Faculty of Sciences

Department of Biological Sciences and Biotechnology

ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF *Azanza garckeana* and *Portulaca Oleracea* ON HELA CELLS

by

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ABBREVIATIONS AND ACCRONYMS

ABC-	ATP Binding Cassette Protein
ABTS	2,2'-Azino-Bis 3-Ethylbenzothiazoline-6-Sulfonic acid
ATP	Adenosine Triphosphate
COX-2	Cyclooxygenase 2
DMEM	Dulbecco 's modified Eagle Medium
DPPH	2,2-Diphenyl-1-Picryl Hydrazyl
EDTA	Ethylenediaminetetracetic Acid
ERK	Extracellular Signal Regulated Kinase
FBS	Fetal Bovine Serum
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography Mass Spectrometry
GPH	Gaborone Private Hospital
GPx	Glutathione Peroxidase
GST	Glutathione-S-Transferase
HPV	Human Papillomavirus
IPMS	Integrated Patient Management Systems
L-glut	L-glutamine
MAPK	Mitogen Activated Protein Kinase.
MEK	Mitogen Activated Protein Kinase Kinase.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-$_k$B	Nuclear Factor Kappa B
NHL	National Health Laboratory
PBS	Phosphate Buffered Saline
Pen-strep	Penicillin-streptomycin

PMH	Princess Marina Hospital
QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase.
TE	Trolox Equivalent
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

DECLARATION REGARDING THE WORK AND COPYRIGHT

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ABSTRACT

Mortality and incidence rates of cervical cancer are on the rise in sub-Saharan Africa, particularly in Botswana. Current cervical cancer management strategies such as chemotherapy and radiation are not fully effective as they bring about major side effects and issues such as drug resistance. Indigenous plants of Botswana have been used in ethnomedicine to treat conditions related to cancer. Two known indigenous plants; *Azanza garckeana* and *Portulaca oleracea* have been found to contain phytochemicals which contribute to their antioxidant and anticancer properties. The aim of this study was to evaluate the antioxidant and anti-proliferation potential of extracts derived from *Portulaca oleracea* and *Azanza garckeana*. The study started with the derivation of extracts from the leaves, stems and roots of these two plants using six different solvents; methanol, ethanol, chloroform, distilled water, hexane and isopropanol in order of their polarity. Phytochemical screening of the extracts was then done to determine different secondary metabolites present. Total phenolic and flavonoid content of the different extracts was determined followed by the determination of the antioxidant activity of the extracts. GC-MS analysis was then conducted to detect different bioactive compounds present in the extracts.

Anti-proliferation activity was determined by the cell viability assay; MTT to determine the IC_{50} values of the different extracts. It was found out that methanol effectively extracted antioxidant compounds.

P. oleracea stem and leaf methanolic extract had high total phenolic and flavonoid content of 16.25 ± 0.48 TE mg/100 g, 16.39 ± 0.51 TE mg/100 g, 45.81 ± 1.02 QE mg/100 g and 41.70 ± 3.69 QE mg/100 g respectively whereas *A. garckeana* leaf methanolic had high total phenolic and flavonoid content of 14.00 ± 1.00 TE mg/100 g and 52.03 ± 2.44 QE mg/100 g respectively.

It was also found out that chloroform extracts had good antioxidant activity as the *P. oleracea* stem chloroform extracts gave a high antioxidant compounds concentration of 687.50 ± 0.88 TE Mmol/g and radical scavenging activity of 97.83 % ± 1.59 due to the presence of antioxidant compounds in the extracts. Methanolic and ethanolic extracts displayed anti-proliferative activity towards HeLa cells used in study and the extracts affected cell viability in a dose dependent fashion. *A. garckeana* leaf ethanolic extracts reduced cell viability by 92.00 % at 400 µg/ml extract concentration, *P. oleracea* leaf methanolic extracts reduced cell viability by 89.00 % at 400 µg/ml extract concentration, *A. garckeana* leaf methanolic extracts reduced cell viability by 91.00 % and *P. oleracea* stem methanolic extracts reduced cell viability by 69.00 % at 400 µg/ml extract concentration.

The IC_{50} values of the extracts which measured the cytotoxicity degree of the extracts were found to be 329.31 µg/mL ± 54.69, 176.44 µg/mL ± 25.49 µg/mL, 190.47 µg/mL ± 46.10, 242.09 µg/mL ± 23.60, 22.78 µg/mL ± 10.37 for *P. oleracea* leaf methanolic extract, *A. garckeana* leaf methanolic extract, *A. garckeana* leaves ethanolic extract, *P. oleracea* stem methanolic extract and cisplatin (anti-cancer drug) respectively.

