KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

COMPARATIVE STUDIES ON THE *IN VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF METHANOLIC AND HYDRO-ETHANOLIC EXTRACTS FROM EIGHT EDIBLE LEAFY VEGETABLES OF GHANA

A Thesis Submitted to the Department of Chemistry,
Kwame Nkrumah University of Science and Technology, Kumasi,
In Partial Fulfillment of the Requirements for the Award
of the Master of Philosophy Degree in Chemistry

BY

Joseph Francis Morrison

SUPERVISOR:

DR. SYLVESTER K. TWUMASI

JUNE 2009

DECLARATION

I, Joseph Francis Morrison, do hereby declare that this thesis is a product of a research work I carried out towards the M. Phil. degree except references to other people's work which have been duly acknowledged, and that neither the whole nor part of this thesis has been presented for another degree or programme elsewhere.

…… ……..

Joseph Francis Morrison
(Candidate, PG 96550-06)

Date

Certified by:

…… ……..

Dr. Sylvester K. Twumasi
(Supervisor)

Date

Certified by:

…… ……..

Dr. Samuel Osafo Acquaah (Head of Chemistry Department) Date

DEDICATION

This thesis is dedicated to the memory of my late Mum, Madam Adjoa Essoun, whose sacrifices and support throughout my formative years have inspired me to be a better person in all aspects of life; and also to my late Dad, Mr. Henry Morrison and Step-Mum, Madam Elizabeth Mensah, whose selflessness nature and care ensured that I had university education. I appreciate your acts of kindness. I really love you.

ACKNOWLEDGEMENT

I wish to convey my deepest gratitude to my supervisor, Dr. Sylvester K. Twumasi, for his inspiration, patience, support, and tutelage during the years I spent working towards the Master of Philosophy degree from Kwame Nkrumah University of Science and Technology. Working with Dr. Twumasi has been a treasured and memorable experience for me.

I would like to thank Dr. Evans Adei and Dr. Samuel Osafo Acquaah (all of Chemistry Department, KNUST) for their academic and fatherly counsel.

I would also want to express my appreciation to Professor P. Y. Boateng (Head of Horticulture Department, KNUST) and Mrs. G. Timpo, of Horticulture Department, KNUST, Kumasi for their assistance in the authentication of the selected leafy vegetables.

I would also want to express my profound gratitude to the following individuals:

1. Dr. Eric Woode (Head of Pharmacology Department, KNUST) and Mr. Patrick Amoateng (Ph.D. student of Pharmacology Department, KNUST) for the supply of the reagents for the antioxidant tests and the GraphPad software.
2. Mr. Thomas Ansah (Senior Technician, Pharmacology Laboratory, Department of Pharmacology, KNUST) and Mr. Prosper Sedziafa (Senior Technician, Microbiology Laboratory, Department of Pharmaceutics, KNUST) for the supply of the bacteria and the reagents for the antibacterial activity tests.

3. Mr. Teddy Delali Blutse (Technical Instructor, Biochemistry Department, KNUST), Miss Diana Benedicta Amoh and Mr. Ernest Amoah Ampah (all final year Biochemistry students-2009 graduating class) for helping with glasswares, equipment, and other reagents for the experimental work.

Also, special thanks are due Mr. Justice E. Ofori, Mr. Christopher Amoako, Mr. John K. Mensah, Mr. Robert Appiah, and Mr. Kwame Asante (all of Apostolic Church of God 7th Day, Ghana) for their financial support and encouragement.

Several people who have had a colossal influence on my life deserve thanks as well, so I would like to express my gratitude to Mrs. Evelyn Owusu Bempah, Mrs. Mary Antwi, Madam Lydia Obeng, Madam Beatrice Serwaa, Mrs. Kate Addae, Mrs. Scholarstica Ofori, Madam Adwoa Yeboah, and Mrs. Philomena Appiah (all of Apostolic Church of God 7th Day, Ghana) for their motherly counsel and support.

My most sincere appreciation goes out to my wonderful friends, Eric Twum Barimah, Michael Yanney, Edmond Bernard Nyame-Appiah, Richard Tia, Desmond Owusu Debrah, Stephen Osei-Tutu, Kwame Osei Boateng, Elvis Baidoo, Linda Mensah Sarpong, and Zelda Yvonne Abban for their loyalty, companionship and support.

ABSTRACT

Epidemiological studies indicate that consumption of fruits and vegetables has the ability to inhibit the damaging activities of free radicals and other pathogens in the human body. Eight edible leafy vegetables of Ghana namely: *Xanthosoma sagittifolium*, *Hibiscus sabdariffa*, *Solanum macrocarpon*, *Talinum triangulare*, *Corchorus olitorius*, *Laportea aestuans*, *Ipomoea batatas*, and *Amaranthus cruentus* were assessed for their *in vitro* antioxidant and antibacterial properties. The experimental results indicate that hydro-ethanol is an effective solvent for extracting the phytoconstituents of the leafy vegetables. The total antioxidant capacity (TAC) and total phenol content (TPC) in the methanol extracts (METE) and hydro-ethanol extracts (HETE) from the selected leafy vegetables within the measured concentration range (0.1 - 3.0 mg/mL) decreased in the order *X. sagittifolium* > *I. batatas* > *L. aestuans* > *T. triangulare* > *H. sabdariffa* > *C. olitorius* > *S. macrocarpon* > *A. cruentus*. A high and positive correlation was observed between TPC and TAC in both the METE and HETE from all the selected leafy vegetables. The selected leafy vegetables showed strong antioxidant properties with respect to their free radical scavenging activity and Fe^{3+} reduction ability with hydro-ethanol extracts indicating higher antioxidant potential compared with their respective methanol extracts. In addition, the extracts

from the selected leafy vegetables exhibited a strong antibacterial activity against the growth of *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus* with the exception of extracts from *H. sabdariffa, C. olitorius, S. macrocarpon,* and *A. cruentus* which showed no activity against the growth of *B. subtilis*.

TABLE OF CONTENTS

Chapter Two

Chapter Four

Chapter Five

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

TAE	Tannic Acid Equivalent
AA	Ascorbic Acid
SE	Standard Error
SEM	Standard Error Mean
ROS	Reactive Oxygen Species

ROI	Reactive Oxygen Intermediates
IELVsG	Indigenous Edible Leafy Vegetables of Ghana
SET	Single Electron Transfer
HAT	Hydrogen Atom Transfer
BHT	Butylated Hydroxytoluene
BHA	Butylated Hydroxyanisol
DPPH	2,2-Diphenyl-1-pricrylhydrazyl
AAE	Ascorbic Acid Equivalent
RSA	Radical Scavenging Activity
F-C	Folin-Ciocalteu
TAC	Total Antioxidant Activity

TPC	Total Phenol Content
METE	Methanolic Extracts
HETE	Hydro-Ethanolic Extracts
ANOVA	Analysis of Variance
EC_{50}	The concentration of agonist that gives a response half way between Bottom and Top
nPG	n-Propyl gallate
SM	*Solanum macrocarpon*
CO	*Corchorus olitorius*
AC	*Amaranthus cruentus*
XS	*Xanthosoma sagittifolium*
TT	*Talinum triangulare*

LA	*Laportea aestuans*
HS	*Hibiscus sabdariffa*
IB	*Ipomoea batatas*
MIZ	Mean Inhibition Zone
MIC	Minimum Inhibitory Concentration
NA	No Activity

Chapter One

1 INTRODUCTION

Generation of oxygen radicals, such as superoxide radical $^{\bullet}O_2^-$, hydroxyl radical ($^{\cdot}OH$) and non-free radical species, such as H_2O_2 and singlet oxygen ($^1O_2$) are associated with cellular and metabolic injury, ageing, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (Ames, 1983; Stadtman, 1992). Free radicals can be generated by metabolic pathways within body tissues and can also be introduced into the body by external sources such as food, drugs as well as environmental pollution.

Previous epidemiological studies have consistently shown that consumption of fruits and vegetables has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancers (Gerber *et al.,* 2002; Kris-Etherton *et al*., 2002; Serafini, Bellocco, Wolk, & Ekstrom, 2002) and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Di Matteo & Esposito, 2003) as well as inflammation and problems caused by cell and cutaneous aging (Ames, Shigenaga, & Hagen, 1993).

During the last decade, the concept of health promotion has become a legitimate part of health care. Epidemiological evidences links intake of ascorbic acid (AA) and other antioxidant micronutrients to health, in virtue of their capability of trapping reactive oxygen species (ROS) which cause damages to biological systems (Elsayed, 2001).

Promoting the consumption of diets with antioxidants, especially deriving from natural sources, is becoming a more effective strategy in combating chronic diseases.

In this regard, several studies have been directed toward the evaluation of antioxidant properties of many naturally occurring botanicals and herbs, potentially useful as nutriceutical ingredients (Farrukh *et al.,* 2002).

Green leafy vegetables are popularly used for food in many countries of the world, being a rich source of *β*-carotene, ascorbic acid, minerals, and dietary fibre (Negi *et al.*, 2001). Epidemiological evidence has clearly shown that diets based on fruits and vegetables, with high contents of natural antioxidants, contribute to reduced mortality from cardiovascular and cerebrovascular diseases (Alia *et al*., 2003). Many workers (Lockeett *et al.*, 2000; Akindahunsi and Salawu, 2005; Edeoga *et al*., 2006; Hassan and Umar, 2006; Ekop, 2007) have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries. Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids, whose antioxidant activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants (Salah *et al*., 1995; Van Acker, Van den Vijgh, & Bast, 1996).

The immune system becomes vulnerable to oxidative stress especially during certain diseased states, as well as during ageing, and therefore there is the need to boost its antioxidant abilities (Devasagayam & Sainis, 2002). Antioxidants in the body preserve an adequate function of immune cells against homeostatic disturbances (De la Fuente & Victor, 2000). Studies to date have demonstrated that phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, antibacterial, and antiviral effects (Waladkhani & Clemens, 1998).

Food-borne diseases are still a major problem in the World, even in well-developed countries (Mead and others, 1999). A variety of microorganisms also cause food spoilage which is one of the most important concerns of the food industry. So far, many pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, and *Campylobacter jejuni* have been reported as the causal agents of foodborne diseases and/or food spoilage (Betts *et al*, 1999 and Deak and Beuchat1996). Hence, chemical preservatives are currently employed to prevent the growth of food spoiling microbes in the food industry (Sagdıc and Ozcan, 2003).

However, due to consumer concerns about the safety of food containing synthetic chemicals used as preservatives, there is a growing interest in the use of natural antibacterial compounds, like extracts of herbs and spices, for preservation of foods, as these possess characteristic flavours and sometimes show antioxidant activity as well as antimicrobial activity **(**Smid and Gorris, 1999).

In this project work, comparative studies on the *in vitro* biological activity on methanolic and hydro-ethanolic leaf extracts from eight indigenous edible leafy vegetables of Ghana (IELVsG) are to be carried out for their antioxidant properties in different antioxidant property determination assays and also for their antimicrobial activities against common food-borne pathogens. The selected leafy vegetables are, *Ipomoea batatas, Xanthosoma sagittifolium*, *Hibiscus sabdariffa*, *Solanum macrocarpon*, *Laportea aestuans*, *Talinum triangulare*, *Corchorus olitorius* and *Amaranthus cruentus* or *Amaranthus hybridus*.

1.1 Aims and Objectives

This project work seeks to compare the *in vitro* bioactivity with respect to antioxidant property and antimicrobial activity on the methanolic and hydro-ethanolic leaf extracts from the selected leafy vegetables of Ghana.

Specific Objectives are:

1. to determine the phytoconstituents present in the leaf extracts from the selected leafy vegetables
2. to determine the antioxidant property on the methanolic and hydro-ethanolic leaf extracts from the selected leafy vegetables in different antioxidant property determination assays

3. to evaluate the antimicrobial activity on the methanolic and hydro-ethanolic leaf extracts from the selected leafy vegetables against common human, animal, and food-borne pathogens namely: *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa, and Staphylococcus aureus* (all bacteria) .

1.2 Justification of Specific Objectives

The selected leafy vegetables are reported to contain high levels of micronutrients and vitamins; have medicinal uses, and are mostly used in soup and stew preparations in most African countries (Dokosi, 1998). Recent investigations on the phytochemical screening and antioxidant indices on some of the selected leafy vegetables indicates that they could be potentially useful as natural antioxidants (Akindahunsi and Salawu, 2005 a,b; Odukoya *et al.,* 2007 and Salawu *et* al., 2006). The present study on the *in vitro* antioxidant and antibacterial activities on the selected leafy vegetables is necessitated by lack of extensive research information on the selected leafy vegetables especially in Ghana. This study aims to compare the antioxidant and antibacterial activities of the selected leafy vegetables to provide useful information on their bioactivity potential which could guide consumers in deciding on which leafy vegetables to utilize in their diets due to their strong bioactivity.

Chapter Two

2 LITERATURE REVIEW

This chapter reviews the selected leafy vegetables, their nutritional and health benefits, antioxidants and free radicals, antioxidant determination methods, and antimicrobial activity.

2.1 Overview of the Selected Leafy Vegetables

Below is a review of the occurrence, description, agronomy, nutrition, and health benefits of the selected indigenous leafy vegetables namely: *Ipomoea batatas* (L.) Lam, *Xanthosoma sagittifolium* (L.) Schott, *Hibiscus sabdariffa* (Linn.), *Solanum macrocarpon* (Linn.), *Laportea aestuans* (Linn.) Chev., *Talinum triangulare* (Jacq.) Wild., *Amaranthus cruentus* (Linn.) Thell. Or *Amaranthus hybridus* (Linn.) and *Corchorus olitorius* (Linn.).

2.1.1 *Ipomoea batatas* (L.) Lam

Family: Convolvulaceae

Common Names: Sweet Potato, Spanish Potato

Vernacular Names: Santom (Fante), Ntommo Aborodwobaa (Twi)

Occurrence

Ipomoea batatas is an ancient root crop native to Tropical America. The perennial vines have been cultivated for as long as 5,000 years.

Description

This plant is a herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose color ranges between red between red, purple, brown and white. Its flesh ranges

from white through yellow, orange, and purple. The pinkish-white flowers with a deep purple throat are up to 2.5 inches (6.4 cm) across.

Agronomy

It grows best at an average temperature of 24°C, abundant sunshine and warm nights. Annual rainfalls of 750-1000 mm are considered most suitable, with a minimum of 500 mm in the growing season. The crop is sensitive to drought at the tuber initiation stage 50–60 days after planting and is not tolerant to water-logging, as it may cause tuber rots and reduce growth of storage roots if aeration is poor. Depending on the cultivar and conditions, tuberous roots mature in two to nine months. They are mostly propagated by stem or root cuttings or by adventitious roots called "slips" that grow out from the tuberous roots during storage. They grow well in many farming conditions and have few natural

enemies; pesticides are rarely needed. Sweet potatoes are grown on a variety of soils, but well-drained light and medium textured soils with a pH range of 4.5-7.0 are more favourable for the plant. They can be grown in poor soils with little fertilizer (Dokosi, 1998).

Nutritional and Health Benefits

Besides simple starches, sweet potatoes are rich in complex carbohydrates, dietary fiber, beta carotene (a vitamin A equivalent nutrient), vitamin C, and vitamin B6. Despite the name "sweet", it may be a beneficial food for diabetics, as preliminary studies on animals have revealed that it helps to stabilize blood sugar levels and to lower insulin resistance. The young leaves and vine tips of sweet potato leaves are widely consumed as green vegetable in West African countries (Guinea, Sierra Leone and Liberia for example), as well as in northeastern Uganda, East Africa (Dokosi, 1998). According to Food and Agriculture Organisation (FAO) leaflet No. 13 - 1990, sweet potato leaves and shoots are a good source of vitamins A, C, and B2 (riboflavin), and lutein.

2.1.2 Xanthosoma sagittifolium (L.) Schott

Family: Araceae

Common Names: Cocoyam

Vernacular Names: Mankani (Akan) for corms; Kontomire (Akan) for cocoyam leaves

Occurrence

It is a native of West Indies and Central America where several varieties occur. It reached West Africa from West Indies in 1843, probably by West Indian Missionaries and spread inland from the coast. In Ghana, it is believed to have reached the Ashanti at the beginning of the 20th century (Dokosi, 1998).

Description

It is a stout herbaceous plant, tuberous rhizomes, erect, semi-erect or horizontal, up to 3 m high, leaf-blade up to 35 cm broad, 30-50 cm long, bottom nerves nearly marginal at petiole, basal lobes not overlapping, petiole up to 60 cm long; peduncle up to 40 cm long, spathe to 20 cm long, spadix slightly longer than spathe; fruits rarely produced. It is distinguished in part by its usually large leaves, with fleshy petioles to 6 feet long and broad arrowhead-shaped blades to 3 feet long (and nearly as wide at the blade base). These leaves arise from a broad corm; offshoots appear on short rhizomes extending from the corm. The main underground stems are generally referred to as corms and may be white, pink or yellow (Dokosi, 1998).

Fig. 2.2 Leaves of *Xanthosoma sagittifolium*

Agronomy

It is mostly cultivated for both corms and leaves. In Ghana, it is cultivated in forest areas as rain-fed crop, in fairy moist soil conditions where the annual rainfall varies between 1000 and 1500 mm. irrigation may be required during dry periods, particularly during the actively growing period. A high level of soil organic material is essential for early

maturity of the underground stems. Fertilizers containing nitrogen may be required as supplementary surface dressing at intervals during the early growing period. A pH range of 5.5 – 6.5 is preferable. The plant is sensitive to high temperatures and will tolerate some degree of shade, although optimum growth is obtained from plants grown is full sunlight. Altitudes up to 800-1000 m are generally suitable. Propagation is normally by means of small corms, with the tops of the young plants removed and inserted as cuttings. Rooted cuttings or corms are planted on low ridges 75-90 cm apart. The more vigorous forms may require wider spacing. It has a remarkable ability for re-growth. It does not thrive well if covered by weeds. Harvesting of the leaves can start 4 weeks after planting whereas the corms take 240-420 days after planting to mature.

Nutritional and Health Benefits

The leaves of x*anthosoma sagittifolium* (per 100 g edible portion) are reported to contain beta carotene and ascorbic acid. It contains crystals of calcium oxalate, steroidal saponin-like compounds, tannins, prussic acid. The leaves of x*anthosoma sagittifolium* are also used in soup and stew preparations.

2.1.3 *Hibiscus sabdariffa* (Linn.)

Family: Malvaceae

Common Name: Roselle

Vernacular Names: Suule (Hausa), Rarna (Hausa)

Occurrence

A native of West Indies cultivated in Ghana, especially in the Northern, Upper East and Western Regions and also in certain parts in Southern Ghana by settlers from Northern Ghana; distributed from Senegal to Nigeria (Dokosi, 1998).

Description

Roselle is a robust many-branched annual or perennial herb or woody-based subshrub that gets 4-7 ft tall and almost as broad. The dark green leaves are about 15 cm across and deeply dissected into 5 narrow lobes. The stems, branches, leaf veins, and petioles (leaf stems) are reddish purple. The hibiscus-like flowers are yellow and about 7.6 cm across. At the bottom of each flower, enclosing the bases of the five petals is a fleshy bright red cup-like structure called a calyx. The calyx is about 2.5 cm in diameter.

Fig. 2.3 Leaves of *Hibiscus sabdariffa*

Nutritional and Health Benefits

The green leaves are used like a spicy version of spinach. In Ghana, the green leaves are eaten as a potherb. The green leaves are picked, dried and are kept in pots and used as potherb in the dry season. Fresh calyces are removed from the fruit and cooked and used as soup. The dry calyces

can be prepared in the same way as tea or by putting the petals in hot water and boiling. The petals can be left in a jug of cold water for few hours, flavourings may be added and taken as drink (Dokosi, 1998). The dried leaves are used in cold beverages called 'Sobo' in Nigeria. The red calyces of the plant are increasingly exported to America and Europe, where they are used as food colourings. Roselle is associated with traditional medicine and is considered to have antihypertensive properties to and it is reported to be used to treat urinary tract infections. The calyces and leaves of *Habiscus sabdariffa* are used as antiscorbutic and diuretic. In East Africa, the calyx infusion, called "Sudan tea", is taken to relieve coughs. It has been generally used in folk medicine as a diuretic, mild laxative, and treatment for cardiac and nerve diseases and cancer. Roselle juice, with salt, pepper, and molasses, is taken as a remedy for biliousness. A lotion made from leaves is used on sores and wounds. In India, a decoction of the seeds is given to relieve dysuria, strangury and mild cases of dyspepsia. In Thailand, Roselle is drunk as a tea, believed to also reduce cholesterol. *Habiscus sabdariffa* is rich in phenolic compounds with marked physiological activities. Hibiscus flowers contain gossypetin, glucoside, bibiscin, hibiscus anthocyanin and Hibiscus protocatechuic acid, which may have diuretic and effects, reducing blood pressure, and stimulating intestinal peristalsis (Olatunde Farombi, 2003).

2.1.4 *Solanum macrocarpon* (Linn.)

Family: Solanaceae

Common Name: African eggplant

Vernacular Names: Gboma (Ewe), Aturopo (Twi)

Occurrence

It is cultivated in farms and gardens and sometimes occurs as escape in waste places in Ghana. It is also cultivated in other African countries such as Egypt, Mali, Sierra Leone, Republic of Togo and Nigeria. It is thought to probably originated from Africa (Dokosi, 1998).

Description

A semi-woody, glabrous, armed or unarmed with spines, annual, up to 60 cm high, branches stout; leaves oblong, sub-obtuse tip, cuneate at the base, slightly lobed, petiole to 8 cm long; flower bluish-purple to 25 mm in diameter; fruit green tinged with purple of white, yellow when ripe.

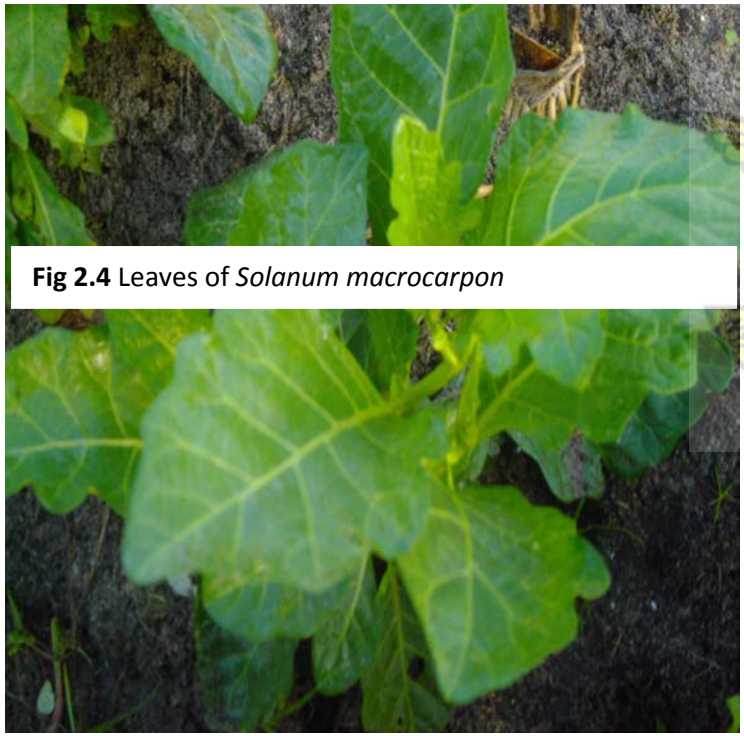


Fig 2.4 Leaves of *Solanum macrocarpon*

Agronomy

In most African countries, *Solanum marcrocarpon* is produced in the high-rainfall or humid coastal zones and semi-arid and savanna areas. Seeds are sown in a nursery and spaced at 20 cm between rows. Seedlings can be transplanted after 4-6 weeks. Soil fertility is important, and when preparing beds, 15:15:15 NPK fertilizers are applied at a rate of 80-100 kg/ha. First harvest of the leaves usually takes place about 1 week after flowers appear.

