources (FAO 2012; Nyadanu & Lowor 2015). Most of the studies are focused on the analysis of the main constituents and it is difficult to distinguish if the protective effects are caused by antioxidants or other lesser known compounds which are present and have potentially great significance level (Škrovánková et al. 2012).There is therefore a relevant need to increase interest in cultivation, domestication, conservation and consumption of these neglected crops, as well as characterization of biological activities and chemical analyses of secondary metabolites in plant extracts (Chivenge et al. 2015; Joshi et al. 2020; Mugiyo et al. 2021).

1.2.6. Categories and Nutritional Value

NUS are a wide and diverse group of crops that includes cereals, root and tuber crops, leguminous crops, leafy vegetables, oil seeds and fruits. They cover all macro and micronutrients, thus significantly contribute to human health and nutrition (Chivenge et al. 2015; Joshi et al. 2020). The most important crops among neglected cereals are millets (pearl, foxtail and finger millet). These are mainly cultivated by smallholder farmers in arid and semi-arid areas due to their adaptation to drought and high tolerance to pests and diseases. Millets contain higher oil and vitamin A content than maize and other staple cereals, which makes them suitable crops for combating hidden hunger in some regions (Chivenge et al. 2015; Hossain & Maitra 2021). They are also rich in dietary fibre, bioactive components and have a low glycemic index (Hossain & Maitra 2021). Root and tuber crops belonging to NUS are for example taro (*Colocasia esculenta*) and arrowroot (*Maranta arundinaceae*). *C. esculenta* is a great source of carbohydrate, vitamins A and C and protein, improving the diet of smallholder farmers (Chivenge et al. 2015). The rhizomes of *M. arundinaceae* are a source of well-digestible starch (Amante et al. 2020). Some of the NUS legumes are bambara groundnut (*Vigna subterranea*) and cowpea (*Vigna unguiculata*). *V. subterranea* is due to its drought tolerance ideal for cultivation in arid and semi-arid areas. It is cultivated mainly for consumption in local communities, playing an important role as a source of protein and also in rotations with cereal crops because of the ability to fix nitrogen. *V. unguiculata* is also a drought tolerant crop, which can be utilized as a grain legume or leafy vegetable. Leaves contain several vitamins and minerals, whereas grains are a source of protein (Chivenge et al. 2015; Li et al. 2020; Hossain & Maitra 2021). Examples of NUS leafy vegetables are amaranth (*Amaranthus* spp), wild mustard (*Brassica juncea*) and wild watermelon (*Citrullus lanatus*). *Amaranthus* is easily adapted to various climatic conditions, is drought tolerant and able to grow on a wide range of soils. Its leaves are high in protein, dietary fibre, lipids, vitamins and minerals (Chivenge et al. 2015; Hossain & Maitra 2021). *B. juncea* withstands diverse environmental conditions and is of great importance in the nutrition of rural areas, due to the content of several vitamins, iron, calcium and other nutrients in the leaves and high content of oil and protein in the seeds (Chivenge et al. 2015; Hossain & Maitra 2021). *C. lanatus* has edible leaves and fruits, which are also an important source of water in some arid areas during dry months. There are many NUS fruits, which are essential for the nutrition of rural communities for their content of sugars, vitamins, minerals, protein, carbohydrates and oils (Chivenge et al. 2015). Among those we can find for instance a large number of *Annona* species, *Syzigium* species, starfruit (*Averrhoa carambola*) and tamarind (*Tamarindus indica*). Fruits of *Annona* species are rich in vitamins and nutrients and are locally used for production of juices, ice creams and other food products. Some can be also used as prevention of cardiovascular diseases, arthritis, cancer and a treatment for diarrhoea. Fruits of *Syzigium* species are usually eaten fresh and are source of sugar, minerals, carbohydrates and antioxidants, especially vitamin C. They are also used as medicine, as they contain anti-diabetic compounds and blood purifying agents (Dandin & Kumar 2016). *A. carambola* is mostly grown in a small scale in home gardens and is rich in vitamin C (Hossain & Maitra 2021). *T. indica* is a highly drought tolerant plant, its fruit is rich in vitamin C and tartaric acid and seeds are used for extraction of oil, starch and protein (Dandin & Kumar 2016).