The anti-proliferative and antioxidant activity of the extracts of *P. oleracea* and *A. garckeana* was speculated to be due to the presence of antioxidant and anticancer compounds in the extract such as; 10-acetoxy-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester ,octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, 3(2*H*)-benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-,(*E*), cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-, 3-O-Methyl-D-glucose, cyclononasiloxane and octadecamethyl.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

In Botswana, cervical cancer is the most prevalent cancer in women between the ages of 15-45, with the incidence rates anticipated to be 374 women diagnosed with the disease per year [1]. Cervical cancer has been identified as a prevailing cause of cancer mortality in middle-and low-income countries [2]. Additionally, among all age groups, cervical cancer has greater incidence amongst young women [2]. It has been speculated that 75 % of cancer deaths globally will arise in lower-middle income countries by 2030 [3].Cervical cancer is characterized by the development of tumours around the cervical region in the body. There are two types of cervical cancer: squamous cell carcinoma and adenocarcinoma [4]. These two types of cervical cancer are distinguished based on the site of development of the cancer with cervical squamous cell carcinoma arising from the outer part of the cervix whereas adenocarcinoma arises from glandular cells in the inner part of the cervix [4]. The common risk factors associated with cervical cancer are the human papillomavirus (HPV), the use of oral contraceptives, multiple concurrent partners, early onset of sexual intercourse and smoking [2,5].

All different types of cancer, including cervical cancer, have common features which are referred to as hallmarks. Firstly, cancer cells can maintain proliferation signalling in which they do not regulate the way that growth promoting signals are made and released as opposed to normal cells [6]. This causes abnormality in the division and growth of the cancer cells [6].

Secondly, cancer cells can escape growth suppressors and become insensitive to anti-growth signals [6].To strictly manage cell division, cells have certain systems and processes that ensures that cells are not overstimulated to continue growing and dividing [6]. Furthermore, normal cells use tumour suppressor genes and contact inhibition mechanism to ensure that overstimulation does not occur. However, in cancer cells, the tumour suppressor genes are downregulated which masks their function, and therefore the cells continue growing disproportionately [6]. Cancer cells can also be insensitive to anti-growth signals by bypassing contact inhibition in which the cells continue to grow regardless of filling up the spaces they are in and touching adjacent cells [6].

Another feature of cancer cells is their ability to avoid apoptosis which is programmed cell death [6]. Cancer cells can bypass the apoptosis mechanism as they become insensitive to anti-growth signals such as tumour suppressor genes [6]. These tumour suppressor genes, such as the p53 and Rb, are downregulated in cancer cells whereas anti-apoptotic proteins such as the Bcl-2 are upregulated, and this paves way for the inhibition of apoptosis in cancer cells [6]. Moreover, cancer cells have an unlimited replicative potential [6]. This occurs due to the maintenance of telomere length. This is because cancer cells influence enzymes responsible for increasing the length of telomeres [6,7]. Consequently, cancer cells proceed with cell division and override senescence, a state where the cell acquires the ability to acquire genome instability [6,7].

Moreover, cancer cells portray the ability to undergo persistent angiogenesis in which the cells produce new blood vessels in order to obtain a constant supply of oxygen and nutrients [6-8]. The cancer cells will then continue to proliferate as they have a good supply of oxygen and nutrients. The other hallmark which describes the brutality of cancer is the ability to invade nearby tissues and metastasize [6].

Cancer cells can form their own environment known as a tumour microenvironment to manoeuvre their way to another tissue by invading blood vessels and are incorporated into the circulatory systems enabling them to replicate and survive [6-8]. Cancer cells are also specialized by having a deregulated metabolism [6]. The cells have developed systems to harness energy efficiently and effectively. The efficiency and effectiveness of this is through the Warburg effect in which the cells enhance glycolysis and lactic acid fermentation in the cytosol and inhibit the mitochondria from completing normal aerobic respiration [7,8].

Therefore, the cells shift from complete oxidation of glucose to make ATP to converting pyruvate to stimulating the production of more cells [6-8]. In addition, cancer cells can avoid interaction with the immune system, and this can be due to their drug resistance which is seen in several types of cancer [6,7].

It is also worth noting that cervical cancer can be attributed to external factors such as radiation. These external factors trigger oxidative stress in which there is over production of reactive oxygen species (ROS) which leads to oxidative stress [9,10]. The production of free radicals such as ROS has been associated with DNA mutations which eventually leads to the progression of cervical cancer [9-11]. Upon the body encountering HPV infection which have been linked to the onset of cervical cancer, there is an alteration in cell growth, proliferation, and cell death patterns and this eventually leads to tumour progression [9-11].