Nutritional and Health Benefits

It is a potherb of Africa and is widely cultivated on the continent for this purpose. In Ghana, the leaves *Solanum marcrocarpon* are eaten as potherb or in soup. In India and Malaysia the mature acidic fruits are used as a sour relish. In Sierra Leone, heated leaves are chewed to treat throat troubles; in Nigeria fruits are taken as a laxative, and to treat cardiac diseases, while flowers and fruits are chewed to clean the teeth. In Kenya, the juice of boiled roots is drunk to get rid of hookworms, while crushed leaves are taken to treat stomach troubles. The root is used against bronchitis, itches and for body aches; also for asthma and to cure wounds while the seeds are used to treat toothache (Dokosi, 1998).

2.1.5 *Laportea aestuans* (Linnaeus) Chev.

Family: Urticaceae

Common Name: West Indian Woodnettle

Vernacular Names: Hunhon (Twi), Bonhon (Brong), Ahonhon (Akan), Atro (Ewe)

Occurrence

Laportea aestuans is an annual herb and usually found around waste places. It is possibly native to tropical Africa, although it is now widespread as an introduced species throughout both the western hemisphere and eastern hemisphere tropics and sub-tropics, including USA, Central America and West Indies (Dokosi, 1998).

Description

Annual herb, 1-10 dm, sparsely to densely pubescent with stinging hairs and stipitate-glandular, nonstinging hairs. Leaf blades broadly ovate to nearly orbiculate, 9-20 × 6-16 cm, base rounded or abruptly attenuate, auriculate, margins regularly serrate or dentate, apex short-acuminate. Inflorescences with both staminate and pistillate flowers in same panicle, or proximal panicles with staminate flowers. Staminate flowers ca. 2 mm across; tepals 4-5, equal in length; stamens 4-5, opposite tepals; filaments longer than tepals. Pistillate flowers ca. 0.7 mm; tepals 2-4, appressed, inner pair ca. 1/2 length of ovary; ovary ovoid to ellipsoid; style persistent, hooked and beaklike, ca. 0.2 mm, becoming knoblike in fruit. Fruit about 1 mm long with a residual hooked stigma.

Fig. 2.5 Leaves of *Laportea aestuans*

Agronomy

It is normally found around waste places.

Nutrition and Health Benefits

In Ghana, the leaves are used in preparing soup and taken by women who have recently delivered to revitalize them (as tonics). They are taken as emmernagogue to promote menstrual flow. The poultice is also applied against scorpion stings. Also, the poultice mixed with mashed ripe plantain is locally applied to heal boils

In other African countries, for instance in Nigeria, the Yorubas use the boiled leaves in treating constipation. In Congo, the sap is used for eye infections and for swellings. In Lulonga, they form a remedy for gonorrhoea. Also in Cote d'Ivoire, the liquid obtained by soaking crushed leaves in palm oil is used for curing wounds and for massaging purposes (Dokosi, 1998)

The leaves cause urticaria, but their decoction is used medicinally. A decoction of the aerial parts is used as a diuretic and laxative. The juice from the crushed leaves can be used as an eye-wash for "sore eyes" (Lachman-White and others, 1992)

2.1.6 *Talinum triangulare* (Jacq.) Wild.

Family: Portulacaceae

Common Name: Water leaf

Vernacular Names: Bokoboko, Kotubetsew (Fante), Efan (Akan), Yevugboma (Ewe)

Occurrence

Talinum triangulare is of tropical African origin and is dispersed widely throughout the African subcontinent. It is a naturalized weed, usually of waste places; sometimes cultivated especially in Ghana, Senegal, and Cameroun (Dokosi, 1998).

Description

An erect succulent herb 45 cm high, woody below and with a swollen rootstock; leaves obovate to obovate-lanceolate, apex emarginated and mucronate, base cuneate, up to 5 cm long, 35 mm broad, glabrous and fleashy; flowers pink, sometimes white (hybrids occur) in loose, forking, few-flowered panicles; capsule straw-coloured; seeds small, black (Dokosi, 1998).

Fig. 2.6 Leaves of *Talinum triangulare*

Agronomy

Waterleaf grows best under humid conditions at temperatures of about 30 ^{o}C. Growth is very fast during the rainy season but slows down considerably during the dry season. It grows well under shade and in cloudy weather. Waterleaf can be sown, planted or collected from the wild. Commercially, it is mostly grown through cuttings 10-15 cm long. It is advisable to remove the lowest pair of leaves before planting. Waterleaf may also be propagated by seed. Seeds are tiny (1000-seed weight is about 0.25 g) and can only be collected from fruits which have turned yellow. However, collection of seeds from mature fruits is difficult as they shatter when touched. Under natural conditions, the plant will live for about 4 months before it perishes, mainly due to drought. The plant takes only three weeks from planting to the first harvest and the leaves can be harvested on the average four times from a plant before its growth starts to decline.

Nutritional and Health Benefits

In Ghana, a poultice of the leaves is externally applied when a child develops an enlarged spleen. The poultice is mixed with sooth (carbon) and raw egg and incisions are made on the skin at the appropriate spot and and the preparation externally applied. The patient also eats the cooked leaves as spinach without salt and pepper. It is mostly used in soup and stew preparations. It is also a popular potherb and chicken-feed. As a potherb, it is also eaten by pregnant women their birth date is overdue. It is eaten for curing hernia. A decoction of the root cooked with rhizomes of *Canna indica* (the green variety) is used in preparing palm-nut soup for barren woman, using the hide of a quadruped as meat. In other African countries, the pounded leaves are applied to inflammations and a water leaf infusion has diuretic properties (Dokosi, 1998). It is also used for medicinal purposes, where the shoots are used in the preparations of laxatives and curative portions against measles. In south East Asia, the roots are used as tonic for general weakness, possible substitute for ginseng, to treat inflammation and swelling (Nya and Eka , 2007). Half glass of fresh leaves of *Talinum triangulare* crushed in 2 liters of water, filtered and

stored could be taken before food twice daily to manage hypertension (Aiyeloja and Bello, 2006). In Cameroon, waterleaf is used as a treatment for measles, whera in Assam (India), it is used to treat diabetes. In Indonesia a tonic is made from the fleshy root (Grubben and Denton, 2004).

2.1.7 *Amaranthus cruentus* (Linn.) Thell. Or ***Amaranthus hybridus*** (Linn.)

Family: Amaranthaceae

Common Name: Amaranth or pigweed

Vernacular Names: Alayafu or Aleefu (Hausa), Efan (Akan), Otofamen (Twi)

cruentus **Fig. 2.7** (**b**) Flowered *Amaranthus cruentus* plant

Occurrence

It is commonly cultivated, but is sometimes found as weed in Ghana,Guinea, Nigeria, and Sudan. It is widely naturilised in many parts of tropical Africa. It is also found in Southern Europe, America, and Asia (Dokosi, 1998)

Description

An erect, branched green or reddish annual herb, 30-150 cm high, without spines; leaves obovate-rhomboidal or narrowly elliptic, Cuneate at base; inflorescence terminal, rarely ancillary, often much branched; flowers green or reddish, female perianth-segments 5; bracteoles and fruit longer than oblong female perianth-segements (Dokosi 1998). Taproot is long, fleshy red or pink. The seeds are small and lenticellular in shape; with each seed averaging 1–1.5 mm in diameter and 1000 seeds weighing 0.6–1.2 g (Akubugwo *et al*., 2007).

Agronomy

The most popular method of cultivation is to sow the seeds on a plant bed and harvest it directly by uprooting the whole plant. This method is mostly used in market gardening in West and East Africa. The crop can be harvested 4-5 weeks after sowing the seeds. Usually the first harvest is the main shoot and the farmer allows side shoots to develop. The plant grows well on compost.

Nutritional and Health Benefits

Assessment of the vitamin composition of the leaves of *Amarathus hybridus* L. leaves show the presence of β-Carotene (Vitamin A), riboflavin (Vitamin B2)**,** niacin (Vitamin B3)**,** pyridoxine (Vitamin B6), ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E) (Akubugwo *et al*., 2007). Also according to Akubugwo *et*

al., (2007), phytochemicals such as alkaloid, flavonoids, saponins, phenols, tannins, hydrocyanic acid and phytic acid are present in the leaves of *Amaranthus hybridus* L. In Ghana, the leaves of the plant are eaten as spinach in soup and stew preparations. The juice of the crushed leaves is mixed with the juice of limes and drunk by mothers in labour to hasten childbirth. The juice obtained from warmed leaves serves as an eye-lotion (Dokosi, 1998).

2.1.8 *Corchorus olitorius* (Linn.)

Family: Tiliaceae

Common Name: Bush okra (vegetable jute), Jew's marrow

Vernacular Names: Ayoyo (Kasem), Oturo (Twi), Otur (Fante)

Occurrence

It is usually cultivated on farms and gardens in Ghana and sometimes a weed of waste places. It is found also in Senegal and Nigeria. It is common in the tropics, and in Australia (Dokosi, 1998).

Description

It is an annual/perennial growing to 3.5 m tall. It is glabrous herb, woody at the base; leaves lanceolate or ovate-lanceolate, to 20 cm long, 7 cm broad, sometimes tailed; flowers yellow, cream or pale orange; capsule usually 5-valved, to 6 cm long, stout, rather abruptly narrowed to the ápex.

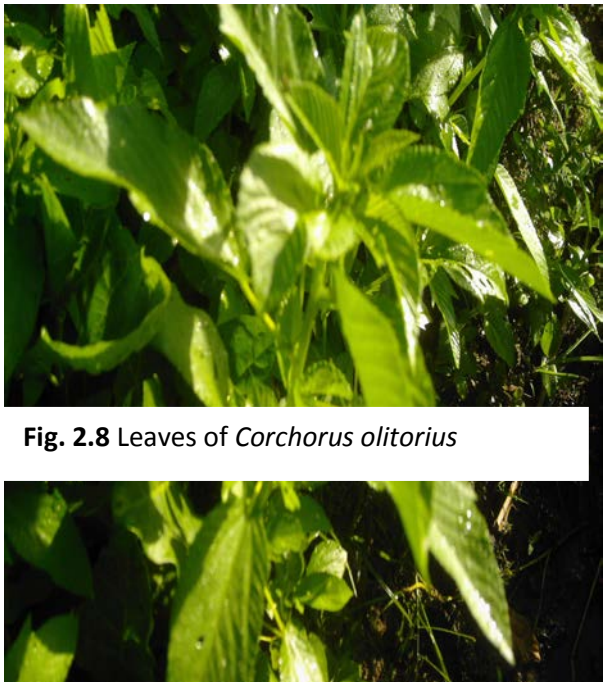


Fig. 2.8 Leaves of *Corchorus olitorius*

Agronomy

The plant prefers light (sandy), medium (loamy), and heavy (clay) soils. The plant can grow in acid, neutral, and basic (alkaline) soils. It cannot grow in the shade and requires moist soils. Farmers sow the seeds directly or transplant in lines.

Nutritional and Health Benefits

In Ghana, young leaves are added to salads whilst older leaves are cooked as a pot-herb. It is also used to induce milk andfor softening and drawing the breast. It is reported to contain a cardioactive glycoside, strophanthidan. In Senegal, it is used internally as a remedy for pains in the intestines and externally as an emollient (Dokosi, 1998). The leaves are demulcent, diuretic, febrifuge, and tonic. They are used in the treatment of chronic cystitis, gonorrhoea and dysuria. A cold infusion is said to restore appetite and strength. The seeds are purgative. Injections of olitoriside, an extract from the plant, markedly improve cardiac insufficiencies and have no cumulative attributes; hence, it can serve as a substitute for strophanthin.

2.2 Antioxidants and Free Radicals

According to the mitochondrial free radical theory of ageing (De Grey, 1999), free radicals are a class of molecule that possesses one or more unpaired electrons. The nature of atomic structure and of the covalent chemical bond (the features that give an atom its valency) are fixed by the rule that electrons occupy orbitals of atoms, such that an orbital can contain zero, one or two electrons, and that electrons carry less energy when they are one of a pair in an orbital than when they are unpaired. Thus, a molecule is only a free radical if it possesses one or more unpaired electrons (**Fig. 2.9**). Thus, compounds which are antioxidants by virtue of their ability to act as reductants in solution tend to be easily oxidized (loss of electrons).

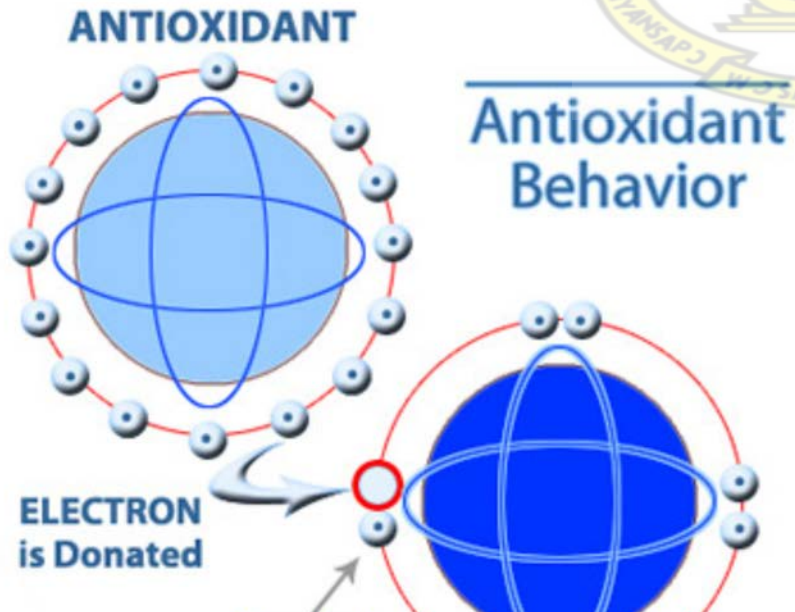


Fig. 2.9 Free Radical Molecule (www.biomatrixone.com)

Antioxidants are added in food during processing to improve food quality and stability. The demand for natural antioxidants recently has increased because of questions about the long-term safety and negative consumer perception of synthetic antioxidants (**Fig. 2.10**) such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT).

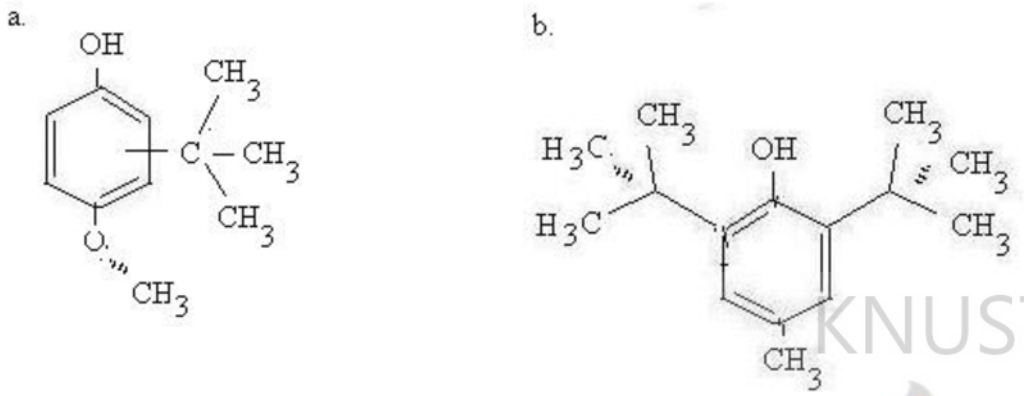


Fig. 2.10 Chemical structures of synthetic antioxidants (a) butylated hydroxyanisol (BHA), and (b) butylated hydroxytoluene (BHT) (modified from chemistry.about.com)

It has been suggested that antioxidants may amend cellular oxidative status and prevent biologically significant molecules such as DNA, proteins, and membrane lipids from oxidative damage and as a result lessen the risk of several chronic diseases including cancer and cardiovascular disease (Zhou and others, 2005). The core dietary antioxidants are vitamins A, C and E, carotenoids, terpenes, and polyphenols, including flavonoids (Stanner and others

2004). A sufficient ingestion of natural antioxidants in food is therefore of great consequence for the defense of macromolecules against oxidative damage (Halliwell and Gutteridge, 1999 and Wallace, 1997).

According to Stratil (2006), the cells most frequently damaged by oxidative stress are unsaturated fatty acids in lipids, cholesterol, different functional polypeptides and proteins, and nucleic acids. Mechanisms of antioxidants consist of free radical quenching, transition metal chelating, reducing peroxide, and simulation of *in vivo* antioxidative enzyme activities (Zhou and Liangli, 2005). In living systems, the antioxidants may elevate the levels of endogenous defenses. The action of antioxidants in foods and biological systems is reliant on the systems' composition, interfacial phenomena, and partitioning properties of the antioxidants between lipid and aqueous phases (Diaz-Reinoso and others 2006). Therefore, in addition to their long term safety and capacity to improve food quality and stability, natural antioxidants may also act as nutraceuticals to terminate free radical chain reactions in biological systems and therefore may provide additional health benefits to consumers.

2.2.1 Some Natural Antioxidants

Most natural antioxidants are from plant sources. Examples are Vitamin E, Vitamin C, beta carotene, and tannins.

Alpha Tocopherol (Vitamin E)

Vitamin E is an indispensable nutrient that functions as an antioxidant in the human body. It is essential for the reason that the body cannot produce its own vitamin E and thus it must be supplied by foods and supplements. Tocopherols are present in oils, nuts, seeds, grains, and leafy vegetables (Miller and Britigan, 1997).

Absorption is believed to be associated with intestinal fat absorption, in which only 40% of the ingested tocopherol is absorbed. Vitamin E (**Fig. 2.11**) was shown to accumulate in adipose fat tissue (Halliwell and Gutteridge, 1992 and Borg, 1993).

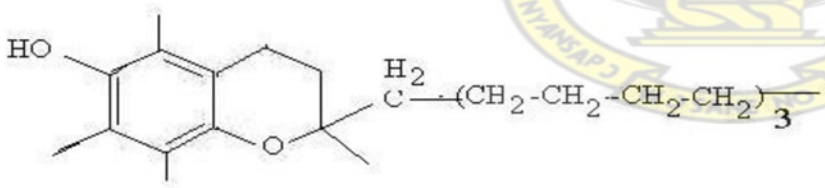


Fig. 2.11 Chemical structure of Vitamin E (modified from www.benbest.com)

Phospholipids of the mitochondria, endoplasmic reticulum, and plasma membranes

possess affinities for alpha tocopherol, and the vitamin tends to concentrate in these sites. Hence, the main function of vitamin E is to prevent the peroxidation of membrane phospholipids, and to avoid cell membrane damage through its antioxidant action. The lipophilic character of tocopherol makes it possible to be located in the interior of the cell membrane bilayers (Halliwell and Gutteridge, 1992 and Borg, 1993).

Ascorbic Acid (Vitamin C)

Ascorbic acid is a water-soluble chain breaking antioxidant that scavenges free radicals and reactive oxygen molecules, which are produced during metabolic pathways of detoxification (Block and Menkes, 1989).

Vitamin C (**Fig. 2.12**) also contributes to the regeneration of membrane-bound oxidized vitamin E. It will react with the alpha-tocopheroxyl radical, resulting in the generation of tocopherol in this process itself being oxidized to dehydroascorbic acid (Ward and Peters, 1995).

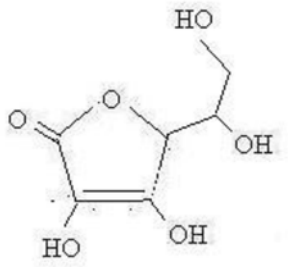


Fig. 2.12 Chemical structure of Vitamin C (modified from www.gma5.com)

Ascorbic acid is present in citrus fruits, potatoes, tomatoes, and green leafy vegetables. Humans must acquire ascorbic acid from dietary sources due to the absence of the enzyme L-gulacolactone oxidase which produces ascorbic acid (Ensimnger and others, 1991).

Beta Carotene

Beta-carotene's capacity to quench singlet oxygen, scavenge free radicals, and protect the cell membrane lipids from the destructive effects of oxidative degradation is the way it functions as an antioxidant. The quenching involves a physical reaction in which the energy of the excited oxygen is transferred to the carotenoid, forming an excited state molecule (Krinsky, 1993).

Precursors of vitamin A, carotenoids (**Fig. 2.13**) are pigmented micronutrients present in fruits and vegetables.

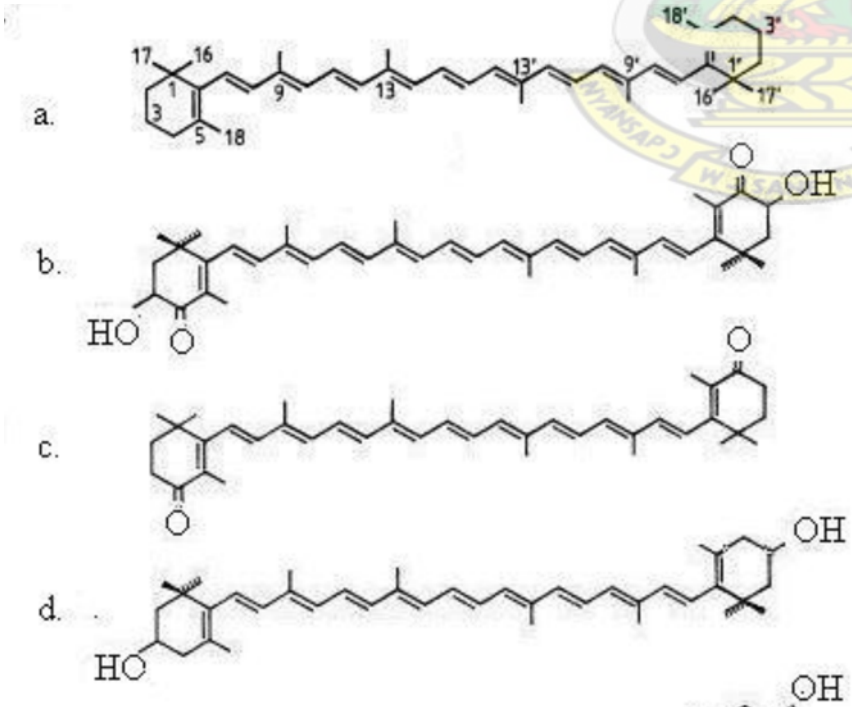


Fig.2.13 Chemical structures of carotenoids: (a)-β-Carotene, (b) astaxanthin, (c) canthaxanthin, (d)- zeaxanthin, (e)-lutein, (f) cryptoxanthin (modified from Straub, 1987)

According to Prior and others (2005), in relation to phenolics, carotenoids are not particularly good quenchers of peroxyl radicals but are exceptional in quenching singlet oxygen, at which most other phenolics are relatively ineffective.

2.2.2 Reactive Oxygen Species (ROS) Or Oxidants

In living organisms, reactive oxygen species (ROS) can be formed in different ways. Normal aerobic respiration could stimulate polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells (Miller and Britigan, 1997). Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides (Miller and Britigan, 1997). ROS can cause lipid peroxidation in foods, which leads to the deterioration of the food and can induce oxidative damage on biomolecules like lipids, nucleic acids, proteins, and carbohydrates. This damage causes ageing, cancer, and many other diseases. ROS have been implicated in more than 100 diseases, including, heart disease, stroke, arteriosclerosis, diabetes, and cancer (Oboh & Rocha, 2008).

Toxicity of the selected oxidants in biological systems

Bulger and Helton (1998) define oxidative stress as the state in which the level of toxic reactive oxygen intermediates (ROI) overcomes the endogenous antioxidant defenses of its host. This results in a surplus of free radicals, which can react with cellular lipids, proteins, and nucleic acids leading to damage

and eventual organ dysfunction. Free radicals play a vital role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by neutrophil granulocytes. The peroxyl radical is the most common free radical in human biology, but the hydroxyl radical, singlet oxygen, superoxide radical, and reactive nitrogen species all are present in biological systems (Wu and others, 2004). They result from molecular oxygen under reducing conditions, because of their reactivity. These same free radicals can have a role in unwanted side reactions causing cell damage. Since free radicals are essential for life, the body has a number of mechanisms to reduce free radical induced damage and to restore damage that does occur to living organisms such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Campos, 2003 and Pinzino, 1999).