1.2.7. Ethnobotanical Evaluation

Ethnobotanical inventories are an important source of information in NUS research. Ethnobotany is a multidisciplinary study of the relationships between plants and people and includes all locally used plants. It is especially important for the conservation of biological diversity, preservation, recovery and diffusion of local knowledge and wisdom and identification and development of new economic products. Data collection is a crucial component of successful field research. Foremost, the flora of selected region should be thoroughly studied from literature and herbaria. The interviews with respondents should be done with considerable tact and care and could be either informal, unstructured, semi-structured or structured. Informal interviews are usually used in the early stages of study, they are not controlled and lack any structure. Unstructured ones are more controlled but still flexible, as they are used for approaching sensitive issues and building initial sympathy with informants. In the semi-structured type, there are already identified questions that need to be followed, usually with an interview guide assistance. Last but not least, the structured type of interview includes fixed questions and is used in the later stages of the study in order to obtain detailed data which can be further used to quantification and statistical analysis. Some of the interview techniques are walking in the vegetation with informant while collecting information on plants and their uses; presenting a checklist of plant names with their photographs and reference specimen to the informant who is asked for their uses; or a group interview where informants can be asked sequentially or simultaneously (Heneidy et al. 2017; Pei et al. 2020; Kumar et al. 2021). Collected information together with the promotion of consumption of wild fruits and vegetables hold enormous potential to partake in solving of many global challenges, such as poverty, malnutrition and improving the overall health status, since they are adapted to local conditions and often rich in vitamins, micronutrients, antioxidants and other bioactive compounds (Kumar et al. 2021). Ethnobotanical studies play a key role in preserving and documenting of the indigenous knowledge as many of the NUS species that are used locally set the basis for the discovery of many modern drugs and food supplements (Heneidy et al. 2017).

1.2.8. Underutilized Crops of Ghana as Sources of Antioxidants

Plants generally accumulate antioxidants as secondary metabolites to ensure protection in hostile environments. Therefore, African plants have higher potential to accumulate these substances then those of northern hemisphere as there is higher occurrence of pathogenic microbes, including bacteria, viruses and fungi and stronger ultraviolet rays of the tropical sunlight (Ndhlala et al. 2018). Africa has enormous biodiversity resources, accounting for a wide variety of valuable food crops and plant species whose potentials have not been fully exploited yet (Borokini et al. 2014; Oloyede & Oloyede 2014; Ndhlala et al. 2018; Baa-Poku & Asante 2020). NUS are mostly grown by smallholder farmers, who carry the knowledge about the utilization and actual value of these crops. This indigenous knowledge is traditionally transferred from generation to generation as a survival tool and should be implemented in the development projects and research on NUS to better understand their role; as they have a high potential to improve poverty alleviation and are important resources for nutrition, food security, several health benefits, management of agricultural risks and income to the rural communities (Chivenge et al. 2015; Nyadanu et al. 2016). Among the NUS that are utilized in Ghana are for example amaranth (*Amaranthus cruentus*), Malabar spinach (*Basella alba*), nalta jute (*Corchorus olitorius*), cocoyam (*Xanthosoma sagittifolium*), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), bambara groundnut (*Vigna subterranea*), breadfruit (*Artocarpus altilis*), chayote (*Sechium edulis*), kola (*Cola milenii*), marula (*Sclerocarya birrea*), African ebony (*Diospyros mespiliformis*) and many others (Aboagye et al. 2007). *A. cruentus* is cooked as spinach and used in soups and other dishes. Its consumption lowers blood pressure, cholesterol levels and help in prevention against cardiovascular diseases. Leaves of *C. olitorius* are used in local cuisine and for preparation of herbal tea which is supposed to be diuretic, purgative and is used as a treatment for fever, pains and tumours. *X. sagittifolium* has edible leaves and roots. Leaves are consumed as vegetable and help in the management of diabetes. Consumption of the roots helps to fight inflammation. Grains of *S. bicolor* and *P. glaucum* are widely used in the preparation of many dishes and *V. subterranea* is important for the high content of protein (Aboagye et al. 2007; Nyadanu & Lowor 2015). *A. altilis* is a good source of protein, vitamin C, thiamin, potassium and other minerals. Its consumption lowers the risk of diabetes. Most varieties are purgative when eaten fresh, therefore they are usually boiled, steamed or roasted. Fruit of *S. edulis* can be eaten raw or cooked and contains considerable amount of vitamins and minerals such as vitamin C, K, folic acid, manganese, copper and zinc (Aboagye et al. 2007; Nyadanu & Lowor 2015; Nyadanu et al. 2016; Brennan 2020). *C. milenii* produces edible fruits which are a great source of magnesium, iron, potassium, zinc and calcium (Borokini et al. 2014); and various plant parts contain bioactive substances such as alkaloids, saponins, tannins,.glycosides, flavonoids and terpenoids (Akinnibosun & Adewumi 2019). The fruits of *S. birrea* and *D. mespiliformis* have high concentrations of minerals such as calcium, potassium and magnesium, vitamins A and C and polyphenols, especially flavonoids (Achaglinkame et al. 2019). Despite the existence of studies on NUS mentioned above, there are still many species traditionally used in Ghana as fruits and vegetables whose antioxidant properties have not been fully explored yet.