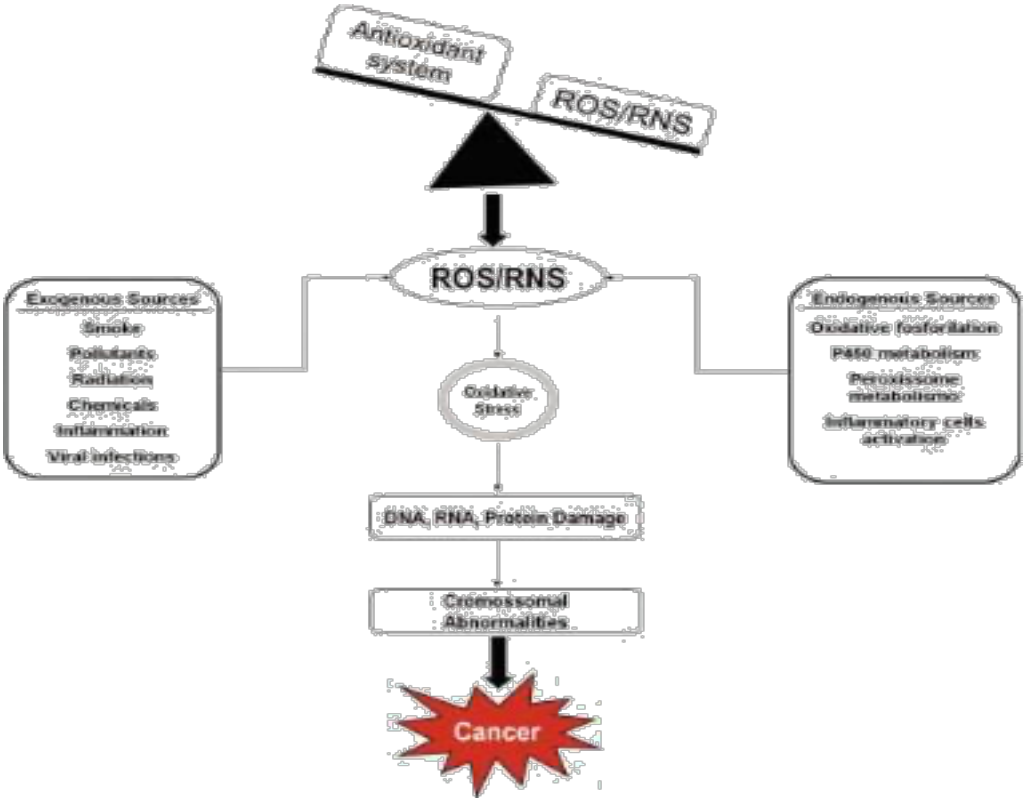


Fig. 1: External and internal factors linked with initiation and progression of cancer .

There are various external sources referred to as exogenous and internal sources referred to as endogenous linked with the production of ROS. Upon accumulation of ROS, oxidative stress is induced leading to DNA, RNA and protein damage and this causes cancer.

Studies have demonstrated that certain natural compounds such as polyphenols, flavanols, carotenoids and steroids have antioxidant properties, making them chemo preventive and chemotherapeutic agents [9,10],[12,13]. Antioxidants have an impeccable feature of scavenging free radicals that the body can over produce. These antioxidants have been marked as a protective measure against HPV persistence and thereby can alleviate cervical cancer [11,12,14].

Sources of these antioxidants are plant extracts from medicinal plants. Key studies have unlocked the antioxidant and anticancer nature of two indigenous plants in Botswana namely *Portulaca oleracea* which is commonly known as the pig weed and "Serepe" in Setswana and *Azanza garckeana* which is commonly known as the African chewing gum or snot apple and "morojwa" in Setswana [13,15-19].

1.2 Statement of the problem

Cervical cancer cases are steadily on the rise in Botswana, and it has been placed as the most common cancer affecting women between the ages of 15-44 years. A 2021 report by ICO/IARC information centre on HPV and cancer has indicated that on average, 374 women are diagnosed with cancer and 207 women succumb to the disease annually [1]. However, current cervical cancer management strategies are not fully effective, necessitating the exploration of alternative cancer management strategies such as the use of phytochemicals derived from medicinal plants.

1.3 Justification of study

Key studies have indicated that phytochemicals; phenols and flavonoids from *Portulaca oleracea* and *Azanza garckeana* can inhibit the proliferation of cervical cancer cells [12,15-19]. These effectively slow down the growth of cancer cells and can be used to develop anticancer drugs, replacing conventional methods such as chemotherapy, hormonal therapy, and surgery. Additionally, conventional cancer treatment methods are not fully effective as cancer relapse can occur.

1.4 Research question

Does *Azanza garckeana* and *Portulaca oleracea* display antioxidant and antiproliferative activity towards HeLa cells ?

1.5 Research Hypothesis

Phytochemicals derived from *P. oleracea* and *A. garckeana* will display antioxidant activity and anti-proliferative activity towards HeLa cells used in study.