The addition of one electron to O_2 yields the superoxide radical ($^{\cdot}O_2^-$) which at physiologic pH rapidly reduces itself ($k' \sim 2 \times 10^5\ M^{-1}\ s^{-1}$) to form the divalent oxygen reduction product, hydrogen peroxide (H_2O_2). Trivalent oxygen reduction *in vitro* occurs via the reaction of H_2O_2 with $^{\cdot}O_2^-$ to produce the hydroxyl radical ($^{\cdot}OH$). However, at physiologic pH, this reaction is of little biologic importance unless a transition metal catalyst (e.g., Fe^{3+}) is present to enhance the reaction rate, yielding $^{\cdot}OH$ via the Haber-Weiss reaction. Besides $^{\cdot}OH$ formation, interactions between H_2O_2 and iron chelates may also lead to the production of the reactive iron peroxocomplex and ferryl ion (Miller and Britigan, 1997). Recently, intense investigation has been directed at another oxidant species, nitric oxide ($NO^{\cdot}$). $NO^{\cdot}$ is not a classic product of O_2 reduction; instead its formation in mammalian cells is dependent on a group of enzymes termed nitric oxide synthases (NOS). These enzymes oxidize L-arginine to L-citrulline and $NO^{\cdot}$.

Below is a summary of some chemical reactions that lead to *in vivo* generation of reactive oxygen species (Miller and Britigan, 1997).

Reaction **Formula (X=halide)**

Haber-Weiss reaction ..... $^{\cdot}O_2^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$ (Metal Reduction)

$H_2O_2 + Fe^{3+} \longrightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$ (Fenton Reaction)

$^{\cdot}O_2^- + H_2O_2 \longrightarrow {}^{\cdot}OH + OH^- + O_2$

2.2.3 Reaction Mechanism of Antioxidants against Free Radicals

There are two reaction mechanisms in which antioxidants can deactivate radicals. The first of these methods is the single electron transfer (SET) assay which detects the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals (Wright *et*. *al*., 2001). According to Prior (2005), SET reactions are usually slow and can equire a lengthy time to reach completion, so the antioxidant capacity calculations are based on percent decrease in product rather than kinetics**.** The second method is the hydrogen atom transfer (HAT), which measures the antioxidant's ability to quench free radicals by hydrogen donation. HAT reactions are solvent and pH independent and are usually quite rapid. The presence of reducing agents, including metals, is a complication in HAT assays and can lead to erroneously high apparent reactivity (Prior *et*. *al*, 2005). There are methods utilizing both HAT and SET mechanisms. The Trolox Equivalent Antioxidant Capacity (TEAC) and DPPH assays are usually classified as SET reactions,

however these two indicator radicals in fact may be neutralized either by direct reduction via electron transfers or by radical quenching via HAT (Jimenez *et*. *al*., 2004).

2.2.4 Antioxidant Assessment Methods

Most natural antioxidants are multifunctional in complex heterogeneous foods and hence their antioxidant activity cannot be assessed by any one method (Frankel and Meyer, 2000 and Sanchez-Moreno, 2002). No single assay will accurately reflect all of the radical foundations or all antioxidants in a mixed or

complex system, and that there are no simple universal methods by which antioxidant capacity can be measured accurately and quantitatively (Prior and others, 2005). Total Antioxidant Capacity Assay, Reducing Power Assay, DPPH Assay and Total Phenol Content Assay are among the most commonly used antioxidant methods with modifications for the evaluation of antioxidant capacities or potentials of plant materials (Amarowicz *et al.,* 2000).

2.2.4.1 DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Assay

DPPH• radical is one of a few stable and commercially available organic nitrogen radicals bearing no similarity to the highly reactive and transient peroxylradicals involved in various oxidative reactions *in vivo* (Huang, 2005 and Wu, 2004).

According to Hseu *et al.,* (2008) the model of scavenging the stable DPPH radical is extensively used for relatively rapid evaluation of antioxidant activities compared to other models.

Principle

This assay is based on the measurement of the reducing ability of antioxidants toward DPPH•. The reducing ability can be evaluated by electron spin resonance or by measuring the decrease of its absorbance. The measurement of the loss of DPPH• colour at 517 nm following the reaction with test compounds is what the antioxidant assays are based on (Prior, 2005). Thus, when DPPH• radical accepts an electron or hydrogen in the presence of a suitable free radical scavenger or antioxidant compound (reducing agent), it changes to its reduced DPPH form (**Fig. 2.14**). The changes in colour from deep violet to light yellow (Residual DPPH) is then measured at 517 nm on a UV/visible light spectrophotometer. The absorbance decreases with increasing free

radical scavenging ability of the antioxidant compound. The % DPPH scavenging effect or % Radical Scavenging Acitivity (% RSA) of the antioxidant compound is calculated using the equation below:

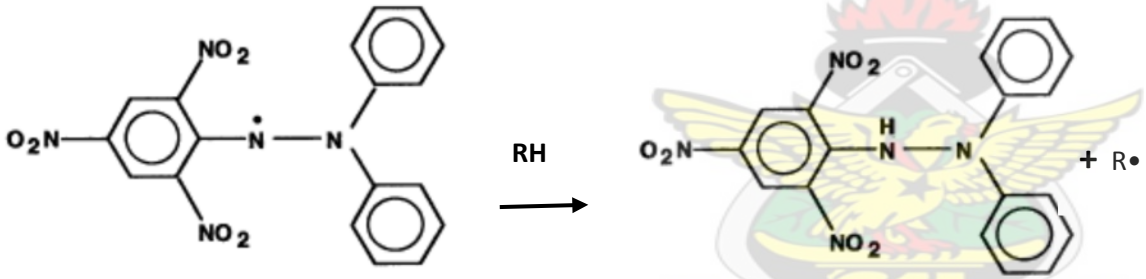


Figure 2.14 (a) Diphenylpicrylhydrazyl (b) Diphenylpicrylhydrazine

(free radical) (nonradical)

Using the DPPH assay has its advantages. It is simple, rapid and needs only a UV-Visible spectrophotometer to carry out. However, it has many disadvantages. Because DPPH is both a radical probe and an oxidant, the assay is not a competitive reaction. DPPH color can be lost due to radical reaction (HAT) or reduction (SET) as well as unrelated reactions, and steric accessibility is a major factor for determining results of the reaction (Prior, 2005).

2.2.4.2 Folin-Ciocalteau or Total Phenol Content Assay

The Folin-Ciocalteu (F-C) method has for many years been used as a means to determine total phenolics in natural products (Prior, 2005). The reaction that takes place is an oxidation/reduction and hence F-C is also considered an antioxidant capacity method. This assay has many variations.

Different reagent concentrations and timing of additions and incubation are frequently varied (Prior, 2005). Also, many studies show the recommended reference standard (gallic acid) being replaced with tannic acid equivalents, caffeic equivalents, vanillic acid equivalents and catechin equivalents, among others. Phenolic compounds can be found in flavonoides, phenolic acids, hydroxycinnamic acid derivatives and lignans.

Principle

Phosphomolybdate-phosphotungstate salts of Folin-Ciocalteu's reagent are reduced by phenolic compounds in alkaline medium giving a blue colouration. The intensity of the blue colouration is then quantified spectrophotometrically at 760 nm. Absorbance increases with increasing phenol content (Prior, 2005).

2.2.4.3 Reducing Power Assay

Principle

The assay depends upon the ability of a test compound to reduce Fe^{3+} to Fe^{2+}. The resultant Fe^{2+} then reacts with ferricyanide ion to form a Prussian blue Iron ferriccyanide complex with maximum absorbance at 700 nm. The greater the reducing power, the greater the intensity of the blue complex and the higher the absorbance (Oyaizu, 1986).

The equations of the reactions are as follows:

Fe^{3+} to Fe^{2+} …… (1)

$K_3 Fe(CN)_6 (aq) + Fe^{2+} (aq) \longrightarrow KFe[Fe(CN)_6] + 2K^+ (aq)$ ……..(2)

2.2.4.4 Total Antioxidant Capacity (TAC) Assay

The TAC Assay is based on the reduction of Molybdate (VI) to Molybdate (V) by the antioxidant compound and subsequent formation of green Phosphate/Molybdate (V) complex at acid pH. The greater the antioxidant capacity, the greater the intensity of the green colour and the higher the absorbance (Prieto *et al.,* 1999).

2.3 Some Phytochemicals

A brief description of the tested phytochemicals and their medicinal properties are reviewed below.

2.3.1 Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are

used as medications and recreational drugs. Examples are the stimulants such as cocaine, caffeine and nicotine; analgesics such as morphine and antimalarials such as quinine.

2.3.2 Saponins

Saponins are a class of chemical compounds found in abundance in various plant species. Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by them being composed of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. Saponins are recommended as dietary supplements and nutriceuticals. They are also used as adjuvants in the production of vaccines.

2.3.3 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of an unripened fruit. The anti-inflammatory effect of tannins helps control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Tannins are also reported to heal burns and stop bleeding. Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhoea, dysentery, fatigue and skin ulcers. Tannins have shown potential antiviral, antibacterial, and antiparasitic effects. Tannins have also been studied for their potential effects against cancer through different mechanisms.

2.3.4 General glycosides

Glycosides are certain molecules in which a sugar part is bound to some other part. Glycosides play numerous important roles in living organisms. Many plants store important chemicals in the form of inactive glycosides; which can be activated with water and an enzyme. This causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. Salicin, an alcoholic glycosides is converted in the body into salicylic acid, which is closely related to aspirin and has analgesic, antipyretic and anti-inflammatory effects. Anthraquinone glycosides are reported to have a laxative effect. Some cyanogenic glycosides are reported to have anti-cancer properties.

2.3.5 Flavonoids

Flavonoids are also commonly referred to as bioflavonoids as most flavonoids are biological in origin. Flavonoids are the most common group of polyphenolic compounds in the human diets and are found mainly in plants. The huge increase in antioxidant capacity of blood seen after the consumption of flavonoid-rich foods is reported to be due to increased uric acid levels that result from expelling flavonoids from the body. The process of getting rid of unwanted compounds induces the so-called Phase II enzymes that also help eliminate mutagens and carcinogens, and therefore flavonoids are of value in cancer prevention. Flavonoids are also reported to induce mechanisms that help kill cancer cells and inhibit tumuor invasion. Epicatechin found in cocoa is reported to improve blood flow and thus seems good for cardiac health.

2.4 Antimicrobial Activity

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans, as well as destroying viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are anti-microbial substances used on non-living objects.

However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to most antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients-those in the hospital to recover quickly. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found. Thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means.

2.4.1 Antibiotics

Antibiotics are generally used to treat bacterial infections. Since 1980, the introduction of new antimicrobial agents for clinical use has declined. Paralleled to this there has been an alarming increase in bacterial resistance to existing antibiotic agents. Antibiotics are among the most commonly used drugs. However, antibiotics are also among the drugs commonly misused by physicians, e.g. usage of antibiotic agents in viral respiratory track infection. The inevitable consequence of widespread and injudicious use of antibiotics has been the emergence of antibiotic-resistant pathogens, resulting in the emergence of a serious threat to global public health.

2.4.2 Antivirals

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host, and therefore, can be used to treat infections. Antiviral drugs work by inhibiting the virus ether before it enters the cell, stopping it from reproducing, or in some cases preventing it from exiting the cell. However, like antibiotics, viruses may evolve to resist antiviral drugs.

2.4.3 Antifungals

An antifungal drug is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush) among other diseases. Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host.

2.4.4 Antiparasitics

Antiparasitics are a class of medications which are indicated for the treatment of infection by parasites such as nematodes, cestodes, trematodes, infectious protozoa, and amoebas.

2.4.5 Non-pharmaceutical antimicrobials

A wide range of chemical and natural compounds are used as antimicrobials. Organic acids such as lactic acid, citric acid and their salts are widely used as antimicrobials in food products.

Traditional healers have long used plants to prevent or cure infectious disease. Large numbers of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal.

2.4.6 Gram-Positive Bacteria and Gram-Negative Bacteria

Gram-positive bacteria are bacteria that are able to retain the violet stain because of the high amount of peptidoglycan in the cell wall. The presence of teichoic acids (and lipoteichoic acids) in their cell walls act as chelating agents. Examples are *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus suis*.

Gram-negative bacteria are those bacteria that cannot retain the crystal violet stain but which take up the counter stain (safranin or funchsin) and appearing red or pink. Examples of Gram-negative bacteria are *Escherichia coli, Pseudomonas aeruginosa, K. Pneumonia* and *S. typhimurium.*

2.4.7 Properties of the Selected Bacterial Strains

The nature, pathogenesis and antibiotic resistance of the selected bacterial strains are reviewed below.

2.4.7.1 *Bacillus subtilis*

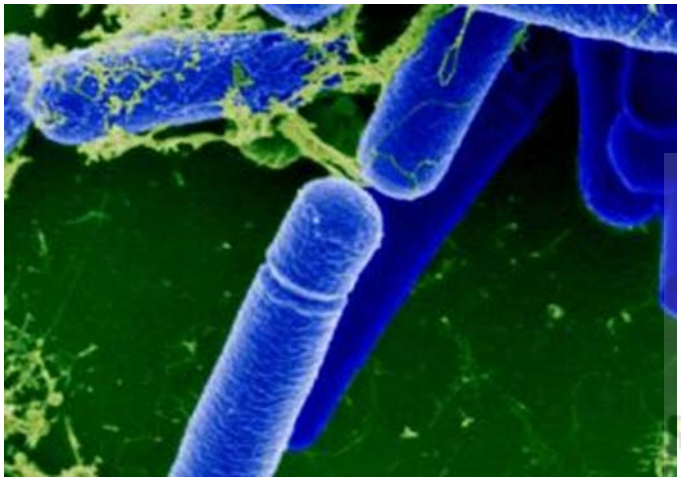


Fig. 2.15 Micrograph of stained *Bacillus subtilis*

Bacillus subtilis, also known as the hay bacillus or grass bacillus is a gram-positive, catalase-positive bacterium commonly found in soils. It is rod-shaped and has the ability to form a tough, protective endospore which allows it to tolerate extreme environmental conditions such as heat, acid, and salt (Nakano and Zuber, 1998).

Pathogenesis

Bacillus subtilis is not considered a human pathogen; however, it may contaminate food and but rarely causes food poisoning. *Bacillus subtilis* spores can survive the extreme heating that is often used to cook food and it is responsible for causing *ropiness* (a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides) in spoiled bread dough (Nakano and Zuber, 1998).

2.4.7.2 *Escherichia coli*

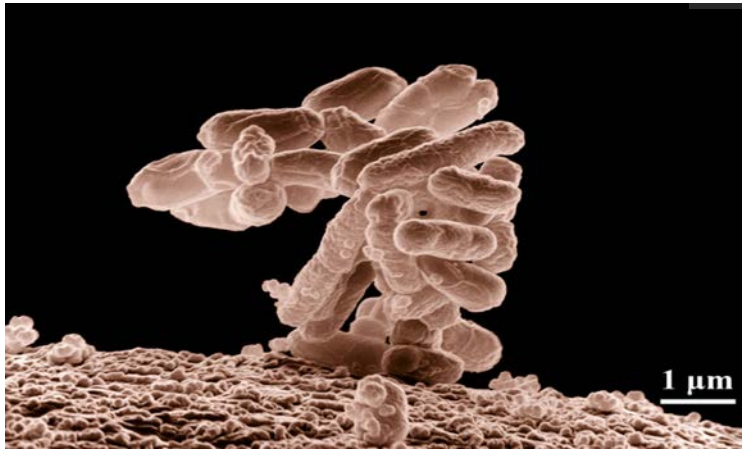


Fig. 2.16 Electron micrograph of a cluster of *E. coli* bacteria

Escherichia coli is a gram negative, facultative anaerobic and non-sporulating bacterium commonly found in the lower intestine of warm-blooded organisms (endotherms). Its cells are typically rod-shaped. *Escherichia coli* also has the ability to survive to survive for brief periods outside the body (Vogt *et al*., 2005).

Pathogenesis

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with good or water or with individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative organism of the human gastrointestinal tract. Most *E. coli* strains are harmless, but some can cause serious food poisoning in humans (Vogt *et al*., 2005).

Antibiotic Resistance

E. coli strains are resistant to many antibiotics that are effective against gram-positive bacteria (Vogt *et* al., 2005).

2.4.7.3 *Pseudomonas aureginosa*

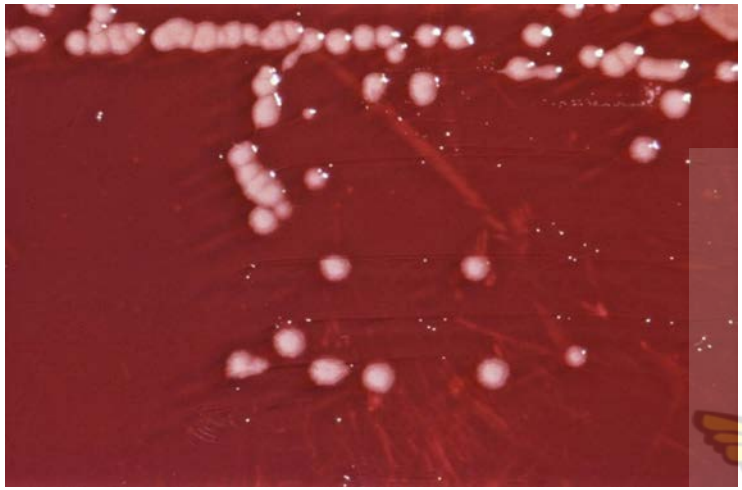


Fig. 2.17 Photograph of *Pseudomonas aureginosa*

Pseudomonas aureginosa is a gram-negative, aerobic, and rod-shaped bacterium. It is an opportunistic human, animal, and plant pathogen. It is found in soils, water, and most man-made environments with little oxygen (Worlitzsch *et al*., 2002)

Pathogenesis

Pseudomonas aureginosa typically infects the pulmonary tract, urinary tract, burns, wounds and also causes other blood infections. It induces symptoms of soft rot in *Arabidopis thaliana* (Thale cress) and *Lactuca sativa* (Lettuce).

Antibiotic Resistance

Pseudomonas aureginosa has low antibiotic susceptibility attributable to a concerted action of multi-drug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. *P. aureginosa* also easily develops acquired resistance either by mutation in chromosomally-encoded genes (Worlitzsch *et al*., 2002).

2.4.7.4 *Staphyloccocus aureus*

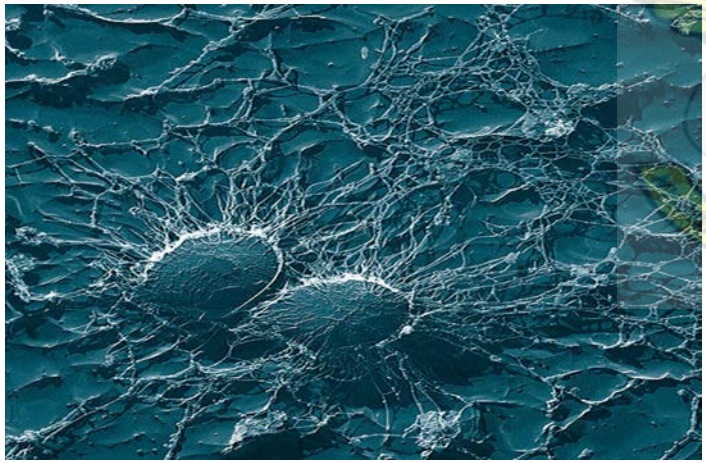


Fig. 2.18 Electron micrograph of stained *Staphyloccocus aureus*

Staphylococcus aureus, also known as golden staph is a spherical, gram-positive, facultatively anaerobic bacterium which appears as grape-like clusters when viewed through a microscope and has large, round, and golden-yellow colonies. It can survive for some hours on dry environmental surfaces (Kluytmans *et al*., 1999).

Pathogenesis

S. aureus is frequently found in the nose skin of a person. It can cause a range of illnesses from minor skin infections, such as pimples, boils, furuncles, carbuncles, scalded skin to life-threatening diseases such as pneumonia, meningitis and endocarditis among others (Kluytmans *et al*., 1999).

Antibiotic Resistance

It has a strong resistance to penincilin and requires a combination therapy with gentamicin to treat serious infections such as endocarditis (Kluytmans *et al*., 1999).

2.4.8 Antimicrobial Evaluation Method - Disc Diffusion Agar Method

The well-known conventional method employed to study antimicrobial activity is the disc diffusion agar method.

Principle

The principle behind this technique is that, when a disc of paper saturated with a sample (or antibiotic) is placed on a nutrient agar previously inoculated with the test microorganism in a Petri disc, the disc imbibes moist content from the agar and creates a gradient. As a result, the sample diffuses radially outward through the agar until its concentration lowers. A clear zone is observed if the sample inhibits the growth of the test microorganism. The extent of inhibition is characteristic of the concentration, solubility, and diffusion rate of the sample. The broader the zone of microbial growth inhibition, the susceptible (less resistance) the test microorganism is (Mousumi Debnath, 2008).

Chapter Three

3 MATERIALS AND METHODS

In this chapter, detailed description of the materials such as equipment, chemical reagents, and samples of selected leafy vegetables used for experimental analysis as well as the experimental and statistical methods used for the study are presented.

3.1 Materials

The chemical reagents and equipment used in the study, sampling, and preparation of the selected leafy vegetables are described below.

3.1.1 Equipment

Soxhlet set-up (500 mL)

UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050)

Centrifuge (Sanyo MSE, MISTRAL 3000E, UK)

Incubator (Gallenkamp model IH, UK).

Rotary evaporator apparatus (BUCHI Rotavapor, R-144)

3.1.2 Chemical Reagents

The entire chemical reagents used were of analytical-reagent grade.

DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate)	Sigma-Aldrich Chemie: Steinheim, Germany
Folin-Ciocalteu Phenol Reagent	Sigma-Aldrich Chemie: Steinheim, Germany
n-Propyl gallate	Sigma-Aldrich Chemie: Steinheim, Germany
Anhydrous Sodium Carbonate	Sigma-Aldrich Chemie: Steinheim, Germany
Trichloroacetic Acid	Sigma-Aldrich Chemie: Steinheim, Germany
Sodium Acetate	Sigma-Aldrich Chemie: Steinheim, Germany

Ascorbic acid	BDH: Poole, England
Sulphuric acid	BDH: Poole, England
Sodium phosphate	BDH: Poole, England
Potassium ferricyanide	BDH: Poole, England
Ferric chloride	BDH: Poole, England
Sodium dihydrogen phosphate monohydrate	BDH: Poole, England
Disodium hydrogen phosphate heptahydrate	BDH: Poole, England
Tannic Acid	BDH: Poole, England
Picric acid	BDH: Poole, England
Hydrochloric acid	BDH: Poole, England
Fehling's solution	BDH: Poole, England
Sodium hydroxide	BDH: Poole, England

3.1.3 Sampling of the Selected Leafy Vegetables

The shoots of the selected leafy vegetables except two were harvested from vegetable farm at Emena, a suburb of Kumasi from September 2008 to October 2008. However, the shoots of *L. aestuans* was collected from a weed-infested area near Ayeduase chief's palace, and that of *I. batatas* from Kumawu-Bodomase – all suburbs of Kumasi within the sampling period of the study. The eight selected leafy vegetables are:

Ipomoea batatas (L.) Lam.

Hibiscus sabdariffa (Linn.)

Laportea aestuans (Linn.) Chev.

Amaranthus cruentus (Linn.) Thell.

Or Amaranthus *hybridus* (Linn.)

Xanthosoma sagittifolium (L.) Schott

Solanum macrocarpon (Linn.)

Talinum triangulare (Jacq.) Wild.

Corchorus olitorius (Linn.)

3.1.3.1 Preparation of Vegetable Samples

The freshly cut leaves of the vegetable samples were washed with distilled water and air-dried with active ventilation at ambient temperature for 2 months after which the leaves were pounded into powdered form using mortar, labeled, and kept in refrigerator for future analysis.