1.3. Selected Plant Species

1.3.1. *Chrysophyllum albidum* G. Don

Chrysophyllum is an abundant genus in the Sapotaceae family, comprising of about 70 species and occurring throughout the tropics. Probably the best-known member of this genus is *Chrysophyllum cainito*, the star apple, which is indigenous to Central America and the West Indies but is also grown in Africa, Philippines and Sri Lanka. However, there are some lesser-known species that are assumed to be indigenous to West Africa. One of them is *C. albidum*, common names are African star apple, white star apple and mululu, a high evergreen forest tree mainly valued for its edible fruit (Redhead 1990; Valíček 2002; Houessou et al. 2012; Fern 2014a). It is often grown as a multipurpose village tree. Its fruit is also frequently collected by native people from the wild and subsequently sold in local markets (Edem et al. 1984; Houessou et al. 2012; Fern 2014a). Trunk of this tree is usually fluted, its bark is thin, pale, exuding latex and is used in folk medicine. The wood is suitable for construction work. Leaves can be oblong-elliptic to elongate obovate elliptic, simple, dark green to pale tawny. Flowers grow in dense clusters, are shortly pedicellate, and 3 mm long and of creamy white colour. Fruits are around 3 cm in diameter, spherical, slightly pointed at the tip, containing 5 seeds. When immature their colour is greenish, turning orange-red or yellow during ripening. The fruit is popular for its sweet-sour flavour and often processed into a soft drink or fermented and distilled for the production of alcoholic beverages (Hemsley 1968; Edem et al. 1984; Orwa et al. 2009; Fern 2014a). Additionally, the physical, chemical and nutritional characterization of the fruits have shown a high commercial potential; especially as a rich source of potassium, calcium, alkaloids, flavonoids and terpenoids and its ascorbic acid content is about 100times that of an orange and 10times that of guava or cashew with 1000-3300 ml/100g (Edem et al. 1984; Houessou et al. 2012; Arueya & Ugwu 2017; George et al. 2018).

1.3.2. *Chrysophyllum perpulchrum* Mildbr. ex Hutch. & Dalziel

Another one of the African species, *C. perpulchrum*, commonly known as monkey star apple, red asanfena or bird-lime tree is a large tree widely distributed in tropical Africa, especially in Cote D'Ivoire, Nigeria, Uganda, Tanzania and Ghana, where it is growing in semi-deciduous forests. Its trunk is slightly fluted, up to 100 cm in diameter, the bark is grey-brown, exuding latex which can be used as a rubber substitute. Leaves are simple, spirally arranged and elliptical to obovate oblong. Flowers occur in axillary fascicles, are broadly ovate and creamy white. Its fruit is a 2 – 4 cm long globose berry, red-brown and hairy, usually containing 5 ellipsoid seeds. Seeds kernel contains 4% oil, with oleic, linoleic, palmitic and stearic acids. The wood has many uses, mainly in construction, furniture and musical instruments manufacture. The bark decoction is used in folk medicine to treat jaundice, asthma and other respiratory diseases. It contains cardiochrysine, an alkaloid which shows depressant activity in the thalamus and hypothalamus, hypotensive and cardiotonic activity (Fern 2014b; PlantUse English contributors 2015). It is also an effective antipyretic used to heal malaria fever in some regions (Go et al. 2021). The fruit is edible and its juice is used to treat malaria in some regions (Philippe et al. 2010).

1.3.3. *Chrysophyllum subnudum* Baker

C. subnudum, common names silvery star apple or adesema, is a medium-sized tree of moist tropics. It is widely spread in lowland semi-deciduous to evergreen forests in Sierra Leone, Liberia, Nigeria, Cameroon, Ghana and other African countries. The trunk is straight and cylindrical, the bark is greyish brown, exuding a sticky latex. Decoction of the bark is in some regions used for treatment of gastrointestinal disorders and as a purgative. Leaves are simple and entire, arranged spirally, blade is elliptical to oblong and hairy. Flowers grow in axillary fascicles, they are regular, broadly ovate and greenish white. Fruit is a 3 cm long globose berry, has greenish to yellowish colour and contains up to 5 ellipsoid and flattened seeds. The sweet-sour fruit is usually harvested from the wild and eaten locally (Fern 2014c; JSTOR 2022a; Prota4u 2022a).