1.6 General aim

To evaluate the antioxidant and anti-proliferation potential of phytochemicals derived from *Portulaca oleracea* and *Azanza garckeana* in HeLa cells, which are cervical cancer cells.

1.7 Objectives

To accomplish the general aim, the following objectives were set.

Objective 1: Extract phytochemicals from leaves, stems, and roots of *P. oleracea* and *A. garckeana* .

Objective 2: Determination of total phenolic and flavonoid content and antioxidant activity of the extracts.

Objective 3: Identification of the specific phytochemical components responsible for the antioxidant and anti-proliferative activity of *Portulaca oleracea* and *Azanza garckeana* extracts.

Objective 4: Determination of the cytotoxic effect of *Portulaca oleracea* and *Azanza garckeana* extracts on HeLa cells.

CHAPTER TWO

LITERATURE REVIEW

2.1 Oxidative stress and cervical cancer

Oxidative stress has been linked with the onset of cervical cancer and the progression of the disease. Oxidative stress occurs when there is an accumulation of reactive oxygen species (ROS) and there is insufficient antioxidant protection [9]. Several enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and catalase function to protect against damage induced by oxidative stress [20,21]. Furthermore, other antioxidant protective measures are non-enzymatic and include natural products derived from plants such as flavonoids [21]. Excessive oxidative stress can trigger DNA damage, causing mutations of tumour suppressor genes which contributes to the overstimulation of the cells to grow: one of the key features of cancer cells [20,21]. This excessive oxidative stress will then aid in a multi-step approach during carcinogenesis.

A study reported that continuous viral HPV infection causes the production of ROS leading to oxidative stress. This makes HPV a major risk factor of cervical cancer [20]. Furthermore, it has been demonstrated that in HPV associated tumours, SOD2, which is an antioxidant enzyme, is overexpressed and this causes oxidative stress. Proteins known as oncoproteins, such as the E6 and E7 also contribute to oxidative stress, thereby leading to cervical cancer by working hand in hand with tumour suppressor factors and cell cycle regulators such as the p53 [9,10,20]. This synergistic relationship causes cells to resist apoptosis and heightens immune evasion [20].

2.2 Current cervical cancer management interventions employed in Botswana

The high mortality rates of cervical cancer cases in Botswana have been attributed to late diagnosis. These cases of high mortality rates can be curbed through easy access to treatment, early presentation of the disease and timely diagnosis [22]. Princess Marina hospital in Botswana has been set as the national referral hospital for southern Botswana and the receiving capacity for cervical cancer cases stands at 100-140 cases per year [22] which is burdensome as the hospital can be overloaded with cases, resulting in late access to treatment. Furthermore, the referral hospital provides the National Health Laboratory (NHL) with information of suspected cancer cases and the NHL provide histopathology services which helps with cervical cancer diagnosis. The channel of diagnosis is done through, firstly, submission of histology requests through the Integrated Patient Management Systems (IPMS) to hospitals where specimens will be transported by hospital staff to the National Health Laboratory [22-24].

Afterwards, the histology results are disseminated from the NHL back to the hospital using the IPMS [22-24]. On the other hand, some patients are referred from private facilities and their histology samples are processed in private laboratories [23] which is costly, and most Batswana citizens cannot afford to acquire such services and are forced to join the long queues at public hospitals.

Upon diagnosis of cervical cancer, patients can go through definitive surgery at Princess Marina Hospital or alternatively in private hospitals. Patients are also offered chemoradiation services at Gaborone Private Hospital (GPH) [22,24]. These incentives are offered by the government of Botswana to all Batswana citizens with all costs covered solely by the government. However, one study indicated that in 2015 and 2016 the one radiation unit in the country was shut down for renovation and patients were referred to other treatment centres in South Africa [22].

This shows the inadequacies in the provision of cervical cancer therapy in the country as it is not a guarantee that the country will be able to provide such, this can contribute to higher mortality cases. Moreover, one study hinted that administration of chemotherapy, surgery and drugs are based on the stage of the cervical cancer. For patients early-stage cervical cancer, the basic treatment was surgery and radiation therapy with or without cisplatin-based chemotherapy [25]. For patients with late-stage cervical cancer, their mode of treatment was a mixture of external beam radiotherapy with simultaneous cisplatin-based chemotherapy and brachytherapy [25]. The study continued to reveal that the survival rate of patients at late stages was at 40%, indicating that the modes of intervention are not always successful [25]. Factors which contributed to survival were haemoglobin concentration levels where lower haemoglobin concentrations led to radio resistance for patients receiving radiotherapy [25-27]. It is paramount that other modes of intervention be considered to ensure higher survival rates of patients with cervical cancer.