3.2 Methods

Below is a description of the methods used for the study on the selected leafy vegetables namely; extraction, screening for phytoconstituents, antioxidant capacity determination assays, and antibacterial activity assay.

3.2.1 Extraction

50 g of the air-dried powdered leaves of the vegetable samples were extracted with 500 mL methanol (99%), 500 mL distilled water-ethanol (98%) mixture (1:1) for 10 hours in soxhlet apparatus. The extracts were evaporated under reduced pressure in a rotary evaporator apparatus (BUCHI Rotavapor, R-144) at approximately 40°C. The concentrated extracts were kept in a desiccator until analyses. The weight percentage yields of the samples were then calculated.

3.2.2 Screening for Phytoconstituents

The following secondary metabolites: saponins, alkaloids, tannins, general glycosides, and flavonoids were screened as described in the following subsections using the method described in Trease and Evans' Pharmacognosy Book (Evans, 1989).

3.2.2.1 Test for saponins

20 mg of the powdered plant sample or extract was moistened with 10 mL of distilled water and boiled over a water bath for 3 mins. The hot content was filtered and the filtrate shaken vigorously. Persistent froth (foam) is an indicative of saponins

3.2.2.2 Test for general glycosides

20 mg of the sample or extract was put into 2 separate beakers and dried at 60^{o}C.

To one beaker: 5 mL of dil. H_2S0_4 was added, boiled, filtered, and cooled. NaOH solution was added to the filtrate and heated with Fehling's solution (A and B) for 3mins. Formation of Reddish – brown ppt. indicates the presence of glycosides.

To the other beaker, 5 mL of distilled water was added, boiled for 3 mins, filtrated, and cooled. The filtrate was alkalinised and heated with Fehling's solution for 3 mins. Absence of precipitate indicates the presence of glycosides.

3.2.2.3 Test for flavonoids

Magnesium turnings were added to alcoholic solution of the extract sample (20 mg). Conc. HCl was dripped afterwards. Brick-red colouration indicates the presence of flavonoids.

3.2.2.4 Test for alkaloids

10 ml of 1% HCl was added to 20 mg extract sample and left to stand for 3 minutes with occasional stirring. The acidified solution was filtered. Hager's regent (saturated aqueous solution of picric acid) was added to 2 mL of the filtrate. Formation of yellow precipitate indicates the presence of alkaloids.

3.2.2.5 Test for Tannins

Test for tannins was carried by adding 6 mL of distilled water to 5 mg of the extract followed by 2 mL ferric chloride solution. The content of the test tube was observed for reddish-black colour which is an indicative of the presence of tannins.

3.2.3 Antioxidant Capacity Determination Assays

The antioxidant potentials of the selected leafy vegetables were evaluated using different antioxidant capacity determination assays such as DPPH Radical Scavenging Assay, Total Phenol Content Assay, Reducing Power Assay and Total Antioxidant Capacity Assay.

3.2.3.1 DPPH Radical Scavenging Assay

Radical scavenging activity of the extracts from the vegetable samples against stable DPPH• radical was determined spectrophotometrically. Radical scavenging activity of the leaf extracts was measured by slightly modified method of Govindarajan *et al*., (2003). The leaf extract (0.1, 0.3, 1.0, 3.0 mg/mL in

methanol) was compared with n-propyl gallate (0.01, 0.03, 0.1, 0.3 mg/mL in methanol) as a reference free radical scavenger. Briefly, 10 ml of the extract was centrifuged at 3000xg using a centrifuge (Sanyo MSE, MISTRAL 3000E, UK) for 10 minutes and the supernatant collected. The supernatant of the extract (1 mL) was added to 3 mL methanolic solution of DPPH (20 mg/L) in a test tube. The reaction mixture was kept at 25^{o}C for 1 hour in an incubator (Gallenkamp model IH, UK). The absorbance of the residual DPPH solution was determined at 517 nm in a UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Methanol (1 mL) was added to 3 mL DPPH solution, incubated at 25^{o}C for 1 hour and used as control. Methanol was used as the blank. The measurements were taken in triplicate. The results were expressed as a plot of % Radical Scavenging Activity against the concentration of the leaf extracts and the EC_{50} determined.

3.2.3.2 Total Phenol Content Assay

The total phenol content ofleaf extracts from the vegetable samples (0.1, 0.3, 1.0 and 3.0 mg/mL) was quantitatively determined by colorimetric assay using Folin-Ciocalteu's reagent (Singleton, 1977) with slight modifications. Tannic acid (0.01, 0.03, 0.1 and 0.3 mg/mL) was used as the reference drug. The leaf extract (1 mL) was added to 1 mL of F-C reagent (diluted five folds in distilled water) in a test tube. The content of the test tube was then mixed and allowed to stand for five minutes at 25^{o}C in an incubator (Gallenkamp model IH, UK). 1 mL of 2 % sodium bicarbonate solution was added to the mixture. The reaction mixture was then incubated at 25^{o}C for 2 hours. The reaction mixture after the incubation period was centrifuged at 3000xg for 10 minutes to get a clear supernatant. The absorbance of the supernatant was then measured at 760 nm using the UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water (1 mL) was

added to 1 mL F-C reagent (diluted five folds in distilled water) as blank and processed in the same way as done for the test extracts and reference drug and used as blank. The measurements were done in triplicate. The content of total phenol content was expressed as Tannic Acid Equivalent (TAE) using the GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

3.2.3.3 Reducing Power Assay

The reducing potential of the extracts (0.1, 0.3, 1.0 and 3.0 mg/mL in methanol) was determined using the method described by Oyaizu (1986), with slight modifications using n-propyl gallate as a reference antioxidant drug. The extract/drug (1 mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% Potassium ferricyanide solution in a test tube. The mixture was incubated at 50^{o}C for 20 minutes. Following this, 1.5 mL of 10% Trichloroacetic acid solution was added to the incubated mixture and centrifuged at 3000xg for 10 minutes using the centrifuge (Sanyo MSE, MISTRAL 3000E, UK). 2.5 mL of the supernatant was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution ($FeCl_{3(aq)}$) in a test tube. The absorbance was then measured at 700 nm using the UV-visible spectrophotometer

(LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water was used in place of the test drug/extract and used as the blank. The absorbance measurements were taken in triplicates. Data was presented as concentration-absorbance curves and the EC_{50} (effective concentration that gives 50% of maximal response) was computed using the GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

3.2.3.4 Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity was evaluated using the method described by Prieto *et al*. (1999). Ascorbic acid was used as the standard antioxidant drug. 3 mL of the extract/standard drug (0.1, 0.3, 1 and 3 mg/mL) was placed in a test tube. 0.3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 95^{o}C

for 90 minutes. After the mixture has cooled to room temperature, the absorbance of each solution was measured in triplicates using the UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050) at 695 nm against a blank. The total antioxidant capacity was expressed as Ascorbic Acid Equivalents (AAE) using the GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

3.2.4 Antibacterial Activity Assay

The antimicrobial activity of the extracts from the vegetable samples was evaluated using the agar well diffusion method described by Mousumi Debnath (2008). Four pathogenic bacterial strains were tested against different concentrations (2.5, 5.0, 7.5, 10.0 mg/mL) of the extracts from the selected leafy vegetables. Certified strains of bacteria namely; *P. aeruginosa, E. coli*, *B. subtilis* and *S. aureus* were obtained from the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi-Ghana.

The prepared agar nutrient broth was inoculated with each of the bacterial strain on sterilized disc plates and grown for 24 hours at 37^{o}C. Wells were made on the cultured disc plates with a sterile cork borer of 4 mm diameter and the wells were impregnated with different extractconcentrations.The disc plates were incubated at 37°C for 24 hours. The zones of inhibition were measured at the end of the incubation period. Microbial growth was determined by measuring in triplicates the diameter of the zone of inhibition and the mean values were calculated.

3.2.5 Statistical Analysis

The EC_{50} (the concentration of agonist that gives a response half way between Bottom and Top) of the extracts and reference antioxidant compounds were analyzed using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

where, *X* is the logar [redacted] at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape. All the experimental data were analyzed statis [redacted] OVA) and "Bonferroni's Multiple Comparison Test" at 95% confidence interval using the software, GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA). Correlation coefficient (r) was used to determine the relationship between two variables (TAE and AAE). All the points on graphical representation of experimental values were expressed as mean±S.E.M. Differences in mean between paired results were accepted as significant at $P < 0.05$.

Chapter Four

4 RESULTS AND DISCUSSION

Summary of the experimental results or findings of the study: extraction yields, phytoconstituents of the selected leafy vegetables, antioxidant potentials and antibacterial activities of the extracts from the selected leafy vegetables (presented in tabular and graphical forms), are thoroughly analyzed and discussed.

4.1 Extraction Yields

The percentage yields of extracts from the selected leafy vegetables using methanol and hydro-ethanol (mixture of water and ethanol) as solvent systems for extraction are presented in **Table 4.1**.

Table 4.1 Methanolic and Hydro-Ethanolic Extraction yields (% $^{w}/_{w}$) of the Selected Leafy Vegetables

Selected Vegetables	(% $^{w}/_{w}$) Yield	
	Methanolic Extract (METE)	Hydro-Ethanolic Extract (HETE)
X. Sagittifolium	8.1	8.7
I. batatas	4.4	7.5
L. aestuans	4.2	5.9
T. triangulare	7.3	8.3
H.sabdariffa	6.2	7.5
C. olitorius	6.7	7.1
S. macrocarpon	7.4	10.3
A. cruentus	6.3	9.4

The extraction yields (**Table 4.1)** of the selected leafy vegetables shows that hydro-ethanol extraction was very effective since the percentage yields of hydro-ethanol extraction on all the leafy vegetable samples were higher that their respective methanol extraction yields. This marginal difference could be due to the polarity differences between the two solvent systems.

4.2 Phytoconstituents

The results of the phytochemical screening for alkaloids, tannins, flavonoids, general glycosides, and saponins are shown in **Table 4.2** (**a**) and (**b**).

Table 4.2 (**a**) and (**b**) Phytoconstituents of the Methanolic and Hydro-Ethanolic Extracts from the selected leafy vegetables

(a) Methanolic Extract (METE)					
	Phytochemicals				
Leafy Vegetable	**Alkaloids**	**Tannins**	**Flavonoids**	**General glycosides**	**Saponins**
X. sagittifolium	+	+	+	+	+
I. batatas	+	+	+	+	+
L. aestuans	+	+	+	+	+
T. triangulare	-	+	+	+	+
H. sabdariffa	-	+	+	+	+
C. olitorius	-	+	+	+	+

S. macrocarpon	+	+	+	+	+
A. cruentus	-	+	+	+	+

(b) Hydro-Ethanolic Extract (HETE)

Leafy Vegetable	**Phytochemicals**				
	Alkaloids	**Tannins**	**Flavonoids**	**General glycosides**	**Saponins**
X. sagittifolium	+	+	+	+	+
I. batatas	+	+	+	+	+
L. aestuans	+	+	+	+	+
T. triangulare	-	+	+	+	+
H. sabdariffa	-	+	+	+	+
C. olitorius	-	+	+	+	+
S. macrocarpon	+	+	+	+	+
A. cruentus	-	+	+	+	+

Results of the phytochemical screening indicate that the leaves of the selected leafy vegetables contain active chemical components namely: tannins, flavonoids, general glycosides, and saponins. All the selected leafy vegetables except *T. triangulare, H. sabdariffa, C. olitorius and A. cruentus* contain alkaloids.

4.3 Antioxidant Potentials

The results of the above-mentioned antioxidant capacity determination assays are presented (in tabular and graphical forms) and discussed below.

4.3.1 Total Phenol Content (TPC) Assay

The results of the total phenol content assay of the extracts from the selected leafy vegetables are expressed as Tannic Acid Equivalent (TAE) and are presented in **Fig. 4.1 (a)** and **(b)** below, **Appendix 2.1** (**a**) to (**h**) and **Table 4.15** to **Table 4.22 of Appendix 1.2**.

(a)

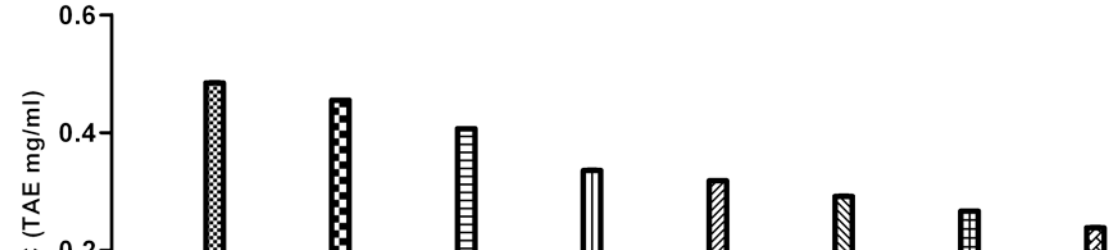


Fig. 4.1 TAE (mg/ml) of extracts from the selected vegetables: (a) METE **(b)** HETE

The total phenol content (TPC) in the METE and HETE from the selected leafy vegetables within the measured concentration range (0.1-3.0 mg/ml) decreases in the order *X. sagittifolium > I. batatas > L. aestuans > T. triangulare > H. sabdariffa >*

C. olitorius> S. macrocarpon> A. cruentus **[Fig. 4.1 (a) and (b)]**. However, Odukoya *et al.* (2007) reported the following order, *C. olitorius* (503.72 mg TAE 100 g^{-1}) >

A. cruentus (406.33 mg TAE 100 g^{-1}) > *S. macrocarpon* (190.07 mg TAE 100 g^{-1}) >

T. Triangular (21.83 mg TAE 100 g^{-1}). Salawu *et* al., (2006) also reported Total flavonoid as quercetin equivalent in the following order, *S. macrocarpon* (3.08 mg l^{-1}) > *C. olitorius* (1.24 mg l^{-1}) > *A. cruentus* (0.33 mg l^{-1}) >*T. triangular* (0.12 mg l^{-1}). The experimental results showed that at the highest extract concentration of 3.0 mg/ml (**Table 4.23, Appendix 1.2)**, *X. sagittifolium* had the highest phenol contents of 0.706 and 0.485 mg TAE ml^{-1} for the HETE and METE respectively while *A. cruentus* showed the least TPCs of 0.339 and 0.238 mg TAE ml^{-1} for the HETE and METE respectively. The results of the study also showed higher TPCs for all the HETE from the selected vegetables compared to the METE. The ANOVA table and Bonferroni's Multiple Comparison Test results showed no significance difference between the results obtained for METE from the selected leafy vegetables (**Table 4.77, Appendix 3.1**). Similar observation was also made of the HETE from the selected leafy vegetables (**Table 4.78, Appendix 3.1**).

Tannic acid is a high phenolic compound and was used as standard

(**Fig. 4.4**, **Appendix 2.1**).

Studies carried out by Manach *et al.,* (2004), Rice-Evans *et al.,* (1996) on dietary medicinal plants and edible leafy vegetables show that phenolic compounds inhibit oxidative stress due to their ability to readily undergo electron-donation reactions with oxidising agents such as ROS. The experimental results therefore suggest that the selected leafy vegetables possess antioxidant ability and can be used as dietary supplements of medicinal importance.

4.3.2 Total Antioxidant Capacity (TAC) Assay

The results of the total antioxidant capacity of the extracts from the selected leafy vegetables are expressed as Ascorbic Acid Equivalent (AAE) and presented in

Fig. 4.2 (**a**) and (**b**) below, **Appendix 2.2** (**a**) to (**h**) and **Table 4.33** to **Table 4.40** of **Appendix 1.4**.

(a)

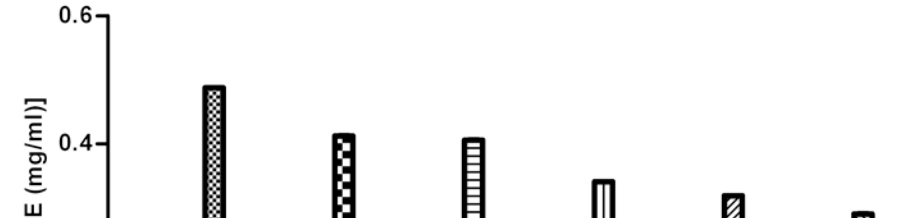


Fig. 4.2 AAE (mg/mL) of extracts from the selected vegetables: (a) METE **(b)** HETE

Total antioxidant capacity is a measure of the ability of substances extracted from food or herbal matrix to delay oxidation process in a controlled system (Miller and Rice-Evans, 1997; Pellegrini *et al.,* 2003). Total antioxidant capacity of the selected leafy vegetables was expressed as equivalents of ascorbic acid. From **Fig. 4.2 (a)** and **(b)**, TAC of the METE and HETE from the selected leafy vegetables within the measured concentration range (0.1-3.0 mg/mL) follows a decreasing order; *X. sagittifolium* >

I. batatas > *L. aestuans* > *T. triangulare* > *H. sabdariffa* > *C. olitorius*>

S. macrocarpon > *A. cruentus*. This trend was similar to that observed for TPC in the selected leafy vegetables. From **Table 4.41** (**Appendix 1.4**), the highest ascorbic acid equivalent was measured for *X. sagittifolium*; 0.488 and 0.681 mg AAE mL^{-1} on the METE and HETE respectively while *A. cruentus* showed the least AAE, 0.228 and 0.312 mg/ml on its METE and HETE respectively. However, Odukoya *et al*., (2007) reported the following order, *C. olitorius* (153.63 mg AA 100 g^{-1}) > *T. triangular* (116.35 mg AA 100 g^{-1}) > *A. cruentus* (52.17 mg AA 100 g^{-1}) > *S. macrocarpon* (38.11 mg AA 100 g^{-1}). Furthermore, Salawu *et* al., (2006) also reported total antioxidant as Gallic acid equivalent in the following order, *S. macrocarpon* (1.60 mg L^{-1}) >

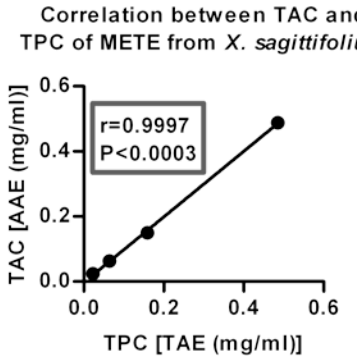
C. olitorius (1.10 mg L^{-1}) > *A. cruentus* (0.55 mg L^{-1}) >*T. triangular* (0.20 mg L^{-1}). The total antioxidant capacities of the HETE from the selected leafy vegetables were generally higher compared with their respective METE. Statistical analysis of the results obtained for the METE from the selected leafy vegetables indicated no significant differences. Similar observation was made for the HETE (**Table 4.79**, **Appendix 3.3** and **Table 4.80**, **Appendix 3.4)**.

Ascorbic acid was used as the reference antioxidant compound (**Fig. 4.5**, **Appendix 2.2)**. Diets high in vitamin C (ascorbic acid) has been associated with improved pulmonary function and reduction in cancer risk (Percival, 1998). Vitamin C is also reported to have neutralizing effect on hydrogen peroxide, hydroxyl, and superoxide radicals (Percival, 1998). The appreciable AAE equivalents of the extracts from the selected vegetables suggest their antiradical potential.

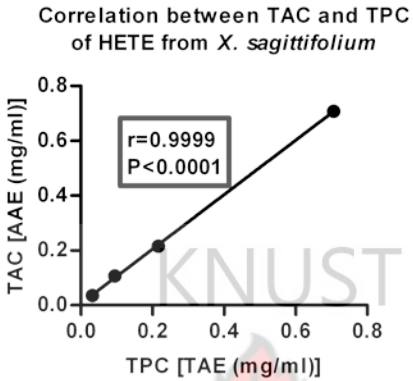
4.3.3 Correlation between Total Phenol Content (TPC) and Total Antioxidant Capacity (TAC)

The correlation between the experimental results of the TPC expressed as Tannic Acid Equivalent (TAE) and TAC expressed as Ascorbic Acid Equivalent (AAE) of the extracts from the selected vegetables are presented in **Fig. 4.3** (**a**) to (**p**).

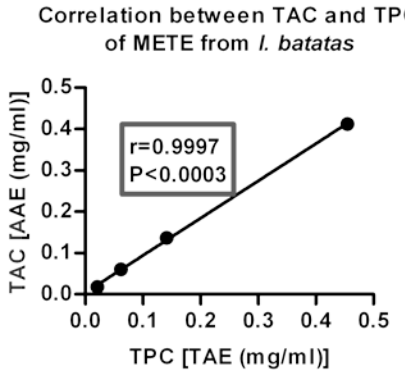
(a)



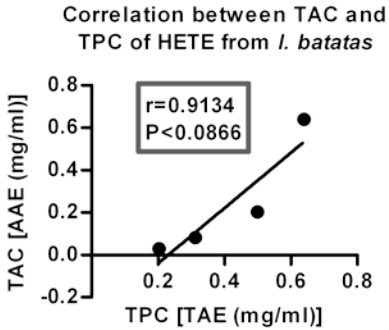
(b)



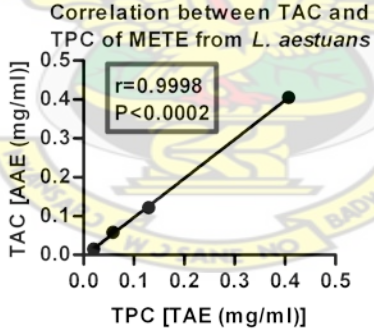
(c)



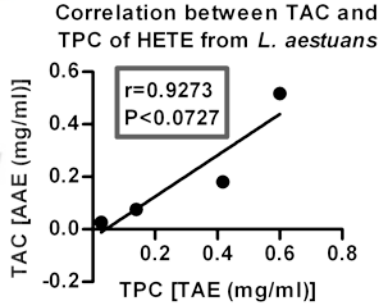
(d)



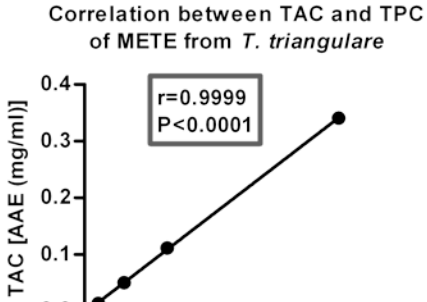
(e)



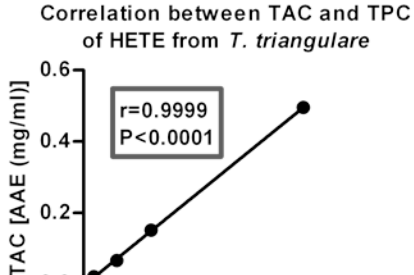
(f)



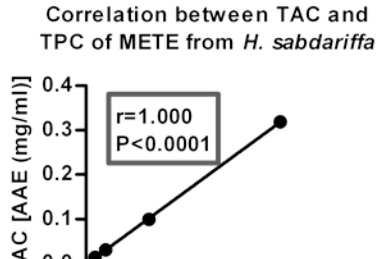
(g)



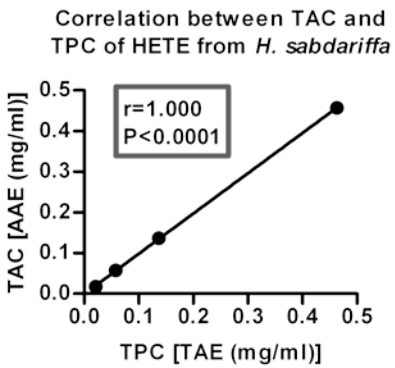
(h)



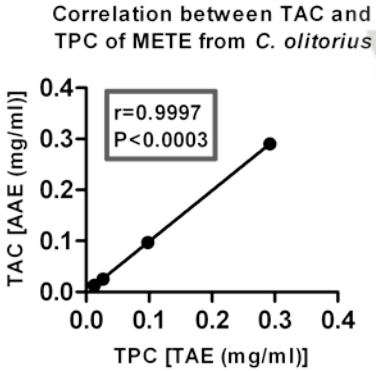
(i)



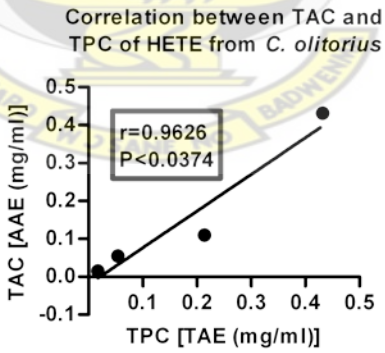
(j)



(k)



(l)



(m)

(n)

Correlation between TAC and

Fig. 4.3 (a) to **(p)**: Correlation between Total Phenol Content (TPC) and Total Antioxidant Capacity (TAC) in the METE and HETE from the selected vegetables

In the study, a high and positive correlation was observed between and TPC and TAC in both the METE and HETE from all the selected leafy vegetables **Fig. 4.3** (**a**) to (**p**). except in the case of *I. batatas* (P <0.0866) *and L. aestuans* (P <0.0727) where relatively poor correlation between TPC and TAC in their HETE were observed. The positive correlation between the TPC and TAC suggests that the phytoconstituents responsible for the total phenol content may also be responsible for the total antioxidant capacity of the vegetable extracts. Moreover, the TAE in all the selected leafy vegetables were higher than their respective AAE. This observation was consistent with the findings of Odukoya *et al.* (2007) except in the case of Odukoya *et al.* (2007), the TAE in *T. triangulare* (21.83 mg TAE 100 g^{-1}) was far lower than its AAE (116.35 mg AA

100 g^{-1}).