1.3.4. *Delpydora gracilis* A. Chev.

Another member of the Sapotaceae family is *D. gracilis*, in Ghana commonly known as kusudama. This shrub or a small tree can be found in the undergrowth in evergreen-forests of Liberia and Ghana. The bark exudates a white latex. The fruit is a globose berry with white juicy pulp, which is collected from the wild and eaten by local people (JSTOR 2022b; Prota4u 2022b).

1.3.5. *Landolphia dulcis* var. barteri (Stapf) Pichon

L. dulcis belongs to the family Apocynaceae. It is a climbing shrub of tropical West Africa, extending from Senegal to Nigeria. Occurring in savannahs in the western regions and in dense forests in the eastern regions. This plant produces stout woody stems with tendrils, which are used for attachment to other plants for support. The fruit is a globose berry of a sweet, acid or sometimes astringent flavour and can be eaten. Various parts of the plant are collected from the wild and used in traditional medicine. The bark contains saponins and shows cardio-tonic action. Leaves contain alkaloids and saponins. A decoction of leafy twigs and bark is used for treatment of serious wounds in some regions. Decoctions of stems and roots can be used externally to treat arthritis and kidney pain. In some regions the roots are considered to be an aphrodisiac (Fern 2014d; JSTOR 2022c).

1.3.6. *Morinda morindoides* (Baker) Milne-Redh.

M. morindoides is an evergreen climbing shrub from the family Rubiaceae, commonly called as Nkonga bululu or Nkama meso. Its native range are forests, often near to the coast, in West Tropical Africa to South Sudan and Angola. Leaves of the plant contain saponins, flavonoids, terpenes, steroids and anthraquinones and show antimalarial, antioxidative and cardioinhibitory activities (Cimanga et al. 2010; Fern 2014e). They are collected from the wild and highly valued as a traditional remedy especially for gastrointestinal disorders, diarrhoea and expelling of intestinal worms. This traditional use is supported by the results from the study of Cimanga et al. (2010) which confirms that samples from leaf extracts of *M. morindoides* exhibit antispasmodic activity, most likely due to the presence of flavonoids. Leaves and roots are also used as a source of dye due to the content of anthraquinone compounds (Fern 2014e).

1.3.7. *Sterculia tragacantha* Lindl

S. tragacantha belongs to the family Sterculiacea and is commonly known as African tragacanth, sterculia, uhobo and abalo. This large tree is widely distributed throughout West Africa. Its leaves are traditionally used for treating pain, edema, diarrhoea, diabetes and infectious diseases in some regions (Onikanni et al. 2021). The study of Onikanni et al. (2021) confirms their therapeutic effect against inflammation, oxidative stress and as a source of bioactive agents for the treatment of diabetes.

In spite of the rich tradition in use of above mentioned species in Ghana, there is still lack of information on their uses as foods as well as on their antioxidant properties.

2. Aims of the Thesis

The main objective of this thesis was gathering of ethnobotanical data and the evaluation of antioxidative effects of NUS traditionally consumed in Ghana as fruits and vegetables.

The specific aims were:

1. Collection and analysis of traditional knowledge on food uses of fruits and vegetables in certain regions of Ghana.
2. Determination of *in vitro* antioxidant activity of extracts from Ghanaian NUS using DPPH and ORAC assays.

3. Methods

3.1. Plant Material

A total of 7 plant species were selected based on ethnobotanical data obtained from literature and collaboration with the CSIR-Plant Genetic Resources Research Institute, Bunso-Ghana. They were collected from the wild in the Eastern (Begoro and Suhum-Koforidua), Western (Ankasa) and Western North (Bibiani and Juaboso) regions of Ghana. Fresh plant material was air-dried in a shady place for several days and then stored in collecting bags and paper packs until use. Local botanist, Dr. S. K. Boateng authenticated the species collected. Voucher specimens will be deposited in the herbarium of the Institute of Tropical and Subtropical Agriculture of the Czech University of Agriculture in Prague. Phohotographs of fruits are presented in the Apendix 1.

3.2. Ethnobotanical Inventory

Semi-structured interviews were conducted among a number of purposively sampled respondents to obtain data on the local names and traditional edible uses of plant species collected which consisted of fruits, fruit parts and vegetables. The survey was conducted during December 2020. The information was collected from 59 persons (23 women, 32 men and 4 adolescents) whose age ranged from 15 to 80 years. Most of the respondents were more than 40 years old and had apt knowledge and background in traditional agriculture. The data were collected via face-to-face interviews, following the classical indications in ethnopharmacobotanical research. The interviews were mainly video recorded and registered in field notebook. The ethnobotanical data including: scientific and local names of plant, family, area of collection, part used and traditional edible use(s) are summarised in Table 1.