2.3 *Portulaca oleracea* as a medicinal plant in cervical cancer management





Fig. 2: *Portulaca oleracea* whole plant which is characterized by small and round fleshy leaves. The shrub is also identified with its yellow leaves and red fleshy stems.

Portulaca oleracea, which is most often referred to as purslane, is a succulent plant characterized by reddish, translucent stems [17,28] which normally grows as a weed in several parts of Botswana during the rainy season. This plant has been shown to be a therapeutic agent against cancer due to its antioxidant and anticancer properties. Several studies have reported that extracts of *Portulaca oleracea* contain several phytochemicals such as flavonoids, steroids and polyphenols which are responsible for the antioxidant and anticancer properties of the plant [15-18,29].

In one study, it was discovered that the administration of *Portulaca oleracea* aqueous extracts to adult male waster albino rats remarkably increased the activity of superoxide dismutase and catalase in the liver, kidney and testes, suggesting that the extracts exhibited antioxidant effects on the tested organs [30].

Another study showed that Homo isoflavone isolated from *Portulaca oleracea* contributed to the antioxidant nature of the plant showing that *Portulaca oleracea* fights against oxidative stress which contributes to cervical cancer by the presence of such compounds [31]. According to one study it, was disclosed that *Portulaca oleracea* had a mild cytotoxic effect against the HeLa cell line due to the presence of water soluble polysaccharides and the sulphated form of the water soluble polysaccharides augment the anti-tumour effect [32]. The study also highlighted that extracts of *Portulaca oleracea* exhibit anti-proliferative effects against HeLa cells by the cell cycle arrest during the S-phase [32]. The aqueous extract of the plant demonstrated an anti-proliferative and apoptotic effect in a dose and time dependent fashion after MTT assay [32]. One other key study highlighted that *P. oleracea* extracts bring about G2/M-phase growth arrest in HeLa cells causing a reduction in growth rate. It was also shown that *P. oleracea* work in synchrony with cisplatin to induce apoptosis by raising intracellular oxidative pressure in the HeLa cells [33]. Additionally anti- tumour effects were exhibited through the over expression of the caspase 3 and 9 apoptotic proteins after treatment of HeLa cells with *P. oleracea* extracts combined with cisplatin [33]. However, the study did not reveal the synergistic effect of combining extracts from different plant parts of *P. oleracea* which may indicate that the plant extracts do not exhibit powerful antioxidant and anticancer effect when combined with cisplatin.

Similarly, previous studies did not indicate the biochemical composition in different plant parts of *P. oleracea* and therefore it cannot be concluded that *P. oleracea* is successful in cancer management as treatment would have to be administered using all plant parts in *vivo* determine the success rate of the plant extracts in cervical cancer management.

2.4 *Azanza garckeana* as a medicinal plant in cervical cancer management





Fig. 3: *Azanza garckeana* plant which is characterized by large and yellow creased leaves. It is also seen with its round green fruits which are referred to as the African chewing gum.

The *Azanza garckeana*, which is popularly known as the African chewing gum, is a small shrub known for its edible fruit and it is characterized by its creased and large green leaves with yellow flowers [19]. Several studies have exploited the pharmacological activity and value of this plant. One study reported that methanolic extracts of this plant's fruit pulp had significant amounts of phytochemicals particularly phenols, flavonoids and alkaloids when compared to the ethyl acetate extract which had a greater amount of tannins [19]. The study continued to reveal that the highest amount of phenolics was measured at 34.32 ± 2.34 mg/100 g of dry sample when compared to ethyl acetate which had a phenolic composition of 25.34 ± 0.32 mg/100 g of dry sample [19]. This suggests that methanol has an excellent capability of extracting high amounts of phytochemicals. Another study determined the antioxidant potential of stem bark extracts of *A. garckeana* and reported that methanolic extracts of stem bark extracts of the plant displayed a significant DPPH radical scavenging activity of $IC_{50} < 100$ µg/mL [13].

Moreover, *A. garckeana* seeds extracts have demonstrated its anticancer potential against ovarian cancer due to the prevalence of flavonoids which constricts the action of oestrogen synthase, an enzyme which formulates oestrogen [17,34]. Another investigation profiled several active components present in the hydro ethanol extracts of fruits of *A. garckeana* identified components such as hexadecenoic acid and 9,12-tetradecadien-1-ol which have been attributed to the anti-tumour and anti-proliferative nature of the plant against several cancer cells [35].Traditionally *A. garckeana* leaves, stems and roots have been used for the treatment of menstrual disorders and treatment of sexually transmitted diseases in Nigeria, Malawi and Kenya [36-38]. This infers their anti-tumour and anti-inflammatory activity which has been reported in other studies. However, the studies have focused mainly on the pharmacological importance of *A. garckeana* fruits and have not focused much on stem-bark extracts.