4.3.4 DPPH Radical Scavenging Assay

The experimental results for the Radical Scavenging Activities (RSA) of the METE and HETE extracts from the selected leafy vegetables against DPPH free radical are presented in **Table 4.3** below and **Appendix 2.3 (a) to (j)**.

Table 4.3 Radical Scavenging Activity (EC_{50}) of the methanolic and hydro-ethanolic extracts from the selected leafy vegetables against DPPH radical

	EC_{50} (mg/mL)	
Test Samples	**Methanolic Extract (METE)**	**Hydro-Ethanolic Extract (HETE)**
n-Propyl gallate	0.01513	0.01513

X. Sagittifolium	0.1294	0.1186
I. batatas	0.1746	0.1307
L. aestuans	0.2326	0.1586
T. triangulare	0.1656	0.1799
H. sabdariffa	0.1745	0.1212
C. olitorius	0.1635	0.1452
S. macrocarpon	0.1541	0.1380
A. cruentus	0.1364	0.1378

The results revealed that the METE and HETE from the selected vegetables exhibited appreciable ability to scavenge the stable $DPPH^{\bullet}$ free radical in a dose-dependent manner from 0.1-3.0 mg/ml.

The smaller the EC_{50} value the greater the Radical Scavenging Activity (RSA) of the extract. It could be deduced from **Table 4.3** that the RSA of the reference antioxidant compound, n-Propyl gallate showed approximately fifthteen times potency (EC_{50}, 0.01513 mg/ml) as compared with the values obtained all the extracts from the selected leafy vegetables.

From the **Table 4.3**, the order of RSA of the METE from the selected leafy vegetables was as follows: *X. sagittifolium* > *A. cruentus* > *S. macrocarpon* > *C. olitorius* >

T. triangulare > *H. sabdariffa* > *I. batatas* > *L. aestuans*.

This means that METE from *X. sagittifolium* showed the strongest radical scavenging activity against DPPH free radical (EC_{50}, 0.1294 mg/mL) and the least radical scavenging activity was exhibited by *L. aestuans* (EC_{50}, 0.2326 mg/mL).

The HETE from the selected leafy vegetables showed a different trend. The RSA order was as follows: *X. sagittifolium* > *H. sabdariffa* > *I. batatas* > *A. cruentus* >

S. macrocarpon > *C. olitorius* > *L. aestuans* > *T. triangulare*.

The strongest radical scavenging activity was again demonstrated by the HETE of *X. sagittifolium* (EC_{50}, 0.1186 mg/mL) and the HETE from *T. triangulare* showed the least RSA (EC_{50}, 0.1799 mg/mL).

Generally, the HETE from the selected leafy vegetables showed a high RSA than the METE from the leafy vegetables. Statistical analysis of the experimental results showed no significance differences between the EC_{50} values obtained for the METE from the selected leafy vegetables as well as for their HETE (**Appendices 3.11 and 3.12**).

The experimental results clearly show that the selected vegetables are very promising as natural antioxidants. This assertion is confirmed by earlier reports that tropical edible leafy vegetables have strong antioxidant activity (Akindahunsi and Salawu, 2005 a,b; Odukoya *et al.,* 2007 and Salawu *et* al., 2006).

4.3.6 Reducing Power Assay

The results of the ability of the METE and HETE extracts from the selected leafy vegetables to reduce F^{3+} to Fe^{2+} are presented in **Table 4.4** below and **Appendix 2.4 (a)** to **(j)**.

Table 4.4 F^{3+} Reducing Potencies (EC_{50}) of the methanolic and hydro-ethanolic extracts from the selected leafy vegetables

	EC_{50} (mg/mL)	
Test Samples	**Methanolic Extract (METE)**	**Hydro-Ethanolic Extract (HETE)**
n-Propyl gallate	0.2104	0.2104
X. sagittifolium	2.535	5.374

I. batatas	2.778	1.005
L. aestuans	1.150	0.6894
T. triangulare	4.518	4.091
H. sabdariffa	5.148	5.028
C. olitorius	3.615	14.12
S. macrocarpon	9.808	5.618
A. cruentus	14.80	10.68

As shown in **Fig. 4. (a)** to **(j)** of **Appendix 2.4**, the reducing power of the test samples correlated well with increasing concentrations. A lower EC_{50} value corresponds to a greater reducing ability. From **Table 4.4**, the decreasing order of the reducing power of the METE from the selected vegetables at the 3.0 mg/mL was as follows:

L. aestuans > *X. sagittifolium* > *I. batatas* > *C. olitorius* > *T. triangulare* > *H. sabdariffa* >*S. macrocarpon* > *A. cruentus* with EC_{50} values of 1.150, 2.535, 2.778, 3.615, 4.518, 5.148, 9.808, and 14.80mg/mL respectively.

For the HETE from the selected vegetables the reducing power follows the order:

L. aestuans > *I. batatas* > *T. triangulare* > *H. sabdariffa* > *X. sagittifolium* >

S. macrocarpon > *A. cruentus* > *C. olitorius* with their respective EC_{50} values of 0.6894, 1.005, 4.091, 5.028, 5.374, 5.618, 10.68, and 14.12 mg/mL. Comparing the EC_{50} values of the extracts, HETE showed a high reducing power than the METE. However, the reducing power of the reference antioxidant compound, n-Propyl gallate

(EC_{50}, 0.2104 mg/ml) was relatively more pronounced compared with all the extracts from the selected vegetables. The results clearly indicate that the leaves of the selected vegetables have reductive properties and can act as antioxidants as previously reported by Salawu *et al.* (2006).

4.4 Antibacterial Activity

The antibacterial activities on the methanolic and hydro-ethanolic extracts from the selected vegetables against selected micro-organisms are shown in **Table 4.5 (a)** and **(b)** below and **Table 4.69** to **Table 4.76** of **Appendix 1.9**.

Table 4.5 (a) and **(b)** Antibacterial activities on the methanolic and hydro-ethanolic extracts (10 mg mL^{-1}) from the selected leafy vegetables against the selected bacteria strains

(a)Methanolic Extract (METE)

Vegetable Samples	*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. aureus*	
	MIZ (mm)	MIC (mg/mL)	MIZ (mm)	MIC (mg/mL)	MIZ (mm)	MIC (mg/ml)	MIZ (mm)	MIC (mg/mL)
X. sagittifolium	6.7	2.2	5.0	2.5	9.3	2.2	4.7	2.2
I. batatas	15.3	2.9	17.7	3.0	12.3	3.3	10.7	2.8
L. aestuans	6.0	3.2	4.0	3.2	3.0	3.3	1.0	3.2
T. triangulare	7.7	2.4	5.5	2.8	8.2	2.5	6.8	2.9
H. sabdariffa	NA	-	18.3	2.0	7.7	2.8	7.0	2.1
C. olitorius	NA	-	10.7	2.9	14.4	2.2	8.4	2.0
S. macrocarpon	NA	-	6.3	2.7	8.4	1.5	9.3	2.9
A. cruentus	NA	-	NA	-	3.0	3.3	3.0	2.7

(b) Hydro-Ethanolic Extract (HETE)

Vegetable Samples	*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. aureus*	
	MIZ (mm)	MIC (mg/mL)	MIZ (mm)	MIC (mg/mL)	MIZ (mm)	MIC (mg/mL)	MIZ (mm)	MIC (mg/mL)
X. sagittifolium	8	2.6	7.3	1.9	13.7	2.5	4.7	2.2
I. batatas	15.1	3.0	7.3	3.3	11.7	3.1	6.3	3.1
L. aestuans	9.4	3.2	9.3	3.1	3.3	3.1	4.3	3.3
T. triangulare	6.3	2.9	5.0	3.3	7.0	3.0	6.0	3.1
H. sabdariffa	NA	-	22	1.1	10.3	1.6	7.6	2.0
C. olitorius	NA	-	6.7	2.8	7.7	2.1	9.7	2.1
S. macrocarpon	NA	-	3.5	3.3	9.3	1.5	5.3	2.7
A. cruentus	NA	-	NA	-	2.0	3.2	3.0	2.7

MIZ=Mean Inhibition Zone; MIC=Minimum Inhibitory Concentration; NA=No Activity

The phytochemical screening results [**Table 4.2** (**a**) and (**b**)] revealed that the selected leafy vegetables contain saponins, phenolic compounds, and general glycosides which are known to possess antimicrobial and pesticide properties (Okwute, 1992). The antibacterial activities of *X. sagittifolium*, *I. batatas*, *L. aestuans*, and *S. macrocarpon* may also be due to their alkaloid constituents since according to Clark (1981); alkaloids inhibit the growth of certain bacteria and fungi.

The METE and HETE from the vegetable samples except the METE and HETE from *A. cruentus*, at the highest concentration of 10 mg/mL were effective against *P. aeruginosa, E. coli* and *S. aureus*; but the METE and HETE from *H. sabdariffa*, *C. olitorius*, *S. macrocarpon* and *A. cruentus* showed no activity against *B. Subtilis*

[**Table 4.2** (**a**) and (**b**)].

High activity against *B. subtilis* was demonstrated by the METE and HETE from *I. batatas* with inhibition zones of 15.3 mm and 15.1 mm respectively while appreciable activity was also observed for the METE and HETE from *X. sagittifolium* with MIC values of 2.2 and 2.6 mg/mL respectively.

The METE from *H. sabdariffa* showed a high activity against *E. coli* (MIZ, 18.3 mm and MIC, 2.0 mg/mL) compared with the other vegetable extracts. Similar results were obtained for the HETE from *H. sabdariffa* but with much activity than the METE (MIZ, 22.0 mm and MIC, 1.1 mg/mL). However, METE and HETE from *A. cruentus* showed no activity on the growth of *E. coli*.

The highest activity against the growth of *P. aeruginosa* was demonstrated by the METE from *C. olitorius* (14.4 mm) and HETE from *X. sagittifolium* (13.7 mm). METE from *S. macrocarpon* also exhibited activity with MIC of 1.5 mg/mL. However, the least activity against *P. aeruginosa* was shown by the METE and HETE from *L. aestuans* (3.0 mm and 2.0 mm respectively).

Strong inhibition on the growth of *S. aureus* was observed for the METE from *I. batatas* (MIZ, 10.7 mm) and HETE from *C. olitorius* (MIZ, 9.7 mm). METE from *C. olitorius* also showed appreciable activity against *S. aureus* (MIC, 2.0 mg/mL). Zakaria *et al.,* (2006) reported a similar result with respect to activity of methanolic extract from *C. Olitorius* against *S. aureus* at concentration above 40,000 ppm. Moreover, the least activity on the growth of *S. aureus* was exhibited by the METE from *L. aestuans* and HETE from *A. cruentus*; with inhibition zones of 1.0 mm and 3.0 mm respectively.

Bonferroni's Multiple Comparison Test results on the METE from the selected leafy vegetables showed no significant differences between the means of the MIZs and MICs and similar results were obtained for the HETE (**Appendices 3.13** to **3.28)**. Generally, the antibacterial activities on the HETE from the vegetable samples were generally effective compared with results for their respective METE [**Table 4.2** (**a**) and (**b**)].

Chapter Five

5. CONCLUSION AND RECOMMENDATION

The findings of the study are summarized below as conclusions and recommendations emanating from the research findings are made.

5.1. Conclusion

Hydro-ethanolic extraction gave high percentage extract yield from all the selected leafy vegetables which suggest that hydro-ethanol is a preferred solvent system for extracting the phytoconstituents in the selected leafy vegetables.

The phytochemical screening of the selected vegetables showed that the leaves of the selected leafy vegetables contain saponins, tannins, general glycosides, and flavonoids. All the selected leafy vegetables except *T. triangulare, H. sabdariffa, C. olitorius*, and *A. cruentus* contain alkaloids.

The *in vitro* antioxidant and antibacterial analyses on the extracts from the selected indigenous edible leafy vegetables of Ghana showed that the selected vegetables exhibited a strong antioxidant and antibacterial activity. Extracts from the selected leafy vegetables all showed appreciable total phenol content, total antioxidant capacity, radical scavenging activity, and reducing ability with slight variations compared with the standard antioxidant compounds employed in the study. The findings of the study agrees to a large extent similar works carried out in Nigeria by Akindahunsi and Salawu, (2005 a,b); Odukoya *et al.,* (2007) and Salawu *et al.,* (2006). This observation suggests that the selected leafy vegetables possess free radical scavenging potential.

The selected leafy vegetables also exhibited a strong antibacterial activity on the tested bacterial strains; *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa, and Staphylococcus aureus*. However extracts from *H. sabdariffa*, *C. olitorius*,

S. macrocarpon and *A. cruentus* showed no activity against *B. Subtilis*.

The bacteria strains used in the study are common human and animal pathogens (Kubmarawa *et al*., 2008) which cause a lot of infections and cause food-poisoning as well. The inhibition of the growth of these bacteria strains by the extracts from the selected leafy vegetables suggests that the selected leafy vegetables possess antibacterial activity.

5.2 Recommendations

Based on the findings of the study, the following suggestions are given as recommendations:

1. Households in Ghana should utilize the selected leafy vegetables in their diets daily since the leafy vegetables contain phytoconstituents that act as strong antioxidant and antibacterial agents.
2. *In vivo* analyses on the selected leafy vegetables should be carried out to ascertain the antioxidant potentials and antibacterial activity of the selected leafy vegetables
3. Antioxidant and antibacterial studies on the selected leafy vegetables using cold maceration extraction method should be carried out to estimate how much antioxidant and antibacterial activity is lost due to heating in soxhlet apparatus.

REFERENCES

Aiyeloja A. A. and Bello O. A. (2006). Ethnobotanical potentials of common herbs in Nigeria:A case study of Enugu state. *Educational Research and Review*. **1** (1): 21

Akindahunsi, A. A. and Salawu, S. O. (2005a). Antioxidant indices of some green leafy vegetables. *Trop. Sci.* **45**: 35-35

Akindahunsi, A. A. and Salawu, S. O. (2005b). Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. *Afr. J. Biotechnol*. **46**: 497-501

Akubugwo, I. E., Obasi, N. A., Chinyere G. C. and Ugbogu A. E. (2007). Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria. *Afr. J. Biotechnol*; **6** (24): 2833-2839

Alia M, Horcajo C, Bravo L, Goya L. (2003). Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutr Res*. **23**: 1251–1267

Amarowicz, R., Naczk, M. and Shahidi, F. (2000). Antioxidant activity of crude tannins of *canola* and rapeseed hulls. *J. Am. Oil Chem. Soc.***77**: 957-961

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings National Academy of Science*, USA; **90**: 7915–7922

Betts, G.D., Linton, P. and Betteridge, R. J. (1999). Food spoilage yeasts: effects of pH, NaCl and temperature on growth. *Food Control*; **10**: 27–33

Block, G. and Menkes, M. (1989). Ascorbic acid in cancer prevention. In: Moon TE, Micozzi MS., editors. *Diet and Cancer Prevention. Investigating the Role of Micronutrients*; New York: Marcel Dekker Inc., 341-388

Bonoli, M., Verardo, V., Marconi, E. and Caboni, M. F. (2004). Antioxidant Phenols in Barley (*Hordeum vulgare* L.) Flour: Comparative Spectrophotometric Study among Extraction Methods of Free and Bound Phenolic Compounds. *Journal of Agricultural and Food Chemistry*; **52**: 5195-5200.

Bulger, E. M. and Helton, W. S. (1998). Nutrient antioxidants in Gastrointestinal Disease. *Gastroentol Clin North Amer*; **27**(2): 403-419

Cadenas, E. and Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine*; **29**: 222-230

Campos, M. G., Webby, R. F., Markham, K. R., Mitchell, K. A. and Cunha, A. P. (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *Journal of Agricultural and Food Chemistry*; **51**: 742-745

Clark, W. S. (1981), Antimicrobial activities of phenolic constituents of *Magnolia grandiflora L. J. Pharm. Sci.* **70**: 951-952

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol*. 564–582

Deak, T. and Beuchat, L. R. (1996). Handbook of Food Spoilage; CRC Press: New York, USADe la Fuente, M., & Victor, M. (2000). Antioxidants as modulators of immune function. *Immunology and Cell Biology*; **78**: 49–54

Devasagayam, T. P. A., & Sainis, K. B. (2002). Immune system and antioxidants, especially those derived from Indian medicinal plants. *Indian Journal of Experimental Biology*; **40**: 639–655

De Grey, A. D. N. J. (1999) . The Mitochondrial Free Radical Theory of Aging, R.G. Landes Company, Austin, Texas, U.S.A., pp 35-85

Diaz-Reinoso, B., Moure, A., Dominguez, H. and Parajo, J. C. (2006). Supercritical CO_2 extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*. **54**: 2441-2469

Di Matteo, V. & Esposito, E. (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzeimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Current Drug Target CNS and Neurological Disorder*. **2**: 95–107

Dokosi, O. B. (1998). Herbs of Ghana. Council for Industrial Research, Accra, Ghana: 50-511

Dykes L. and Rooney, L. W. (2007). Phenolic compounds in cereal grains and their health benefits. *CFW,* **52**(3): 105-111

Elsayed, N. M. (2001). Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. *Nutrition*; **17**: 828-34

Evans, W. C (1989). *Trease and Evans' Pharmacognosy* (13th ed.). Oxford, Gt. Britain: Alden Press, pp 386-548.

Farrukh, A. and Mukhtar, H. (2002). Photochemprevention by botanical antioxidants.

Skin Pharmacol Appl Skin Physiol; **15**: 297-306.

Frankel, E. N. and Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of Science and Food Agriculture*; **80**: 1925-1941

Gerber, M., Boutron-Ruault, M. C., Hercberg, S., Riboli, E., Scalbert, A. & Siess, M. H. (2002). Food and cancer: state of the art about the protective effect of fruits and vegetables. *Bulletin du Cancer*. **89**(3): 293–312

Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, A. K., Mehrotra, S., Pushpangadan, P. (2003). Studies on the antioxidant activities of *Desmodium gangeticum*. *Biol Pharm Bull*. **26** (10): 1424-1427

Grubben G. J. H. and Denton O. A. (2004). Vegetables: Plant Resources of Tropical Africa, *PROTA*; pp 518-521

Halliwell B. and Gutteridge J. M. C. (1999). Oxygen is a toxic gas an introduction to oxygen toxicity and reactive oxygen species. In: Free Radical in Biology and Medicine; Oxford University Press, United Kingdom. pp 1-35

Hseu, Y.-C., Chang, W.-H., Chen, C.-S., Liao, J.-W., Huang, C.-J., Lu, F.-J., Chia, Y.-C, Hsu, H.-K., Wu, J.-J and Yang, H.-L. (2008). Antioxidant activities of *Toona sinensis* leaves extracts using different antioxidant models. *Food and Chemical Toxicology*. 46: 105-114

Huang, D., Ou B. and Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*. **53**: 1841-1856

Krinsky, N. I. (1993). Actions of carotenoids in biological systems. *Annual Review of Nutrition*. **13**: 561-587

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular diseases and cancer. *American Journal of Medicine*; **113**(9B): 71–88

Kubmarawa, D., Khan, M. E., Punah, A. M. and Hassan (2008). Phytochemical Screening and antibacterial activity of extracts from *Pakia clapperotoniana keay* against human pathogenic bacteria. *J.Med. Plant. Res*. **2**(12): 352-355

Liebler, D. C. and McClure, T. D. (1996). Antioxidant reactions of beta-carotene: Identification of carotenoid-radical adducts. *Chemistry Research of Toxicology*; **9**: 8-11

Jansen van Rensburg, W., Voster Ineke, H. J., Van Zijl, J. J. B. and Venter Sonja L. (2007). Conservation of African Leafy Vegetables in South Africa. *AJFAND*. **7** (4): 1-10

Jimenez, A., Selga, A., Torres, J. L. and Julia, L. (2004). Reducing activity of polyphenols with stable radicals of the TTM series. Electron transfer versus H-abstraction reactions in flavan-3-ols. *Organic Letter: American Chemical Society*. **6**: 4583-4586

Kluytmans, J., Van Belkum, A. and Verbrugh, H. (1999). Nasal Carriage of *Staphylococcus aureus*: Epidemiology, Underlying Mechanisms, and Associated Risks. *Clinical Microbiology Reviews*. **10**(3): 505–520

Manach, C., Scalbert, A., Morand, C., Remesy, C. and Jimenez, C. (2004). Polyphenols:Food sources and bioavailability. *Am. J. Clin. Nutr.* **79**:727-747

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Breese, J. S., Shapiro, C., Griffin, P. M. and Tauxe, R. V. (1999). Food related illness and dead in the United States. *Emerg. Infect. Dis.* **5**: 607–625

Miller, N. J. and Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem.***60**: 331-337

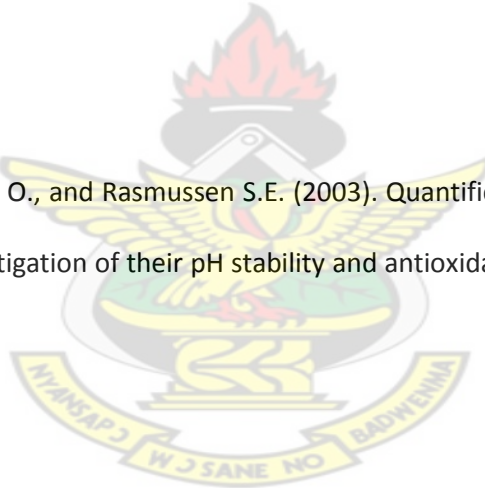
Miller, N. J., Diplock, A. T., Rice-Evans, C. A., Davies, M. J., Gopinathan, V. and Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*; **84**: 407-412

Miller, R. A. and Britigan, B. E. (1997). *Clinical Microbiology Reviews*. **10** (1): 1–18

Mousumi D. (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. *Journal of Medicinal Plants Research*; **2**(2): 45-51

Nakano, M. M. and Zuber, P. (1998). Anaerobic growth of a “strict aerobe” (*Bacillus subtilis*). *Annu. Rev. Microbiol*. **52**: 165-190

Negi, P. S. and Roy, S. K. (2001). Effect of drying conditions on quality of green leaves during long term storage. *Food Res Int*. **34**:283–287



Nielsen, I. L. F., Haren G. R., Magnussen E. L., Dragsted L. O., and Rasmussen S.E. (2003). Quantification of anthocyanins in commercial black currant juices by simple highperformance liquid chromatography. Investigation of their pH stability and antioxidative potency. *Journal of Agricultural and Food Chemistry*; **51**: 5861-5866

Nya, E. J. and Eka, M. J. (2007). Genetic Variability and Heritability of Desirable Metric Characteristics in Talinum triangulare Land-races in South Eastern Nigeria, *Journal of Agronomy*. **6** (3): 459-463

Oboh, G. (2005). Effect of blanching on the antioxidant property of some tropical green leafy vegetables. *Food Sci.Technol*. **38**: 513-517

Oboh, G. and Akindahunsi, A. A. (2004). Change in the ascorbic acid, total phenol and antioxidant activity of some sun-dried green leafy vegetables in Nigeria. *Nutr. Health*. **18**: 29-36

Oboh G., Rocha J. B. T. (2008). Antioxidant and Neuroprotective Properties of Sour Tea (*Hibiscus sabdariffa*, calyx) and Green Tea (*Camellia sinensis*) on some Pro-oxidant-induced Lipid Peroxidation in Brain in vitro. *Food Biophysics*. **3**:382–389

Odukoya, O. A., Inya-Agha, S. I., Sofidiya, M. O. and Ilori, O. O. (2007). Antioxidant activity of selected Nigerian leafy vegetables. *Am. J. Food Technol.* **2** (3); 169-175

Olatunde Farombi E. (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents, *African Journal of* Biotechnology; **2** (12), 662-671

Okwute, S. K. (1992). Plant derived pesticidal and antimicrobial agents for use in agriculture: A review of phytochemical and biological studies on some Nigerian plants. *J. Agric. Sci. Technol.* **2**(1); 62-70

Oyaizu, M., (1986). Studies on the products of browning reaction preparedfrom glucose amine, *Jpn. J. Nutr.* **44**;307-315

Pellegrini P., Serafini M., Colombi B., Del Rio D., Salvatore S., Bianchi M. and Brighenti F. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition*; **133**: 2812-2819

Percival, M. (1998). Antioxidants. *Clinical Nutrition Insights.* **31**: 1-4

Pinzino, C., Capocchi, A., Galleschi L., Saviozzi F., Nanni B. and Zandomeneghi M. (1999). Aging, free radicals, and antioxidants in wheat seeds. *Journal of Agricultural and Food Chemistry*. **47**: 1333-1339

Prieto, P., Pineda, M., Anguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdaenum complex: specific application to the determination of vitamin E. *Anal. Biochem*. **269**(2): 337-341

Prior, R. L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*. **53**: 4290-4302

Proteggente, A. R., Pannala, A. S., Paganga G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put, F., Dacombe, C. and Rice-Evans, C. A. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research*. **36**: 217-233

Rice-Evans, C. A., Miller, N. J. And Pagang, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Rad. Biol. Med.* **20**: 933-956

Rubaihayo, E. B. (2008), African Crop Science Journal, *Africa Crop Science Conference Proceedings*. **3**: 337-1340

Sagdic, O. and Ozcan, M. (2003). Antibacterial activity of Turkish spice hydrosols. *Food Control*. **14**: 141–143

Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P., & Rice- Evans, C. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics*. **322**(2): 339–346

Salawu, S. O., Akindahunsi, A. A. and Comuzzo P. (2006). Chemical composition and *in vitro* antioxidant activities of some Nigerian vegetables. *J. Pharmacol. Toxicol*. **1**(5): 429-437

Sanchez-Moreno, C. (2002). Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science Technology International*. **8**: 121-137

Serafini, M., Bellocco, R., Wolk, A., and Ekstrom, A. M. (2002). Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology*. **123**(4): 985–991

Singleton, E. B. (1977). Robert Shaw Maclntyre. 1912-1976. *Radiology*. **122**(3):847

Smid, E. J. and Gorris, L.G. M. (1999). Natural antimicrobials for food preservation: Handbook of Food Preservation; Rahman, M.S., Ed.; Marcel Dekker: New York, pp. 285–308

Stadtman, E. R. (1992). Protein oxidation and aging. *Science*. **257**: 1220–1224.