3.3. Chemicals and Reagents

2,2'-azobis (2-methylpropionamidine) dihydrochloride [AAPH], 2,2-diphenyl-1-picrylhydrazyl [DPPH], (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and fluorescein sodium salt were purchased from Sigma-Aldrich (Prague, Czech Republic). Methanol for preparation of extracts and antioxidant activity assay was acquired from Penta (Prague, Czech Republic). Inorganic salts K_2HPO_4 and KH_2PO_4 were obtained from Lach-Ner (Neratovice, Czech Republic).

3.4. Preparation of Extracts

Air-dried plant material was pulverised using a Grindomix apparatus (GM100 Retsch, Haan, Germany). 5 g of each milled plant sample were extracted at room temperature in 150 ml of 99.8% methanol using an orbital laboratory shaker (GFL3005, GFL, Burgwedel, Germany) for 24 h. Each extract was filtered afterwards and concentrated to dryness using a rotary evaporator Hei-VAP Expert (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) in vacuum at 40°C. Dry residues were stored at room temperature until tested. A total of 11 extracts were prepared and the yield of dry residue (%) of each extract determined (Table 1).

3.5. *In vitro* Antioxidant Activity

3.5.1. DPPH Assay

The scavenging activity of the samples on the DPPH free radical was measured according to Sharma & Bhat (2009) with some modifications. Concentrations and volumes of samples, standard and reagent were adjusted to be used in a microplate format. A two-fold serial dilution of each extract in methanol was performed via the automated pipetting platform Freedom EVO 100 (Tecan, Männedorf, Switzerland) in 96-well microtiter plates. Subsequently, 75 μl of methanol and 25 μl of freshly prepared 1 mM DPPH in methanol was manually added to each well using a multichannel pipette (Eppendorf, Hamburg, Germany) in order to start the radical antioxidant reaction. The final concentration range of each extract and Trolox (positive control) in microtiter plate was 0.125 – 256 µg/ml. Plates were incubated for 30 minutes at 25°C in an incubator IPP55plus (Memmert GmbH & Co. KG, Schwabach, Germany). Absorbance was spectrophotometrically measured at 517 nm using a Multimode Reader Cytation 3 (BioTek Instruments, Winooski, VT, USA). All tests were performed as three independent experiments each carried out in triplicate. Results were expressed as half maximal inhibitory concentration (IC_{50}) with standard deviation (±SD) in µg/ml.

3.5.2. ORAC Assay

The oxygen radical absorbance capacity assay (ORAC) was performed to determine samples' ability to protect fluorescein against oxidative degradation by AAPH using a protocol by Ou et al. (2001) which was slightly modified. Stock solutions of AAPH radical (153 mM) and fluorescein (48 nM) were prepared in 75 mM phosphate buffer (pH 7.0). A two-fold serial dilution of each extract was prepared in phosphate buffer in black absorbance 96-well microtiter plates using the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, Switzerland). Subsequently, 150 µL of fluorescein was added into each well and incubated for 10 min at 37°C. Afterwards, the reaction was started by application of 25 µL of AAPH and the plates were incubated for 90 min at 37°C in an incubator IF110plus (Memmert GmbH & Co. KG, Schwabach, Germany). The final concentration range of each sample tested was between 1 and 256 µg/ml. Outer wells of each microtiter plate were filled with 200 µl of distilled water, in order to provide better thermal mass stability. Blank 1 (fluorescein with AAPH in phosphate buffer) and blank 2 (fluorescein in phosphate buffer) were part of each microtiter plate. Trolox was used as a positive control. Fluorescence changes during the incubation were measured using the Multimode Reader Cytation 3 (BioTek Instruments, Winooski, VT, USA) in 1-min intervals with excitation and emission wavelengths set at 485 nm and 528 nm, respectively. All tests were performed as three independent experiments each carried out in triplicate. ORAC was expressed as mean values of IC_{50} ±SD in µg/ml.