2.5 Recent treatment and response of HeLa cells to *P. oleracea* and *A. garckeana* extracts

It has been reported that *P. oleracea* aqueous extracts exhibit antiproliferative effects towards HeLa cells and the cytotoxicity displayed by the extracts is concentration and time dependent [32]. An observation made from one study indicated that an aqueous extract (POL P3b) of *P. oleracea* significantly halted cell proliferation and tumor growth in U14 bearing mice [32]. In another study, it was indicated that polysaccharides extracted from *P. oleracea* targets free radicals and promotes antioxidant activity thereby modulating the immune response which leads to an anticancer effect [31,33].

Furthermore, another study has indicated that the half maximal inhibitory concentration for *P. oleracea* extracts (IC_{50}) on HeLa cells were 139.5 ± 1.26 µg/mL after 48 hrs incubation [33]. The study continued to show that higher extract concentrations are needed to reach notable cytotoxic effect [33]. It was further reported that HeLa cells displayed a diminished cell viability when treated with *P. oleracea* extract concentrations between 100 to 800 µg/mL over periods of 24 to 72 hrs indicating that there was a significant decrease in cell proliferation which was observed in a dose dependent manner[33].

For *Azanza garckeana* extracts, a study has reported that the crude methanol extract of the *A. garckeana* pulp has greater phenols and flavonoids which are noticed for their antioxidant properties[19]. The In vitro assays have effectively shown dose dependent antioxidant activities with IC_{50} indicating efficient scavenging ability against free radicals such as DPPH [36-38].

Another study has shown that an aqueous fruit extract of the *A. garckeana* can obstruct the proliferation of HeLa cells [35]. The study reported that, higher doses of the extract significantly reduce the cell viability of HeLa cell proving that cell viability is affected in a concentration-dependent manner [35].

However, from the studies not all plant parts have been used to treat the HeLa cells therefore the need to explore other plant parts to determine the antiproliferative and antioxidant effect of the plant extracts.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant collection

Azanza garckeana leaves, stems and roots were carefully collected from Serowe (22° 26' 52"S 26° 44' 46"E) whereas *Portulaca oleracea* whole plant was collected from Palapye, Khurumela Ward (23°25'11"S 26°44'0"E). The identity of the plants was verified by the Herbarium Unit of the Botswana National Museum (*A. garckeana* voucher no: 011BRAG.F-BIUST; *P. oleracea* voucher no: 003BRPO.P-BIUST). *Azanza garckeana* was identified as a small shrub with round, simple, alternate, lobed, green leaves with yellow flowers whereas *Portulaca oleracea* was identified by its small, round, fleshy leaves and stems.

3.2 Plant preparation

The leaves, stems and roots of the two plants were separated and washed with tap water to remove dirt . The separated plant parts were dried under direct sunlight for seven days. After drying, the separated leaves, stems and roots were crushed into fine powder using a pestle and mortar for the stems and a waring commercial blender (Model 32BL79, Dynamics corporation, New Hartfort, Connecticut, USA) for the leaves and roots. The fine powder was stored in sample zip-lock bags and kept at -20°C for future use.

3.3 Extraction of phytochemicals

Various plant parts were macerated in Erlenmeyer flasks with 20 g/ 200 mL (w/v) of plant powder : solvent in a non-exhaustive set up. Maceration was used as a method of extraction to ensure preservation of the bioactivity and structure of extracted phytochemicals [39]. Six different solvents were used to extract phytochemicals based on the degree of polarity from non-polar, least polar, moderate polar to highest polar; hexane (non-polar), chloroform(slightly polar), isopropanol (moderately polar), ethanol, methanol and distilled water (most polar). This was done to establish the relationship between extraction efficiency and solvent polarity as mentioned in previous studies [39-41]. Maceration was done for a period of 48 hrs with vigorous shaking using an orbital shaker (OS-350D, Digisystem Laboratory Instrument Inc., New York, USA) set at a speed of 1500 rpm to maximize extraction [42,43].

Extracts were filtered with a funnel and Whatman filter paper of 0.45 µm to remove insoluble material. The remaining solvent was concentrated at 45°C using a rotary evaporator (Heidolph Laborato 4000 efficient, Bavaria, Germany) to acquire the crude extracts. The extracts were dried under a fume hood for a period of two days, and then stored at 4ºC for further use. The extraction yield of the different solvents was determined using the formula;

(weight of the extract after evaporating solvent and drying)/(dry weight of the sample)×100



Fig. 4: Stages in preparing crude extracts of both *A. garckeana* and *P. oleracea*.