Stanner, S. A., Hughes, J., Kelly, C. N., and Buttriss, J. (2002). A review of the epidemiological evidence for the antioxidant hypothesis. *Public Health Nutrition*. **7**(3): 407-422

Stratil, P., Klejdus, B. and Kuban, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables Evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry*. **54**: 607-616

Sun, Y. (1990). Free radicals, antioxidant enzymes and carcinogenesis. *Free Radical Biology and Medicine*. **8**: 583–599

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology and Medicine*. **28**: 1685-1696

Van Acker, S. A. B. E., Van den Vijgh, W. J. F. & Bast, F. (1996). Structural aspects of antioxidant activity of flavonoids. *Free Radical Biology and Medicine*. **20**(3): 331–342

Vogt, R. L., Dippold, L. (2005). Escherichia coli O157:H7 Outbreak Associated with Consumption of Ground Beef. *Public Health Reports.* **120**: 174-178

Waladkhani, A., & Clemens, M. R. (1998). Effect of dietary phytochemicals on cancer development. *International Journal of Molecular Medicine*. **1**: 747–753

Wallace, S. S. (1997). Oxidative damage to DNA and its repair, in Oxidative Stress and Molecular Biology of Antioxidant Defenses. Scandalios JG, editor; Cold Spring Harbor Laboratory Press: Plainview, NY., 49-90

Worlitzsch, D., Tarran, R., Ulrich, M., Schwab, U., Cekici, A., Meyer, K. C., Birrer, P., Bellon, G., Berger J, Weiss, T., Botzenhart, K., Yankaskas, J. R., Randell, S., Boucher, R. C. and Döring G. (2002). Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J. Clin. Invest.* **109**(3): 317-325

Wright, J. S., Johnson E. R. and DiLabio G. A. (2001). Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. *Journal of American Chemical Society*; **123**: 1173-1183

Wu X., Beecher G. R., Holden J. M., Haytowitz D. B., Gebhardt S. E. and Prior R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*. **52**: 4026-4037

Zakaria, Z. A.; Somchit, M. N.; Zaiton, H.; . Mat Jais, A. M.; Sulaiman, M. R.; Farah, W. O.; Nazaratulmawarina, R. and Fatimah, C.A. (2006). The in vitro Antibacterial Activity of Corchorus olitorius Extracts. *International Journal of Pharmacology*. **2**(2): 213-215

Zhou K., Yin J. and Yu L. (2005). Phenolic acid, tocopherol and carotenoid compositions, and antioxidant functions of hard red winter wheat bran. *Journal of Agricultural and Food Chemistry*. **53**: 3916-3922.

http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Corchorus+olitorius (Accessed Date: April 8, 2009)

http://tropilab.com/sol-mac.html (Accessed Date: April 8, 2009)

http://en.wikipedia.org/wiki/Amaranthus_hybridus#Description (Accessed Date: April 8, 2009)

http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Corchorus+olitorius (Accessed Date: April 8, 2009)

www.plantoftheweek.org/week343.shtml (Accessed Date: April 13, 2009)

http://en.wikipedia.org/wiki/Sweet_potato (Accessed Date: April 13, 2009)

http://www.agristarts.com/xan_sagittifolium.htm (Accessed Date: April 13, 2009)

http://www.floridata.com/ref/H/hibi_sab.cfm (Accessed Date: April 9, 2009)

http://en.wikipedia.org/wiki/Hibiscus_sabdariffa (Accessed Date: April 9, 2009)

http://tropilab.com/sol-mac.html (Accessed Date: April 8, 2009)

http://en.wikipedia.org/wiki/Laportea_aestuans (Accessed Date: April 21, 2009)

http://en.wikipedia.org/wiki/Bacillus_subtilis (Accessed Date: May 26, 2009)

http://en.wikipedia.org/wiki/Staphylococcus_aureus (Accessed Date: May 26, 2009)

http://www.bionewsonline.com/i/what_is_staphylococcus_aureus.htm (Accessed Date: May 26, 2009)

http://en.wikipedia.org/wiki/Escherichia_coli (Accessed Date: May 26, 2009)

http://en.wikipedia.org/wiki/Pseudomonas_aeruginosa (Accessed Date: May 26, 2009)

http://en.wikipedia.org/wiki/Antimicrobial (Date Accessed: May 28, 2009)

http://en.wikipedia.org/wiki/Alkaloid (Accessed Date: June 1, 2009)

http://en.wikipedia.org/wiki/Saponin (Accessed Date: June 1, 2009)

http://en.wikipedia.org/wiki/Secondary_metabolite (Accessed Date: June 1, 2009)

http://en.wikipedia.org/wiki/Tannin (Accessed Date: June 1, 2009)

http://en.wikipedia.org/wiki/Flavonoid (Accessed Date: June 1, 2009)

APPENDICES

The appendices of the experimental results are categorized into three sections namely:

Appendix 1 (Table of Experimental Results), **Appendix 2** (Graphical Representation of experimental results) and **Appendix 3** (Statistical Analysis of Experimental Results).

Appendix 1: Table of Experimental Results

Appendix 1.1: Absorbance Measurements for Total Phenol Content (TPC) Assay

Table 4.6 Absorbance of the Tannic acid (reference drug)

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
Tannic acid	0.01	0.184	0.187	0.189
	0.03	0.344	0.346	0.348
	0.1	0.886	0.887	0.887
	0.3	3	3.002	3.004

Table 4.7 Absorbance of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
METE	0.1	0.251	0.252	0.255
	0.3	0.655	0.659	0.652
	1	1.581	1.583	1.585
	3	4.872	4.876	4.871
HETE	0.1	0.346	0.341	0.344
	0.3	0.962	0.965	0.961
	1	2.142	2.141	2.143
	3	6.946	6.942	6.943

Table 4.8 Absorbance of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
METE	0.1	0.231	0.232	0.231
	0.3	0.632	0.63	0.632
	1	1.413	1.412	1.412
	3	4.482	4.481	4.482
HETE	0.1	0.312	0.314	0.314
	0.3	0.499	0.494	0.494
	1	2.019	2.018	2.015
	3	6.291	6.29	6.295

Table 4.9 Absorbance of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
METE	0.1	0.221	0.224	0.22
	0.3	0.6	0.602	0.604
	1	1.392	1.395	1.392
	3	4.111	4.117	4.113
HETE	0.1	0.296	0.294	0.292
	0.3	0.765	0.767	0.769
	1	2.098	2.093	2.095
	3	5.126	5.128	5.124

Table 4.10 Absorbance of the METE and HETE from *T. triangulare*

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
METE	0.1	0.201	0.204	0.20
	0.3	0.538	0.536	0.534
	1	1.097	1.095	1.099
	3	3.312	3.316	3.317
HETE	0.1	0.257	0.253	0.255
	0.3	0.763	0.762	0.766
	1	1.512	1.515	1.517
	3	4.868	4.864	4.86

Table 4.11 Absorbance of the METE and HETE from ***H. sabdariffa***

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
	0.1	0.174	0.178	0.176

METE	0.3	0.344	0.34	0.347
	1	1.034	1.032	1.032
	3	3.142	3.147	3.144
	0.1	0.231	0.235	0.23
HETE	0.3	0.601	0.604	0.607
	1	1.374	1.37	1.377
	3	4.561	4.569	4.564

Table 4.12 Absorbance of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
	0.1	0.151	0.154	0.156
METE	0.3	0.29	0.293	0.295
	1	0.984	0.981	0.984
	3	2.886	2.882	2.889
	0.1	0.197	0.193	0.195
HETE	0.3	0.561	0.562	0.561
	1	1.123	2.125	2.126
	3	4.261	4.264	4.262

Table 4.13 Absorbance of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/mL)	Absorbanc (760 nm)		
	0.1	0.144	0.142	0.14
METE	0.3	0.24	0.243	0.241
	1	0.83	0.835	0.832
	3	2.64	2.636	2.638
	0.1	0.168	0.166	0.167
HETE	0.3	0.501	0.501	0.503
	1	0.914	0.912	0.914
	3	3.747	3.749	3.745

Table 4.14 Absorbance of the METE and HETE from *A. cruentus*

Solvent System	Conc. (mg/mL)	Absorbance (760 nm)		
METE	0.1	0.134	0.136	0.132
	0.3	0.199	0.199	0.197
	1	0.785	0.783	0.782
	3	2.364	2.362	2.366
HETE	0.1	0.145	0.141	0.143
	0.3	0.412	0.41	0.414
	1	0.716	0.712	0.715
	3	3.348	3.343	3.345

Appendix 1.2: TAE (mg/mL) of the METE and HETE from the Selected Leafy Vegetables

Table 4.15 TAE (mg/mL) of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.023	0.023	0.024
	0.3	0.064	0.064	0.064
	1	0.159	0.159	0.159
	3	0.485	0.485	0.484
HETE	0.1	0.032	0.032	0.032
	0.3	0.095	0.096	0.095
	1	0.216	0.216	0.216
	3	0.706	0.706	0.706

Table 4.16 TAE (mg/mL) of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.021	0.021	0.021
	0.3	0.062	0.061	0.062
	1	0.141	0.141	0.141
	3	0.455	0.455	0.455
HETE	0.1	0.203	0.203	0.203
	0.3	0.312	0.314	0.314
	1	0.499	0.494	0.494
	3	0.639	0.639	0.64

Table 4.17 TAE (mg/mL) of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.02	0.02	0.02
	0.3	0.058	0.058	0.058
	1	0.129	0.129	0.129
	3	0.407	0.407	0.407
HETE	0.1	0.027	0.027	0.027
	0.3	0.139	0.140	0.139
	1	0.417	0.417	0.417
	3	0.600	0.602	0.604

Table 4.18 TAE (mg/mL) of the METE and HETE from *T. triangulare*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.018	0.018	0.018
	0.3	0.052	0.052	0.052
	1	0.109	0.109	0.109
	3	0.335	0.336	0.336
HETE	0.1	0.023	0.023	0.023
	0.3	0.075	0.075	0.075
	1	0.152	0.152	0.152
	3	0.494	0.494	0.493

Table 4.19 TAE (mg/mL) of the METE and HETE from *H. sabdariffa*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.015	0.015	0.015
	0.3	0.032	0.032	0.033
	1	0.103	0.103	0.103
	3	0.318	0.318	0.318
HETE	0.1	0.021	0.021	0.021
	0.3	0.058	0.059	0.059
	1	0.137	0.137	0.138
	3	0.463	0.464	0.463

Table 4.20 TAE (mg/mL) of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.013	0.013	0.013
	0.3	0.027	0.027	0.027
	1	0.098	0.097	0.098
	3	0.292	0.291	0.292
HETE	0.1	0.017	0.017	0.017
	0.3	0.054	0.055	0.054
	1	0.214	0.214	0.214
	3	0.432	0.433	0.432

Table 4.21 TAE (mg/ml) of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.012	0.012	0.011
	0.3	0.022	0.022	0.022
	1	0.082	0.082	0.082

	3	0.267	0.266	0.266
HETE	0.1	0.014	0.014	0.014
	0.3	0.017	0.017	0.017
	1	0.090	0.090	0.090
	3	0.380	0.380	0.380

Table 4.22. TAE (mg/ml) of the METE and HETE from *A. cruentus*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.011	0.011	0.011
	0.3	0.017	0.017	0.017
	1	0.077	0.077	0.077
	3	0.238	0.238	0.239
HETE	0.1	0.012	0.012	0.012
	0.3	0.039	0.039	0.039
	1	0.07	0.07	0.07
	3	0.339	0.338	0.339

Table 4.23 TAE of METE and HETE from the selected leafy vegetables (3.0 mg/mL)

Methanolic Extract (METE)	TPC expressed as TAE (mg/mL)
X. Sagittifolium	0.485±0.105
I. batatas	0.455±0.098
L. aestuans	0.407±0.087
T. triangulare	0.336±0.072
H.sabdariffa	0.318±0.070
C. olitorius	0.292±0.064
S. macrocarpon	0.267±0.059

A. cruentus	0.238±0.053

Hydro-Ethanolic Extract (HETE)	TPC expressed as TAE (mg/mL)
X. Sagittifolium	0.706±0.153
I. batatas	0.639±0.097
L. aestuans	0.602±0.131
T. triangulare	0.494±0.106
H.sabdariffa	0.463±0.101
C. olitorius	0.432±0.095
S. macrocarpon	0.380±0.087
A. cruentus	0.339±0.076

Mean±SE

Appendix1.3: Absorbance Measurements for Total Antioxidant Capacity (TAC) Assay

Table 4.24 Absorbance of the Ascorbic acid (reference drug)

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
Tannic acid	0.01	0.071	0.071	0.07
	0.03	0.094	0.093	0.093
	0.1	0.189	0.186	0.187
	3	0.398	0.396	0.397

Table 4.25 Absorbance of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	0.091	0.092	0.091

METE	0.3	0.135	0.136	0.135
	1	0.232	0.232	0.232
	3	0.611	0.612	0.612
HETE	0.1	0.104	0.103	0.103
	0.3	0.184	0.184	0.185
	1	0.308	0.306	0.306
	3	0.859	0.858	0.857

Table 4.26 Absorbance of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.084	0.085	0.083
	0.3	0.131	0.132	0.131
	1	0.218	0.218	0.218
	3	0.526	0.527	0.526
HETE	0.1	0.097	0.096	0.096
	0.3	0.156	0.156	0.155
	1	0.291	0.29	0.29
	3	0.781	0.782	0.782

Table 4.27 Absorbance of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.081	0.081	0.081
	0.3	0.129	0.129	0.128
	1	0.2	0.201	0.201
	3	0.518	0.518	0.519
HETE	0.1	0.094	0.094	0.093
	0.3	0.148	0.149	0.149
	1	0.268	0.267	0.267
	3	0.645	0.644	0.644

Table 4.28 Absorbance of the METE and HETE from ***T. triangulare***

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.079	0.079	0.079
	0.3	0.121	0.120	0.120
	1	0.188	0.189	0.188
	3	0.445	0.446	0.445

	0.1	0.089	0.089	0.088
HETE	0.3	0.139	0.139	0.139
	1	0.234	0.234	0.235
	3	0.619	0.62	0.619

Table 4.29 Absorbance of the METE and HETE from *H. sabdariffa*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	0.079	0.079	0.079
METE	0.3	0.099	0.097	0.099
	1	0.176	0.176	0.175
	3	0.422	0.422	0.421
	0.1	0.084	0.084	0.083
HETE	0.3	0.128	0.129	0.129
	1	0.218	0.218	0.217
	3	0.575	0.576	0.576

Table 4.30 Absorbance of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	0.078	0.078	0.078
METE	0.3	0.093	0.092	0.092
	1	0.173	0.173	0.173
	3	0.391	0.391	0.392
	0.1	0.082	0.081	0.082
HETE	0.3	0.126	0.125	0.125
	1	0.188	0.187	0.187
	3	0.548	0.548	0.548

Table 4.31 Absorbance of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	0.077	0.077	0.077
METE	0.3	0.087	0.086	0.086
	1	0.166	0.168	0.167
	3	0.362	0.362	0.361
	0.1	0.080	0.079	0.08
HETE	0.3	0.118	0.119	0.118
	1	0.165	0.166	0.166

	3	0.491	0.492	0.492

Table 4.32 Absorbance of the METE and HETE from *A. cruentus*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.076	0.076	0.076
	0.3	0.083	0.083	0.084
	1	0.151	0.15	0.15
	3	0.331	0.33	0.331
HETE	0.1	0.077	0.077	0.077
	0.3	0.108	0.107	0.107
	1	0.142	0.141	0.141
	3	0.444	0.445	0.445

Table 4.33 AAE (mg/mL) of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
METE	0.1	0.024	0.025	0.024
	0.3	0.063	0.064	0.063
	1	0.150	0.150	0.150
	3	0.488	0.488	0.488
HETE	0.1	0.036	0.035	0.035
	0.3	0.107	0.107	0.108
	1	0.216	0.216	0.216
	3	0.709	0.708	0.707

Table 4.34 AAE (mg/mL) of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
METE	0.1	0.018	0.019	0.017
	0.3	0.060	0.061	0.060
	1	0.137	0.137	0.137
	3	0.412	0.413	0.412

Appendix1.4: AAE (mg/mL) of the METE and HETE from the Selected Leafy Vegetables

	0.1	0.030	0.030	0.029
HETE	0.3	0.082	0.082	0.081
	1	0.203	0.202	0.202
	3	0.639	0.640	0.640

Table 4.35 AAE (mg/mL) of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
	0.1	0.015	0.015	0.015
METE	0.3	0.058	0.058	0.058
	1	0.121	0.122	0.122
	3	0.406	0.405	0.406

	0.1	0.027	0.027	0.026
HETE	0.3	0.075	0.076	0.076
	1	0.182	0.181	0.181
	3	0.518	0.517	0.517

Table 4.36 AAE (mg/mL) of the METE and HETE from *T. triangulare*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
	0.1	0.014	0.014	0.014
METE	0.3	0.051	0.050	0.050
	1	0.111	0.112	0.111
	3	0.340	0.341	0.341
	0.1	0.022	0.022	0.022
HETE	0.3	0.067	0.067	0.067
	1	0.152	0.152	0.153
	3	0.495	0.496	0.495

Table 4.37 AAE (mg/ml) of the METE and HETE from *H. sabdariffa*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
METE	0.1	0.014	0.014	0.014
	0.3	0.031	0.031	0.030
	1	0.099	0.100	0.100
	3	0.318	0.319	0.319
HETE	0.1	0.018	0.018	0.017
	0.3	0.057	0.058	0.058
	1	0.137	0.137	0.136
	3	0.456	0.457	0.457

Table 4.38 AAE (mg/ml) of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
METE	0.1	0.013	0.013	0.013
	0.3	0.026	0.025	0.025
	1	0.097	0.097	0.097
	3	0.290	0.290	0.291
HETE	0.1	0.016	0.015	0.016
	0.3	0.055	0.055	0.055
	1	0.111	0.110	0.110
	3	0.432	0.432	0.432

Table 4.39 AAE (mg/ml) of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/mL)	TAE (mg/mL)		
METE	0.1	0.012	0.012	0.012
	0.3	0.020	0.020	0.020
	1	0.091	0.093	0.092
	3	0.262	0.261	0.261
HETE	0.1	0.014	0.014	0.014
	0.3	0.048	0.049	0.048
	1	0.090	0.091	0.091
	3	0.381	0.382	0.382

Table 4.40 AAE (mg/ml) of the METE and HETE from *A. cruentus*

Solvent System	Conc. (mg/mL)	AAE (mg/mL)		
	0.1	0.011	0.011	0.011

METE	0.3	0.017	0.017	0.018
	1	0.078	0.077	0.077
	3	0.228	0.228	0.228
HETE	0.1	0.012	0.012	0.012
	0.3	0.039	0.039	0.039
	1	0.070	0.069	0.069
	3	0.339	0.340	0.340

Table 4.41 AAEof the METE and HETE from the selected leafy vegetables (3.0 mg/ml)

Methanolic Extract (METE)	**TAC expressed as AAE (mg/mL)**
X. Sagittifolium	0.488±0.105
I. batatas	0.412±0.089
L. aestuans	0.406±0.088
T. triangulare	0.340±0.073
H.sabdariffa	0.318±0.070
C. olitorius	0.290±0.064
S. macrocarpon	0.261±0.059
A. cruentus	0.228±0.053

Hydro-Ethanolic Extract (HETE)	**TAC expressed as AAE (mg/mL)**
X. Sagittifolium	0.681±0.145
I. batatas	0.621±0.134
L. aestuans	0.517±0.111
T. triangulare	0.462±0.099
H.sabdariffa	0.445±0.097
C. olitorius	0.421±0.093
S. macrocarpon	0.365±0.084
A. cruentus	0.312±0.069

Mean±SE

Appendix 1.5: Absorbance Measurements for Reducing Power Assay

Table 4.42. Absorbance of the n-Propyl gallate (reference drug)

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
n-Propyl gallate	0.01	0.128	0.129	0.128
	0.03	0.392	0.393	0.392
	0.10	1.160	1.161	1.161
	0.30	2.141	2.140	2.142

Table 4.43 Absorbance of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.10	0.201	0.202	0.202
	0.30	0.345	0.346	0.347
	1.00	0.701	0.702	0.704
	3.00	1.231	1.234	1.235
HETE	0.1	0.232	0.235	0.237
	0.3	0.408	0.404	0.402
	1	0.734	0.732	0.731
	3	1.452	1.458	1.456