4. Results and Discussion

4.1. Ethnobotanical Data

Based on the ethnobotanical data collection, *C. albidum* was the most popular fruit among all examined species in this research. Its mesocarp and aril can be consumed fresh and mesocarp together with epicarp can be chewed as a gum. This is consistent with information gathered from the available literature (Edem et al. 1984), who mentioned that mesocarp is commonly eaten fresh and also epicarp is sometimes consumed. The study conducted by Houessou et al. (2012) adds that the fruit may be processed for example into jams. According to our data, the mesocarp of *C. perpulchrum* is eaten fresh or chewed as a gum which corresponds with literature data (Philippe et al. 2010). As it was stated by respondents, the aril of *C. subnudum* is favoured for its pleasant sweet-sour taste and therefore often eaten fresh. Contrastingly, the mesocarp is usually only chewed as a gum because it is less tasty, which is quite new information, as the available data are considerably limited and only mention the general consumption of the fruit (Fern 2014c; JSTOR 2022a). Our data demonstrate, that arils of *D. gracilis, L. dulcis* and *M. morindoides* are locally consumed fresh which is a new fact, as the information from JSTOR (2022b), Prota4u (2022b) and Fern (2014d) mention edible use of *D. gracilis* and *L. dulcis* only briefly. To the best of our knowledge, information about edible use of *M. morindoides* is entirely new as the retrieved study published by Cimanga et al. (2010) refers only to the use of its leaves. Existing data about *S. tragacantha* are also scarce and the study of Onikanni et al. (2021) mentions the traditional use of the leaves only. Therefore, our information obtained about the edible use of the seeds which can be roasted and eaten as peanuts or leaves that can be cooked and eaten as vegetable is new.

Table 1: Ethnobotanical data on collected indigenous Ghanaian fruits and vegetables

Scientific name[a]	Family name[a]	Local name[b]	Area of collection	Part used	Traditional edible uses[b]	EY[c] (%)
Chrysophyllum albidum G.Don	Sapotaceae	Adasaa/ Adesema	Maanfe-Nkwanta (Koforidua-Suhum)	epicarp	chewed as a gum	9.2
				mesocarp	eaten fresh and can be chewed as a gum	0.8
				aril	eaten fresh	8.0
Chrysophyllum perpulchrum Mildbr. ex Hutch. & Dalziel		Asaa/ Atabene	Juaboso-Nkwanta	mesocarp	eaten fresh and can be chewed as a gum	6.0
Chrysophyllum subnudum Baker		Aningene/ Akasa		aril	eaten fresh	9.4
				mesocarp	chewed as a gum	1.8
Delpydora gracilis A. Chev.		Agyinamoa hwoa	Ankasa	aril	eaten fresh	2.2
Landolphia dulcis var. *barteri* (Stapf) Pichon	Apocynaceae	Osono kotodwe	Bibiani-Anwiaso			3.6
Morinda morindoides (Baker) Milne-Redh.	Rubiaceae	Kyeremabua/ Agyinamowa aniwa	Juaboso-Nkwanta/ Begoro Nature Reserve			5.4
Sterculia tragacantha Lindl.	Malvaceae	Foto/ sɔfɔ	Begoro Nature Reserve	seeds	can be roasted and eaten whole like peanuts/ pounded and then cooked with vegetables	7.2
				leaves	cooked as a vegetable/ used in wrapping kenkey	6.2

[a] Scientific names of the species and families are given according to WFO (2021): World Flora Online, http://www.worldfloraonline.org/; [b] ethnobotanical knowledge; [c] EY extract yield

4.2. Antioxidant Activity

In this thesis, the *in vitro* antioxidant activity of 7 plant species from Ghana, namely *C. albidum, C. perpulchrum, C. subnudum, D. gracilis, L. dulcis, M. morindoides* and *S. tragacantha* was analysed using both DPPH and ORAC methods. Among all samples tested, mesocarp of *C. albidum* showed the highest antioxidant activity with the IC_{50} value of 18.54 ± 0.27 µg/ml in the ORAC assay (Figure 1) but only a moderate activity of 95.63 ±13.24 µg/ml in the DPPH assay. Our results of DPPH test are in correspondence with numbers published by George et al. (2018) who reported inhibition ranging from 9.68 to 32.56 % for methanol extracts tested at concentration 20, 40, 60, 80 and 100 µg/ml. However, our results are in slight contrast with the results of DPPH assay conducted by Erukainure et al. (2021) who determined stronger activity with the IC_{50} value of 7.95 ± 0.50 µg/ml. Since the antioxidant activity of the fruit is decreasing during the ripening process (Darko et al. 2021), the variances between our experiments and literature data can be caused by this phenomenon. In the ORAC assay, significant activities were also recorded in the aril and epicarp of *C. albidum* with IC_{50} values of 37.34 ± 6.6 and 58.13 ± 3.42 µg/ml respectively. According to our knowledge, the ability of *C. albidum* to scavenge AAPH free radicals was studied for the first time. The mesocarp of *C. subnudum* showed remarkably high activity in the DPPH assay with the IC_{50} value of 45.18 ± 8.61 µg/ml (Figure 2), whereas in the ORAC assay it showed moderate activity of 75.53 ± 8.12 µg/ml. In the ORAC assay, weak activity was recorded in the arils of *L. dulcis* and *D. gracilis* with values of 175.79 ± 8.38 and 153.27 ± 8.89 µg/ml, respectively. The remaining samples did not show any antioxidant activity in either assay. To the best of our knowledge, this is the first report on antioxidant activity of *C. subnudum*, *L. dulcis* and *D.gracilis*.