Preparation of crude extracts from *A. garckeana* and *P. oleracea* was in five stages, the preparation started with collection of the plant samples followed by drying of the plant samples then grinding of separated parts into fine powder, filtration of extracts after maceration for 48 hrs and lastly solvent evaporation. The prepared crude extracts were used throughout in different experiments.

3.4 Sample preparation for biochemical assays

Fresh stock solutions of 1 mg/mL were prepared. Each stock solution was prepared in a 15 mL tube which was covered with aluminium to retain the extracts, and then stored at -20°C.

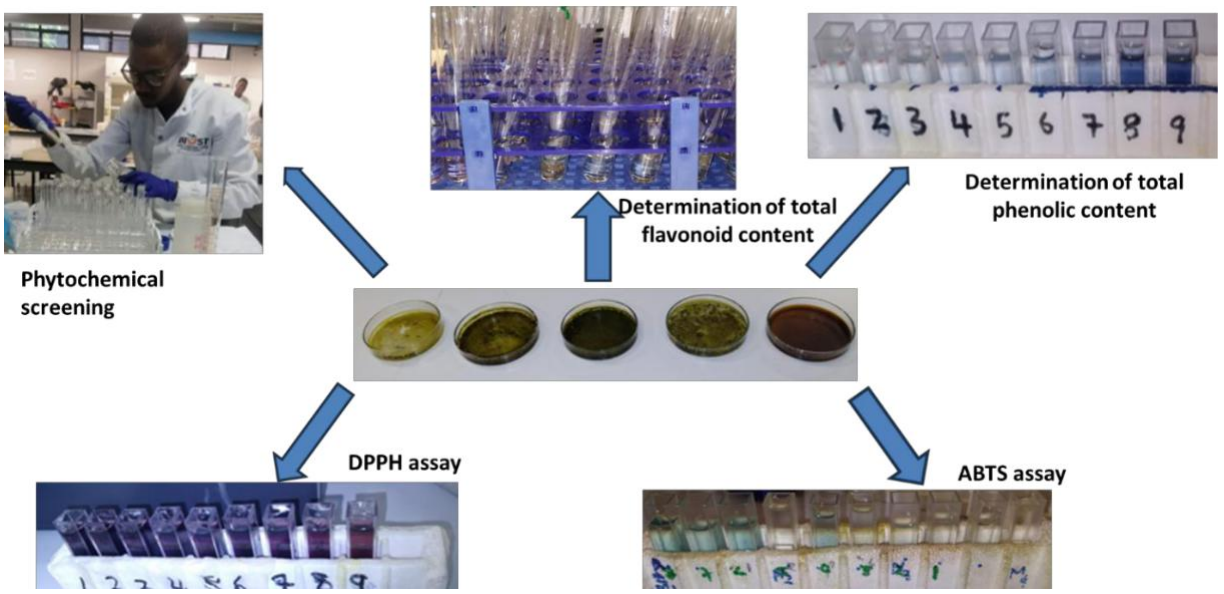


Fig. 5: Overview of the different biochemical assays to determine antioxidant activity of the extracts of both *P. oleracea and A. garckeana* .

The antioxidant activity of the extracts from *P. oleracea* and *A. garckeana* was determined by a series of biochemical assays to determine the phytochemical composition of the extracts. The phytochemical composition of the extracts was determined through qualitative phytochemical screening and determining the total flavonoid and phenolic content. The DPPH and ABTS assays were done to determine if the extracts had antioxidant activity and compare the activity between the different extracts.

3.5 Qualitative phytochemical analysis

Preliminary phytochemical screening was done to qualitatively detect the presence of phenols,flavonoids,saponins,tannins,terpenoids,steroids and cardiac glycosides following methods described Fernández-Poyatos and Loo[1,2].

3.5.1 Test for phenols

In a test tube, 100 µL of 1 mg/mL stock solution of each extract was diluted 10 times with distilled water and then added to 2.5 mL of 5 % ferric chloride. All colour changes were observed, and a blue-green colour observed indicated the presence of phenols.

3.5.2 Test for flavonoids

A volume of 100 µL stock solution (1 mg/mL) of each extract was prepared and diluted 10 times with distilled water. The solutions were mixed with 3.0 mL of 5 % aluminium chloride in a test tube. All colour changes were observed, and a yellow colour depicted the presence of flavonoids.

3.5.3 Test for saponins

Solution of 150 µL of each extract was mixed with 5 mL of distilled water in a test tube. The test tube was vigorously shaken, and the persistent formation of foam indicated the presence of saponins.

3.5.4 Test for tannins

A volume of 100 µL of a stock solution, 1 mg/mL was diluted 10 times with distilled water. The stock solution was mixed with 2 mL of 5 % ferric chloride in a test tube and all colour changes were noted. A blue-black colour indicated the presence of tannins.