Table 4.44 Absorbance of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.1	0.189	0.189	0.187
	0.3	0.300	0.304	0.301
	1	0.678	0.678	0.675
	3	1.213	1.215	1.213
HETE	0.1	0.213	0.214	0.216
	0.3	0.399	0.397	0.398
	1	0.701	0.704	0.706
	3	1.018	1.014	1.012

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.1	0.110	0.113	0.114
	0.3	0.232	0.236	0.234
	1	0.543	0.544	0.547
	3	0.821	0.824	0.820
HETE	0.1	0.189	0.187	0.187
	0.3	0.354	0.351	0.355
	1	0.686	0.688	0.686
	3	0.906	0.901	0.903

Table 4.45 Absorbance of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.1	0.104	0.104	0.102
	0.3	0.199	0.197	0.198
	1	0.403	0.401	0.405
	3	0.812	0.814	0.813
HETE	0.1	0.145	0.147	0.144
	0.3	0.235	0.233	0.231
	1	0.503	0.505	0.504
	3	0.972	0.977	0.975

Table 4.46 Absorbance of the METE and HETE from *T. triangulare*

Table 4.47 Absorbance of the METE and HETE from *H. sabdariffa*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.110	0.111	0.110
	0.3	0.171	0.170	0.172
	1	0.349	0.349	0.347
	3	0.688	0.690	0.689
HETE	0.1	0.138	0.136	0.138
	0.3	0.199	0.197	0.198
	1	0.412	0.412	0.414
	3	0.805	0.804	0.801

Table 4.48 Absorbance of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.071	0.074	0.070
	0.3	0.121	0.123	0.126
	1	0.201	0.204	0.206
	3	0.471	0.469	0.466
HETE	0.1	0.121	0.122	0.124
	0.3	0.207	0.205	0.208
	1	0.296	0.297	0.295
	3	0.607	0.607	0.609

Table 4.49 Absorbance of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.049	0.051	0.053
	0.3	0.091	0.095	0.089
	1	0.167	0.169	0.164
	3	0.361	0.363	0.367
HETE	0.1	0.098	0.096	0.094
	0.3	0.145	0.148	0.146
	1	0.261	0.264	0.259
	3	0.504	0.509	0.502

Table 4.50 Absorbance of the METE and HETE from *A. cruentus*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
METE	0.1	0.032	0.034	0.037
	0.3	0.066	0.068	0.072
	1	0.121	0.126	0.124
	3	0.287	0.285	0.289
HETE	0.1	0.079	0.073	0.070
	0.3	0.104	0.100	0.107

1	0.195	0.192	0.194
3	0.401	0.406	0.402

Appendix 1.6: Absorbance Measurements for DPPH Radical Scavenging Assay

Table 4.51 Absorbance of the n-Propyl gallate (reference drug)

Conc. (mg/mL)	Absorbance (700 nm)		
0.01	1.419	1.42	1.417
0.03	0.821	0.828	0.824
0.10	0.175	0.172	0.171
0.30	0.126	0.124	0.128
Control		1.595	

Table 4.52 Absorbance of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.10	0.957	0.952	0.954
	0.30	0.6.21	0.629	0.623
	1.00	0.463	0.468	0.461
	3.00	0.315	0.312	0.318
HETE	0.1	0.892	0.894	0.897
	0.3	0.545	0.549	0.541
	1	0.352	0.359	0.355
	3	0.224	0.227	0.222

Table 4.53 Absorbance of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.1	0.988	0.981	0.983
	0.3	0.688	0.681	0.685
	1	0.491	0.498	0.494
	3	0.354	0.359	0.351
HETE	0.1	0.909	0.902	0.906
	0.3	0.573	0.571	0.578
	1	0.389	0.383	0.383
	3	0.256	0.258	0.252

Table 4.54 Absorbance of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
METE	0.1	0.951	0.956	0.953
	0.3	0.702	0.708	0.700

	1	0.525	0.529	0.521
	3	0.384	0.381	0.389
	0.1	0.900	0.902	0.905
HETE	0.3	0.605	0.601	0.600
	1	0.412	0.415	0.413
	3	0.285	0.28	0.287

Table 4.55 Absorbance of the METE and HETE from *T. triangulare*

Solvent System	Conc. (mg/mL)	Absorbance (700 nm)		
	0.1	1.037	1.034	1.032
METE	0.3	0.731	0.735	0.738
	1	0.558	0.555	0.55
	3	0.411	0.414	0.419
	0.1	0.925	0.921	0.923
HETE	0.3	0.632	0.629	0.638
	1	0.46	0.469	0.464
	3	0.316	0.318	0.311

Table 4.56 Absorbance of the METE and HETE from *H. sabdariffa*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	1.038	1.030	1.035
METE	0.3	0.732	0.730	0.737
	1	0.592	0.595	0.597
	3	0.418	0.415	0.412
	0.1	0.940	0.943	0.947
HETE	0.3	0.639	0.636	0.631
	1	0.496	0.493	0.491
	3	0.356	0.351	0.359

Table 4.57 Absorbance of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	1.032	1.031	1.039
METE	0.3	0.739	0.732	0.734
	1	0.604	0.601	0.607
	3	0.431	0.433	0.436

	0.1	0.952	0.954	0.958
HETE	0.3	0.686	0.681	0.683
	1	0.543	0.545	0.541
	3	0.412	0.416	0.418

Table 4.58 Absorbance of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	1.054	1.051	1.057
METE	0.3	0.743	0.746	0.749
	1	0.620	0.623	0.621
	3	0.442	0.445	0.448
	0.1	0.951	0.954	0.952
HETE	0.3	0.686	0.681	0.684
	1	0.542	0.544	0.548
	3	0.421	0.423	0.426

Table 4.59 Absorbance of the METE and HETE from *A. cruentus*

Solvent System	Conc. (mg/mL)	Absorbance (695 nm)		
	0.1	1.086	1.082	1.08
METE	0.3	0.78	0.784	0.782
	1	0.638	0.634	0.631
	3	0.495	0.496	0.491
	0.1	0.991	0.994	0.997
HETE	0.3	0.731	0.733	0.735
	1	0.571	0.579	0.574
	3	0.475	0.471	0.477

Appendix 1.7: % Radical Scavenging Activity (RSA) of the METE and HETE from the Selected Leafy Vegetables

Table 4.60 %RSA of the n-Propyl gallate (reference drug)

Solvent System	Conc. (mg/mL)	%RSA		
	0.01	11.03	10.97	11.16
n-Propyl gallate	0.03	48.53	48.09	48.39
	0.10	89.03	89.23	89.28
	0.30	92.10	92.23	91.97

Table 4.61 %RSA of the METE and HETE from *X. sagittifolium*

Solvent System	Conc. (mg/mL)		%RSA	
METE	0.10	40.00	40.31	40.19
	0.30	61.07	60.56	60.94
	1.00	70.97	70.65	71.10
	3.00	80.25	80.44	80.06
HETE	0.1	44.08	43.95	43.76
	0.3	65.83	65.58	66.08
	1	77.93	77.49	77.74
	3	85.96	85.77	86.08

Table 4.62 %RSA of the METE and HETE from *I. batatas*

Solvent System	Conc. (mg/mL)		%RSA	
METE	0.1	38.06	38.50	38.37
	0.3	56.87	57.30	57.05
	1	69.22	68.78	69.03
	3	77.81	77.49	78.00
HETE	0.1	43.01	43.45	43.20
	0.3	64.08	64.20	63.76
	1	75.61	75.99	75.99
	3	83.95	83.82	84.20

Table 4.63 %RSA of the METE and HETE from *L. aestuans*

Solvent System	Conc. (mg/mL)		%RSA	
METE	0.1	40.38	40.06	40.25
	0.3	55.99	55.61	56.11
	1	67.08	66.83	67.34
	3	75.92	76.11	75.61
HETE	0.1	43.57	43.45	43.26
	0.3	62.07	62.38	62.38
	1	74.17	73.98	74.11
	3	82.13	82.45	82.01

Table 4.64 %RSA of the METE and HETE from *T. triangulare*

Solvent System	Conc. (mg/mL)	%RSA		
METE	0.1	34.98	35.17	35.30
	0.3	54.17	53.92	53.73
	1	65.02	65.20	65.52
	3	74.23	74.04	73.73
HETE	0.1	42.01	42.26	42.13
	0.3	60.38	60.56	60.00
	1	71.16	70.60	70.91
	3	80.19	80.06	80.50

Table 4.65 %RSA of the METE and HETE from *H. sabdariffa*

Solvent System	Conc. (mg/mL)	%RSA		
METE	0.1	34.92	34.42	35.11
	0.3	54.11	54.23	53.79
	1	62.88	62.70	62.57
	3	73.79	73.98	74.17
HETE	0.1	41.07	40.88	40.63
	0.3	59.94	60.13	60.44
	1	68.90	69.09	69.22
	3	77.68	77.99	77.49

Table 4.66 %RSA of the METE and HETE from *C. olitorius*

Solvent System	Conc. (mg/ml)	%RSA		
METE	0.1	35.30	35.36	34.86
	0.3	53.67	54.11	53.98
	1	62.13	62.32	61.94
	3	72.98	72.85	72.66
HETE	0.1	40.31	40.19	39.94
	0.3	56.99	57.30	57.17
	1	65.96	65.83	66.08
	3	74.16	73.92	73.80

Table 4.67 %RSA of the METE and HETE from *S. macrocarpon*

Solvent System	Conc. (mg/ml)	%RSA		
METE	0.1	33.92	34.11	33.73
	0.3	53.42	53.22	53.04
	1	61.13	60.94	61.07

	3	72.28	72.10	71.91
	0.1	40.38	40.19	40.31
HETE	0.3	56.99	57.30	57.12
	1	66.02	65.89	65.64
	3	73.60	73.48	73.29

Table 4.68 %RSA of the METE and HETE from *A. cruentus*

Solvent System	**Conc. (mg/ml)**	**%RSA**		
	0.1	31.91	32.16	32.29
METE	0.3	51.10	50.85	50.97
	1	60.00	60.25	60.44
	3	68.97	68.90	69.22
	0.1	37.87	37.68	37.49
HETE	0.3	54.17	54.04	53.92
	1	64.20	63.70	64.01
	3	70.22	70.47	70.09

Appendix 1.8: Antibacterial Activities of the METE and HETE from the Selected Leafy Vegetables

Table 4.69 Effect of different solvent concentration of METE and HETE from *I. batatas* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. aureus*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)
METE	10	15.3		17.7		12.3		10.7	
	7.5	9.4	2.9±3.4	10.6	3±4.1	4.7	3.3±2.9	6.3	2.8±2.3
	5	3.2		2		0		2.7	
	2.5	00		0		0		0	
HETE	10	15.1		7.3		11.7		6.3	
	7.5	10.3	3.0±3.6	2.7	3.3±1.7	9.3	3.1±3.1	1.7	3.1±1.4
	5	1.7		0		0		0.8	
	2.5	0		0		0		0	

MIC = Minimum Inhibition Concentration; Mean±SE

Table 4.70 Effect of different solvent concentration of METE and HETE from *L. aestuans* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. aureus*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)
METE	10	6		4		3		1	
	7.5	3	3.2±1.4	2	3.2±1.0	1	3.3±0.7	0.5	3.2±0.2
	5	0		0		0		0	
	2.5	0		0		0		0	
HETE	10	9.4		9.3		3.3		4.3	
	7.5	3.3	3.2±2.2	6.3	3.1±2.3	3	3.1±0.9	1.7	3.3±1.0
	5	0		0		0		0	
	2.5	0		0		0		0	

MIC = Minimum Inhibition Concentration; Mean±SE

Table 4.71 Effect of different solvent concentration of METE and HETE from *S. macrocarpon* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginc*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MI

METE	10	0	6.3		8.4
	7.5	0	4.7	2.7±1.4	6.6
	5	0	2.3		4.7
	2.5	0	0		3.3
HETE	10	0	3.5		9.3
	7.5	0	1.3	3.3±0.8	5.7
	5	0	0		4.8
	2.5	0	0		2.6

MIC = Minimum Inhibition Concentration; Mean±SE

Table 4.72 Effect of different solvent concentration of METE and HETE from *C. olitorius* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginosa*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (m
METE	10	0		10.7		14.4	
	7.5	0		6.8	2.9±2.4	9.7	2.2
	5	0		2.3		5.5	
	2.5	0		0		2	
HETE	10	0		6.7		7.7	
	7.5	0		5.3	2.8±1.5	5	2.1
	5	0		2.3		2.6	
	2.5	0		0		1	

MIC = Minimum Inhibition Concentration; Mean±SE

Table 4.73 Effect of different solvent concentration of METE and HETE from *T. triangulare* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginosa*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (m
METE	10	7.7		5.5		8.2	
	7.5	4.7	2.4±1.5	3	2.8±1.2	3.3	2.5
	5	2		1.7		2.3	
	2.5	1		0		1	
HETE	10	6.3		5		7	
	7.5	3.8	2.9±1.4	2	3.3±1.2	3.3	3.0
	5	1.2		0		1	
	2.5	0		0		0	

MIC = Minimum Inhibition Concentration; Mean±SE

Table 4.74 Effect of different solvent concentration of METE and HETE from *X. sagittifoliun* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginosa*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (r
METE	10	6.7		5		9.3	
	7.5	4.3	2.2±1.3	3	2.5±1.1	6	2.2
	5	3.3		1.7		3.3	
	2.5	1.3		1		2.3	
HETE	10	8		7.3		13.7	
	7.5	5	2.6±2.0	4.7	1.9±1.2	8	2.5
	5	1.7		3.3		14.7	
	2.5	1.3		2.3		2.7	

MIC = Minimum Inhibition Concentration; Mean±SE

Table 4.75 Effect of different solvent concentration of METE and HETE from *H. sabdariffa* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. aureus*	
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)
METE	10	0		18.3		7.7		7.0	
	7.5	0		11.7	2.0±3.2	4.4	2.8±1.6	5.0	2.1±1.3

	5	0	8		2		3.0	
	2.5	0	3.3		0		1.0	
HETE	10	0	22		10.3		7.6	
	7.5	0	17.3	1.1±2.9	7.2	1.6±1.6	5.4	2.0±1.4
	5	0	12.6		5.4		3.3	
	2.5	0	8.7		2.7		1.3	

MIC = Minimum Inhibition Concentration; Mean±SE

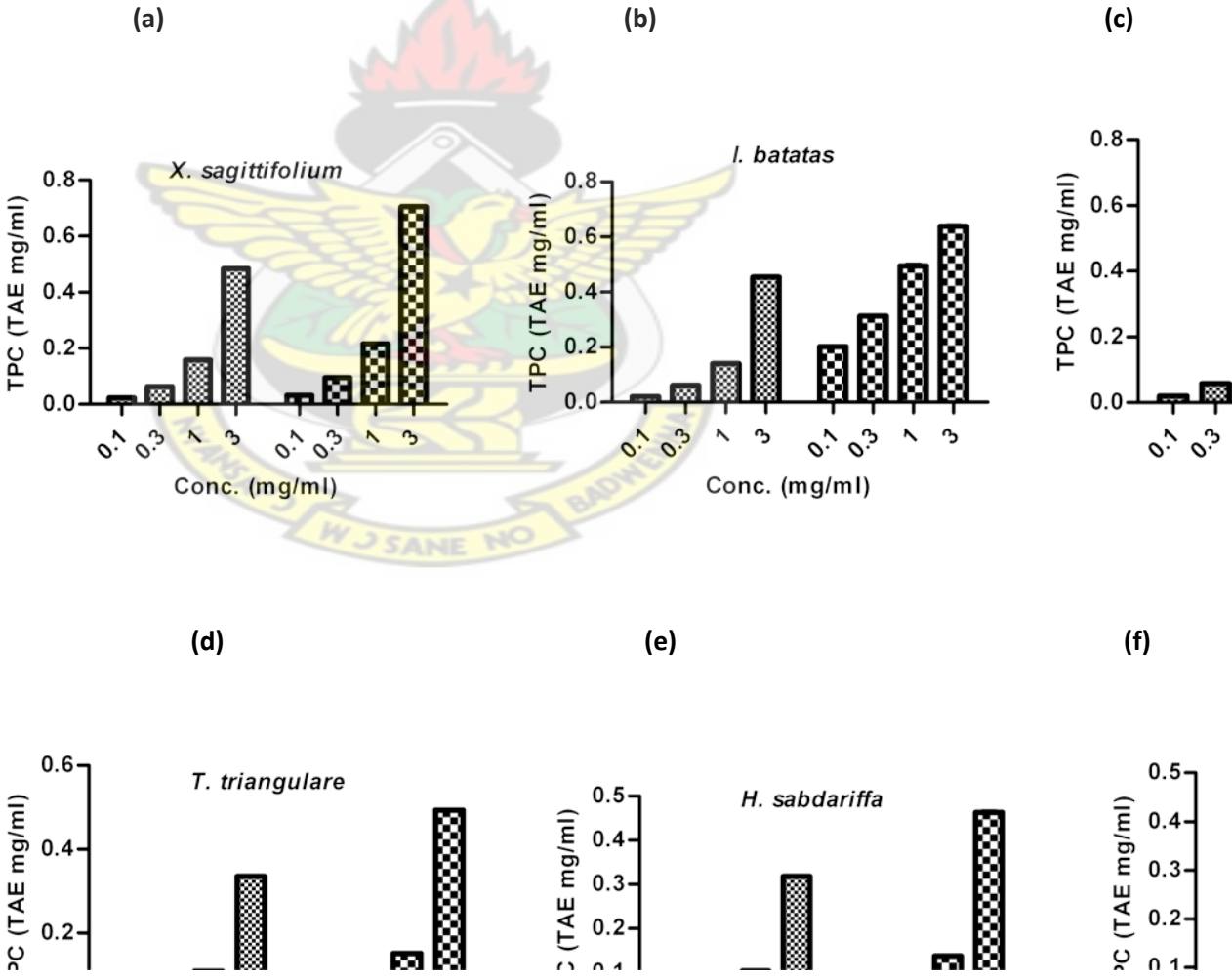
Table 4.76 Effect of different solvent concentration of METE and HETE from *A. cruentus* on bacterial growth (reading after 24 h of growth)

Solvent system	Conc. of Extracts (mg/ml)	*B. subtilis*		*E. coli*		*P. aerug*
		Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)	MIC (mg/ml)	Mean Inhibition Zone (mm)
METE	10	0		0		3
	7.5	0		0		0.7
	5	0		0		0
	2.5	0		0		0
HETE	10	0		0		2
	7.5	0		0		1
	5	0		0		0
	2.5	0		0		0

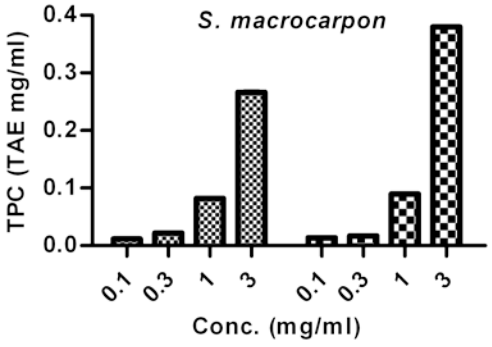
MIC = Minimum Inhibition Concentration; Mean±SE

Appendix 2: Graphical Representation of Experimental Results

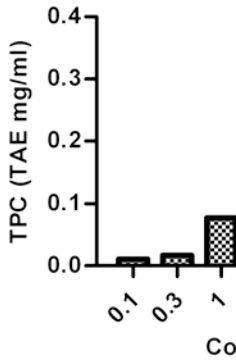
Appendix 2.1 (**a**) to (**h**): TAE (mg/ml) of the METE and HETE from the Selected Leafy Vegetables



(g)



(h)



METE HETE

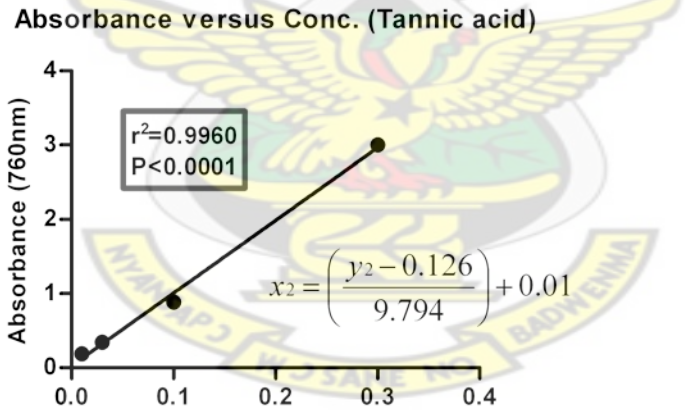
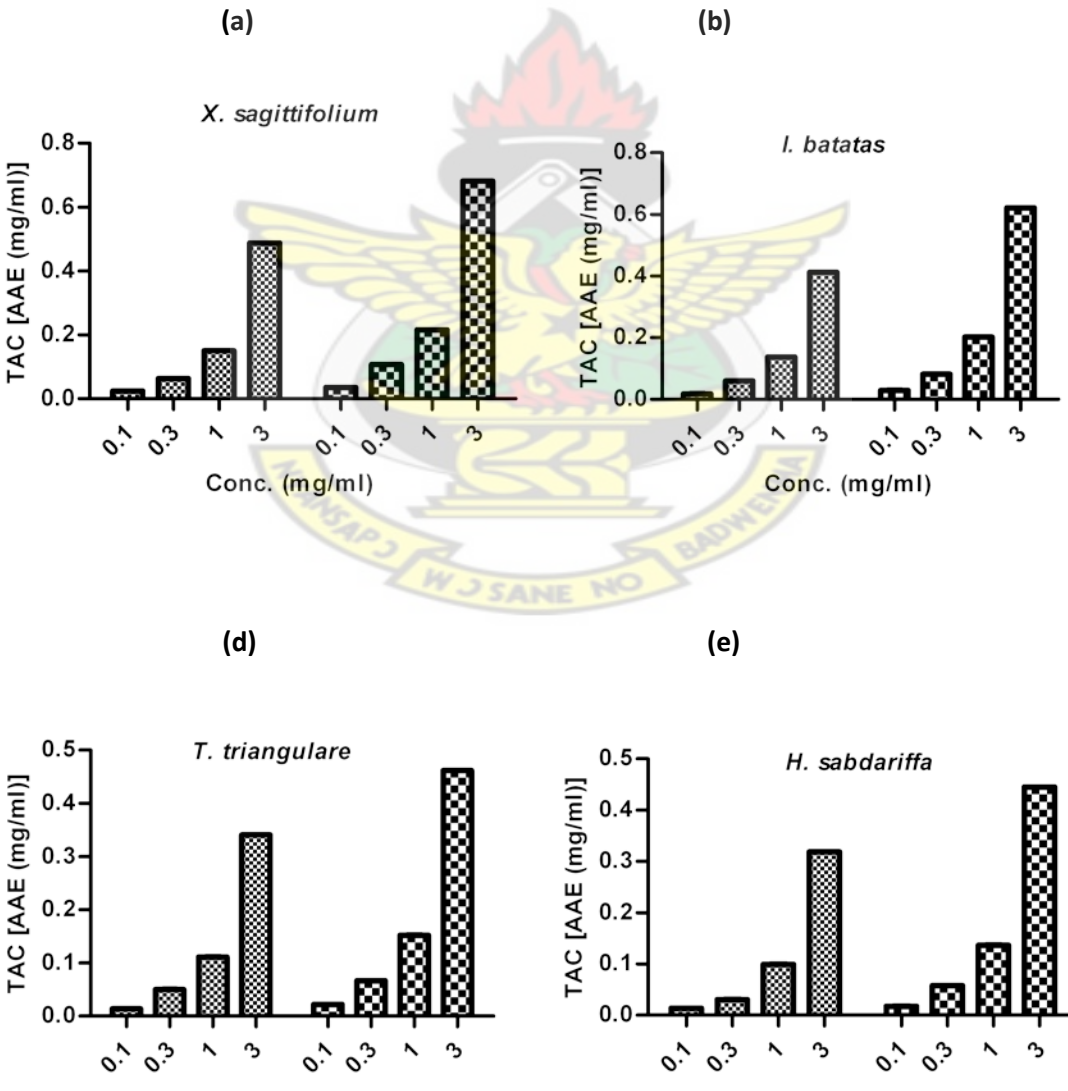
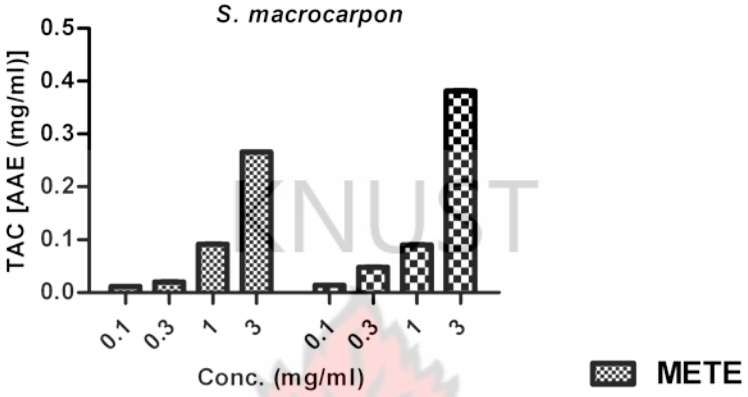