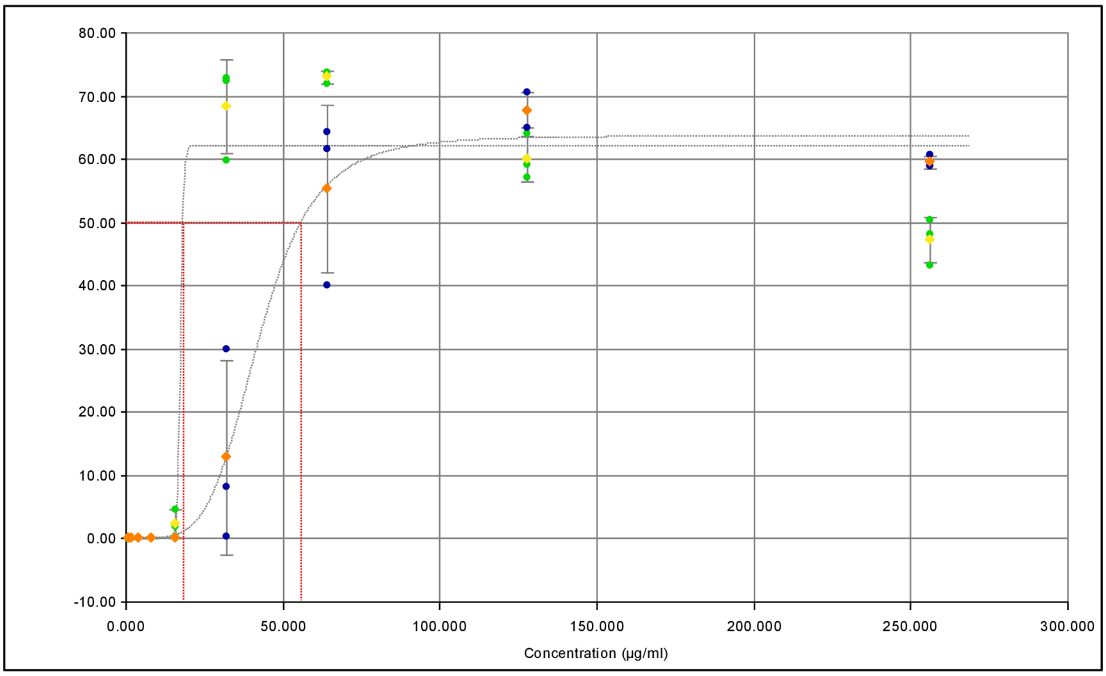

Figure 1: Dose-response curve and half maximal inhibitory concentration value of methanol extracts from the epicarp and mesocarp of *C. albidum* (ORAC assay). All the values are expressed as mean ± standard deviation; n = 3 (one experiment).