Absorbance versus Conc. (Tannic acid)
Absorbance (760nm)
r²=0.9960
P<0.0001
$x_2 = \left(\frac{y_2 - 0.126}{9.794} \right) + 0.01$
0
1
2
3
4
0.0
0.1
0.2
0.3
0.4

Fig. 4.4 Regression Curve of the Standard Tannic Acid

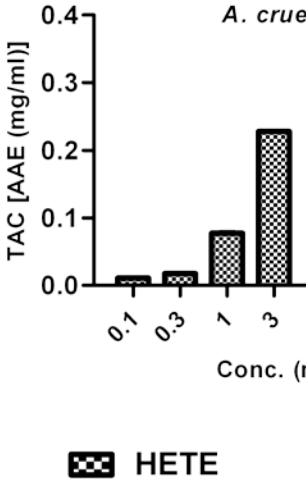
Appendix 2.2 (**a**) to (**h**): AAE (mg/ml) of the METE and HETE from the Selected Leafy Vegetables



(g)


S. macrocarpon
TAC [AAE (mg/ml)]
0.5
0.4
0.3
0.2
0.1
0.0
0.1
0.3
1
3
0.1
0.3
1
3
Conc. (mg/ml)
METE

(h)


A. crue
TAC [AAE (mg/ml)]
0.4
0.3
0.2
0.1
0.0
0.1
0.3
1
3
Conc. (
HETE

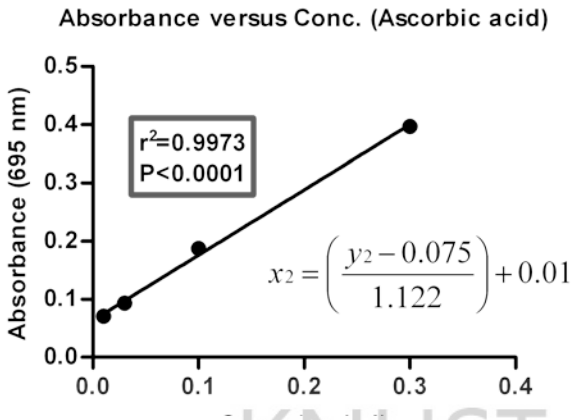
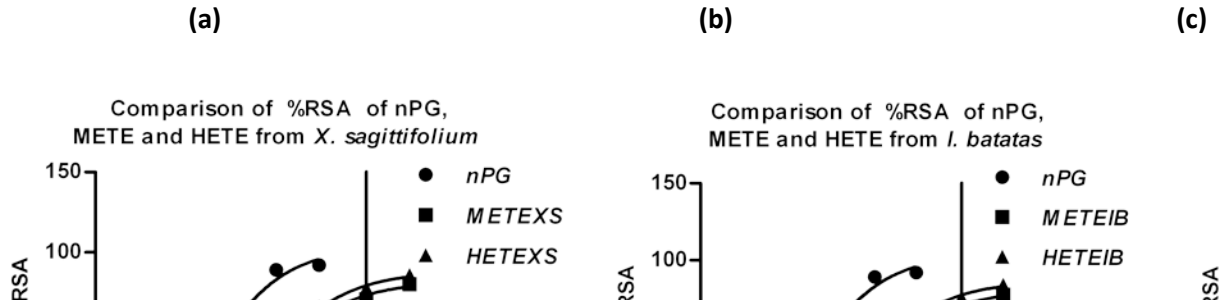
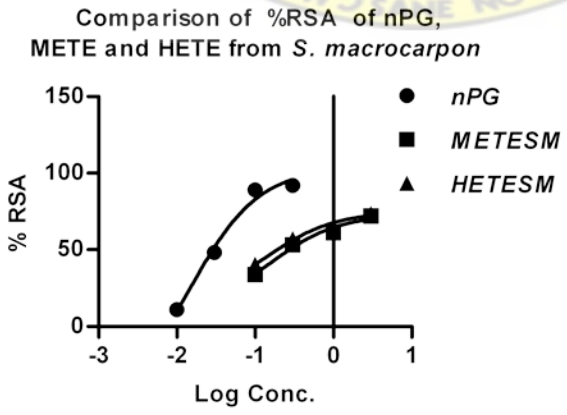


Fig. 4.5 Regression Curve of the Standard Ascorbic Acid

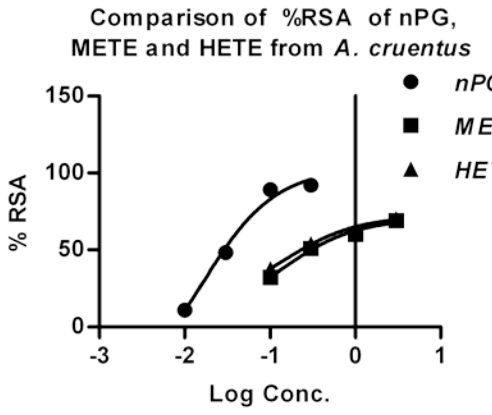
Appendix 2.3(a) to (j): Comparison of Radical Scavenging Activity of nPG, METE and HETE from the Selected Leafy Vegetables against DPPH free radical



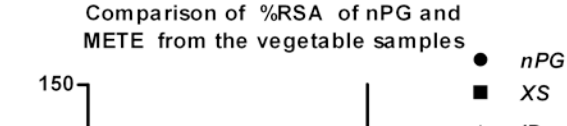
(g)


Comparison of %RSA of nPG,
METE and HETE from S. macrocarpon
nPG
METESM
HETESM
% RSA
150
100
50
0
-3
-2
-1
0
1
Log Conc.

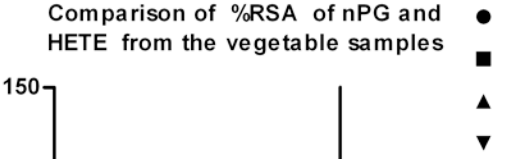
(h)


Comparison of %RSA of nPG,
METE and HETE from A. cruentus
% RSA
150
100
50
0
-3
-2
-1
0
1
Log Conc.

(i)

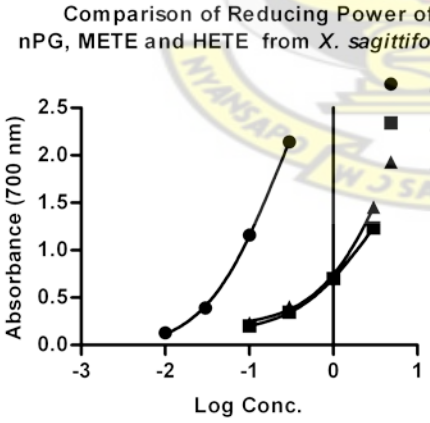

Comparison of %RSA of nPG and
METE from the vegetable samples
nPG
XS
150

(j)

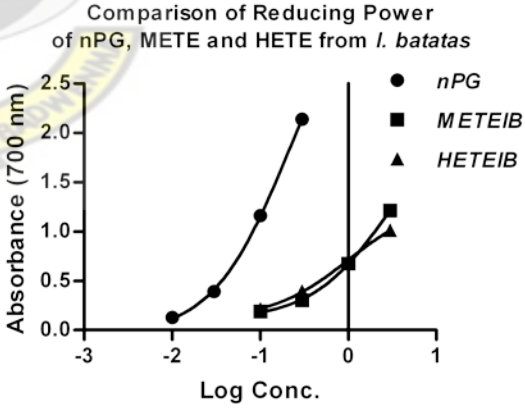

Comparison of %RSA of nPG and
HETE from the vegetable samples
150

Appendix 2.4 (**a**) to (**j**): Comparison of Fe^{3+} Reducing Power of nPG, METE and HETE from the Selected Leafy Vegetables

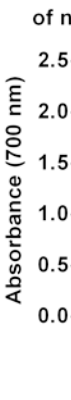
(a)



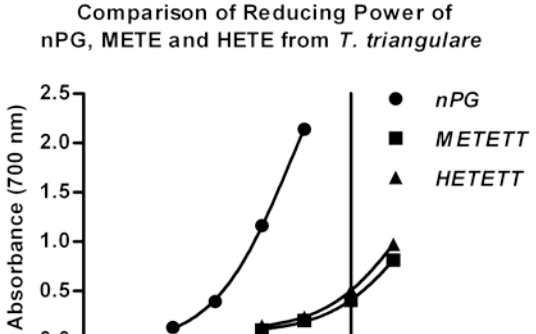
(b)



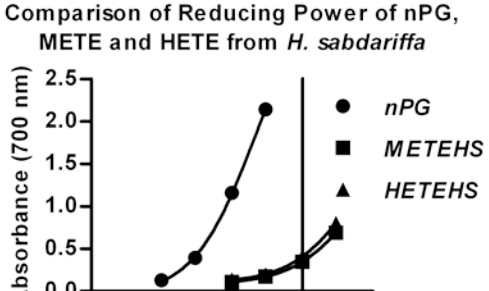
(c)



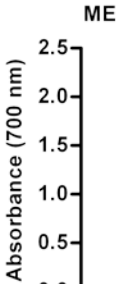
(d)



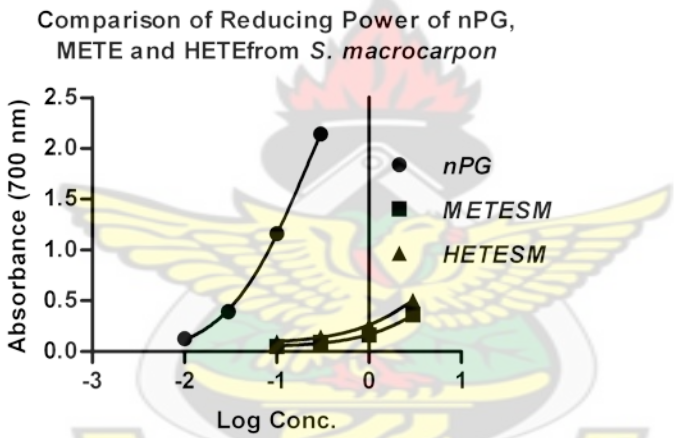
(e)



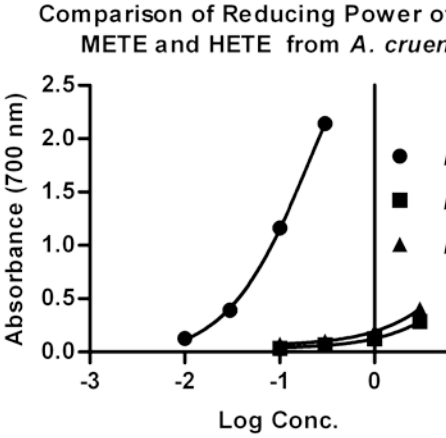
(f)



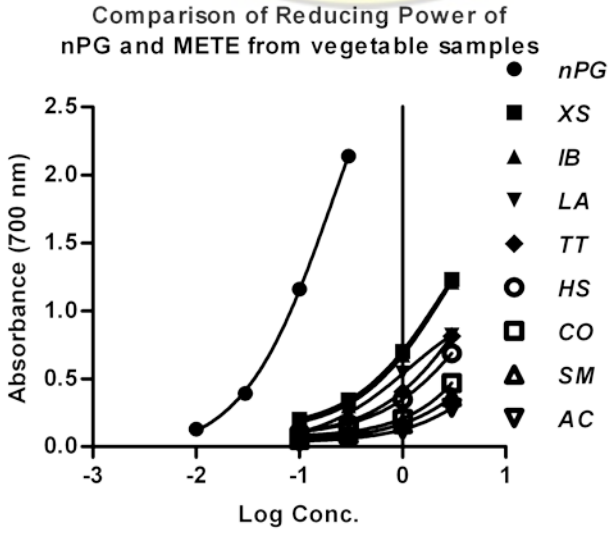
(g)


Comparison of Reducing Power of nPG,
METE and HETEfrom S. macrocarpon
Absorbance (700 nm)
Log Conc.
nPG
METESM
HETESM

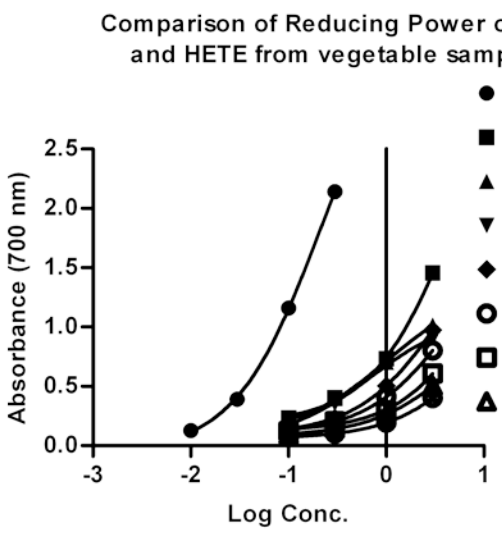
(h)


Comparison of Reducing Power o
METE and HETE from A. cruen
Absorbance (700 nm)
Log Conc.

(i)

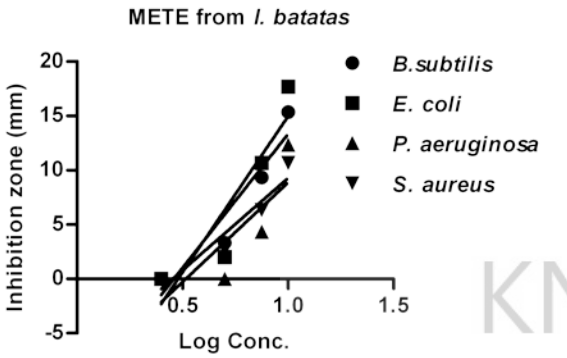

Comparison of Reducing Power of
nPG and METE from vegetable samples
Absorbance (700 nm)
Log Conc.
nPG
XS
IB
LA
TT
HS
CO
SM
AC

(j)

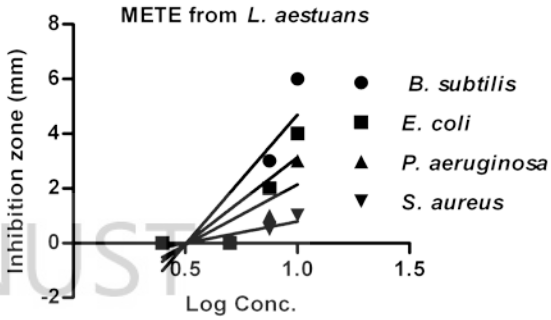

Comparison of Reducing Power o
and HETE from vegetable samp
Absorbance (700 nm)
Log Conc.

Appendix 2.5 (**a**) to (**p**): Minimum Inhibitory Concentrations (MICs) of the METE and HETE from the Selected Leafy Vegetables against the Selected Bacterial Strains

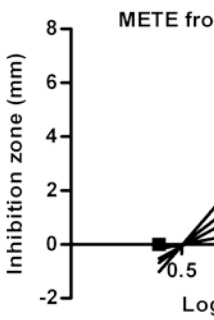
(a)



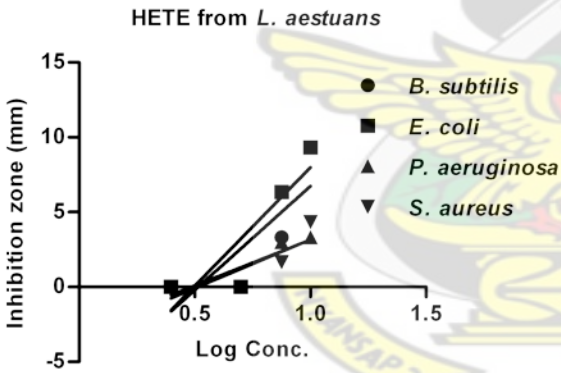
(b)



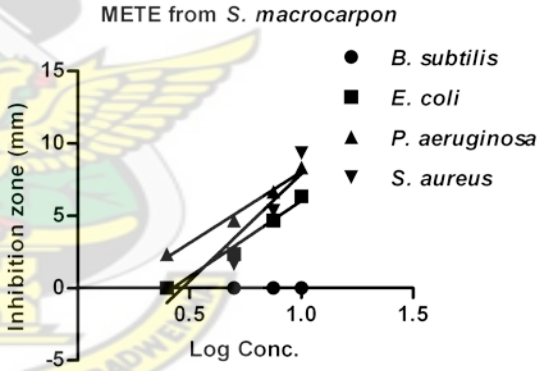
(c)



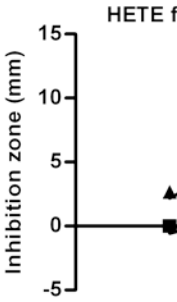
(d)



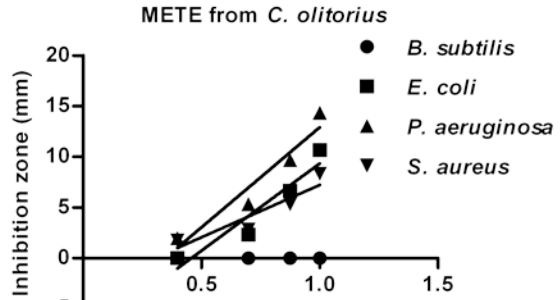
(e)

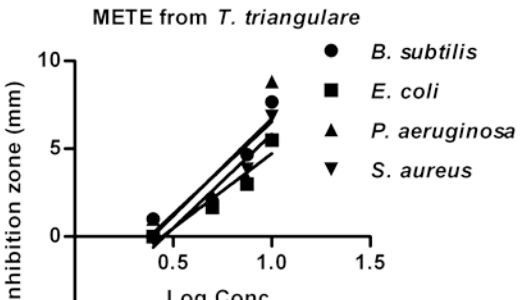


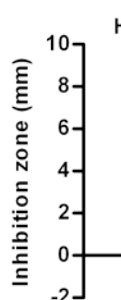
(f)



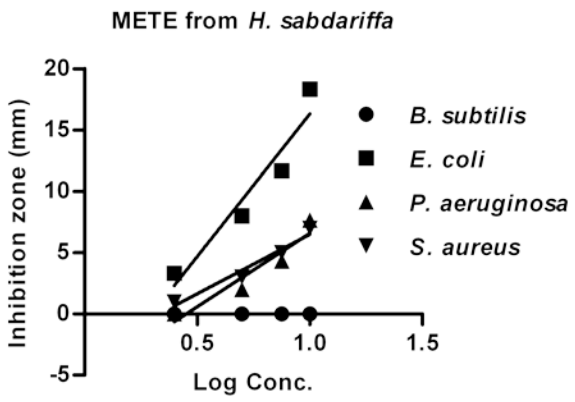
(g) **(h)** **(i)**


METE from C. olitorius
B. subtilis
E. coli
P. aeruginosa
S. aureus
Inhibition zone (mm)
20
15
10
5
0
0.5
1.0
1.5

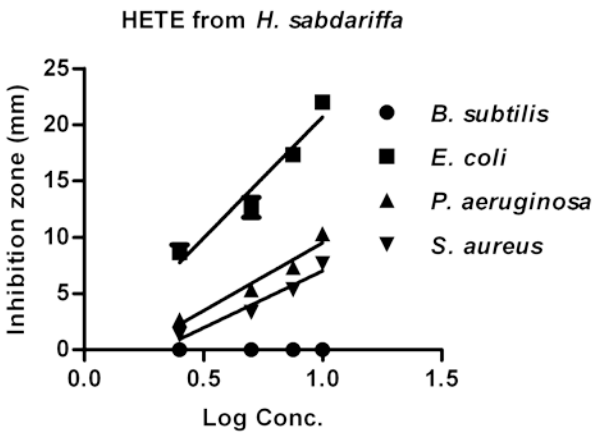

METE from T. triangulare
B. subtilis
E. coli
P. aeruginosa
S. aureus
Inhibition zone (mm)
10
5
0
0.5
1.0
1.5


Inhibition zone (mm)
10
8
6
4
2
0
-2

(m)


METE from H. sabdariffa
Inhibition zone (mm)
20
15
10
5
0
-5
0.5
1.0
1.5
Log Conc.
B. subtilis
E. coli
P. aeruginosa
S. aureus

(n)


HETE from H. sabdariffa
Inhibition zone (mm)
25
20
15
10
5
0
0.0
0.5
1.0
1.5
Log Conc.
B. subtilis
E. coli
P. aeruginosa
S. aureus

Appendix 3.9: Reducing Power (EC_{50}) of Methanolic Extracts (METE) from the selected leafy vegetables

log(agonist) vs. response							
Best-fit values	nPG	XS	IB	LA	TT	HS	
BOTTOM	-0.05367	0.1264	0.1062	0.01576	0.07076	0.07919	0.0
TOP	3.684	2.168	2.241	1.134	1.929	1.735	5.
LOGEC50	-0.6769	0.4039	0.4438	0.06066	0.6550	0.7117	1.
EC50	0.2104	2.535	2.778	1.150	4.518	5.148	3(
Span	3.738	2.042	2.135	1.118	1.858	1.656	5.

Appendix 3.10: Reducing Power (EC_{50}) of Hydro-Ethanolic Extracts (HETE) from the selected leafy vegetables

log(agonist) vs. response							
Best-fit values	nPG	XS	IB	LA	TT	HS	
BOTTOM	-0.05367	0.1895	0.1109	0.04670	0.09367	0.09686	0.1
TOP	3.684	3.719	1.314	1.106	2.176	1.988	2.
LOGEC50	-0.6769	0.7303	0.002087	-0.1615	0.6118	0.7014	1.
EC50	0.2104	5.374	1.005	0.6894	4.091	5.028	14
Span	3.738	3.529	1.203	1.060	2.083	1.891	2.

Appendix 3.11: Radical Scavenging Activity (EC_{50}) of Methanolic Extracts (METE) from the selected leafy vegetables against DPPH Free Radical

log(agonist) vs. response							
Best-fit values	nPG	XS	IB	LA	TT	HS	
BOTTOM	-51.08	8.449	14.52	23.95	10.61	12.21	1
TOP	103.3	81.76	80.43	79.00	76.38	75.48	7
LOGEC50	-1.820	-0.8881	-0.7581	-0.6333	-0.7810	-0.7582	-0.
EC50	0.01513	0.1294	0.1746	0.2326	0.1656	0.1745	0.
Span	154.3	73.31	65.92	55.05	65.77	63.27	6

Appendix 3.12: Radical Scavenging Activity(EC_{50}) of Hydro-Ethanolic Extracts (HETE) from the selected leafy vegetables against DPPH Free Radical

log(agonist) vs. response							
Best-fit values	nPG	XS	IB	LA	TT	HS	
BOTTOM	-51.08	7.090	10.63	17.68	20.13	9.857	1
TOP	103.3	87.97	86.17	84.70	82.54	78.91	7
LOGEC50	-1.820	-0.9258	-0.8838	-0.7997	-0.7449	-0.9167	-0.
EC50	0.01513	0.1186	0.1307	0.1586	0.1799	0.1212	0.
Span	154.3	80.88	75.54	67.02	62.41	69.06	5