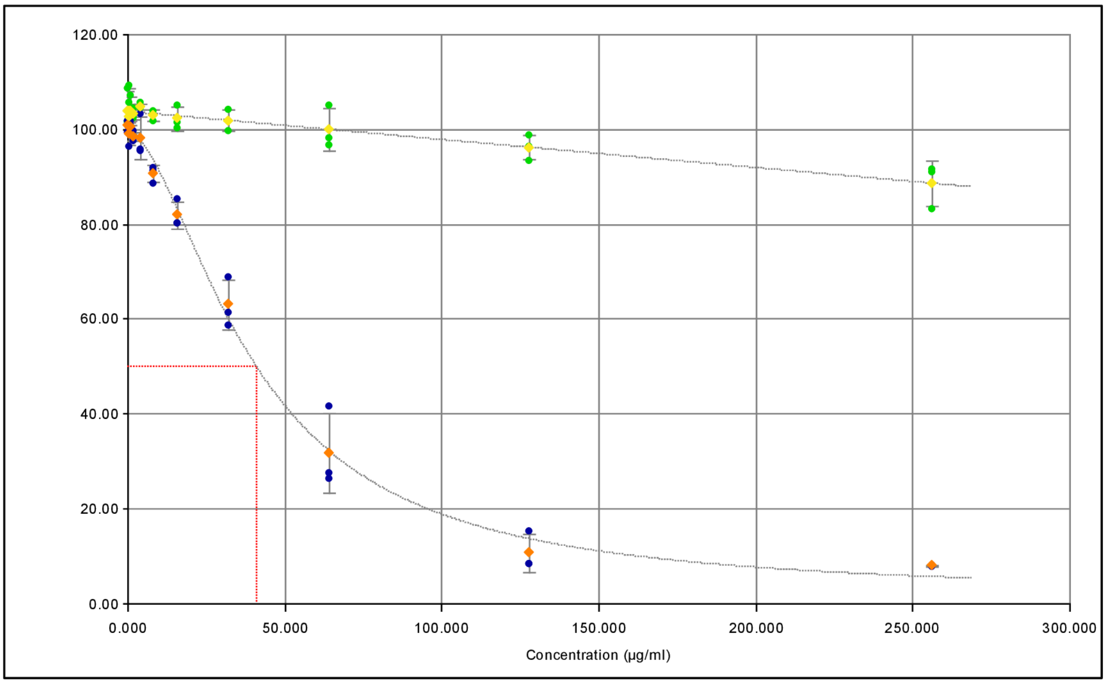

Figure 2: Dose-response curve and half maximal inhibitory concentration value of methanol extract from mesocarp and aril of *C. subnudum* (DPPH assay). All the values are expressed as mean ± standard deviation; n = 3 (one experiment).

Table 1: Antioxidant activity of plant extracts from underutilized species of fruits and vegetables of Ghana

Sample	Plant part used	DPPH IC50 (µg/ml)	ORAC IC50 (µg/ml)
S. tragacantha	seeds	>256	>256
	leaves	>256	>256
C. subnudum	seed pulp	>256	>256
	fruit pulp (mesocarp)	45.18 ± 8.61	75.53 ± 8.12
C. albidum	fruit (epicarp)	135.20 ± 6.13	58.13 ± 3.42
	fruit pulp (mesocarp)	95.63 ±13.24	18.54 ± 0.27
	seed pulp	184.48 ± 6.41	37.34 ± 6.6
C. perpulchrum	fruit pulp (mesocarp)	>256	>256
M. morindoides	seed pulp (aril)	>256	>256
L. dulcis	seed pulp (aril)	>256	175.79 ± 8.38
D. gracilis	seed pulp (aril)	>256	153.27 ± 8.89
Trolox		9.28 ± 0.18	17.33 ± 0.26

5. Conclusion

In this thesis, ethnobotanical data on traditional use of underutilized Ghanian crops were gathered and methanol extracts prepared from their fruits and vegetables were evaluated of antioxidative effects using *in vitro* DPPH and ORAC assays. The results showed that *C albidum* was the most commonly consumed fruit of all selected species. According to our best knowledge, this is the first report on the specific edible uses of *C. subnudum*, *D. gracilis, L. dulcis, M. morindoides* and *S. tragacantha*. Furthermore, various fruit parts of *C. albidum* and *C. subnudum* produced high antioxidant activity in both assays. These findings may serve as a theoretical background for further development of new food products (e.g. supplements and nutraceuticals) for the prevention of some oxidative stress-associated diseases. However, further research of their chemical composition, pharmacological effects and toxicological safety is required. In addition, the study contributed to conservation of traditional knowledge on underutilized fruits and vegetables of Ghana.

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7. Appendices

List of the Appendices:

Appendix 1: Photographic illustrations of selected Ghanaian NUS species

Fig. 1: ***C. albidum*** **fruit**

(Original photo by A.A. Abakah)

Fig. 2: ***C. subnudum*** **fruit**

(Original photo by A.A. Abakah)

Fig. 3: ***C. perpulchrum***

(Original photo by A.A. Abakah)

Fig. 4: ***M. morindoides***

(Original photo by A.A. Abakah)

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Fig. 5: ***D. gracilis*** **fruit I**

(Original photo by A.A. Abakah)

Fig. 6: ***D. gracilis*** **fruit II**

(Original photo by A.A. Abakah)

Fig. 7: ***L. dulcis*** **fruit I**

(Original photo by A.A. Abakah)

Fig. 8: ***L. dulcis*** **fruit II**

(Original photo by A.A. Abakah)