3.5.5 Test for terpenoids

A dilution of a stock solution was prepared by diluting a 1 mg/mL stock solution 10 times with distilled water. The prepared dilution was mixed with 2.5 mL concentrated sulphuric acid in a test tube. All colour changes were observed, and a blue-green colour indicated presence of terpenoids.

3.5.6 Test for steroids

A dilution of the plant extracts stock solutions was prepared by diluting a 1 mg/mL stock solution 10 times with distilled water. A volume of 200 µL of the diluted plant extracts was mixed with 2 mL acetic anhydride and 3 mL concentrated sulphuric acid in a test tube. All colour changes were observed, and a green-blue colour indicated presence of steroids.

3.5.7 Test for cardiac glycosides

Prepared dilutions of plant extracts of 250 µL were mixed with 2 mL of glacial acetic acid followed by the addition of a few drops of 5 % ferric chloride solution. Concentrated sulphuric acid was carefully layered under the test tube containing the mixture and any colour changes were observed. A brown ring showed the presence of cardiac glycosides.

3.6 Quantitative phytochemical analysis

3.6.1 Determination of Total Phenolic Content

Total phenolic content was determined following revisions of the Folin-Ciocalteu method by Singleton and Rossi [44]. Trolox was used for standardization to prepare the calibration curve. Six different concentrations of Trolox (0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.4 and 0.8) mg/mL were prepared from a stock concentration of 1 mg/mL to obtain the standard curve. The different concentrations were further diluted ten times through the aliquoting of 250 µL of the standard to 2.5 mL of distilled water in a test tube.

Aliquot volumes of 100 µL of the seven different concentrations were added to test tubes followed by the addition of 1 mL 10 % (v/v) Folin-Ciocalteu reagent. The mixture was vortexed and incubated for 5 min in the dark. After incubation, 0.8 mL of sodium carbonate 7.5 % (w/v) was added and the resulting mixture was incubated for 1 hr in the dark at room temperature.

Absorbance was measured at 765 nm with a UV-Vis spectrophotometer (Evolution 204, Thermo Fisher Scientific, Wisconsin, USA) against a methanol blank and the standard several concentrations.

The wavelength of the absorbance was selected based on the complex produced by phenolic compounds reducing the Folin-Ciocalteu reagent under alkaline conditions to form a blue complex [44]. The same steps were repeated for the test samples (plant extracts) in which seven different concentrations (0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.4, 0.8) mg/mL of each of the six different plant extracts from the three different plant parts for both *A. garckeana* and *P. oleracea* were prepared. Results were expressed as mg Trolox Equivalent (TE) per 100 g dry weight of plant. All analyses were performed in triplicates. Total phenolic content was calculated using the following equation;

$$\text{TPC (mg/g)} = TE\ from\ calibration\ curve \times volume\ of\ extract\ solution\ in\ mL / weight\ of\ extract\ in\ g$$

3.6.2 Determination of total flavonoid content

Total flavonoid content of *P. oleracea* and *A. garckeana* extracts was determined following revisions of a protocol by Gallo *et al*. [41]. A calibration curve was made through preparing seven concentrations of the quercetin standard (0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.4, 0.8) mg/mL. The concentrations were further diluted ten times by aliquoting 250 µL to 2.5 mL of distilled water in a test tube.

Aliquot volumes of 250 µL of the seven different concentrations were added to the test tubes followed by the addition of 0.15 mL of 5 % sodium nitrite (w/v) which aids in enabling the formation of a flavonoid-aluminium complex [31].

The mixture was incubated for five minutes, followed by the addition of 0.3 mL of 10 % aluminium chloride (w/v).

A Sodium hydroxide solution (1.0 M) of volume, 1.0 mL was added, and the mixture was vortexed. Absorbance was measured at 510 nm with a UV-Vis spectrophotometer (Evolution 204, Thermo Fisher Scientific, Wisconsin, USA). The absorbance measurement was selected based on the formation of a flavonoid and an aluminium metal ion complex under acidic conditions which is yellow orange in colour [31]. The same steps were repeated for the test samples plant extracts) in which seven different concentrations (0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.4, 0.8) mg/mL of each of the six different plant extracts from the three different plant parts for both *A. garckeana* and *P. oleracea* were prepared. Results were expressed as mg Quercetin Equivalent (QE) per 100 g dry weight of the plant.

All analyses were performed in triplicates. Total flavonoid content was calculated using the following equation;

$$\text{TFC (mg/g)} = QE\ from\ calibration\ curve \times volume\ of\ the\ extract\ solution\ in\ mL / Weight\ of\ extract\ in\ g$$

3.7 Screening of extracts for antioxidant potential

3.7.1 Evaluation of antioxidant activity using DPPH assay

The DPPH (2,2-diphenyl-1-picryl hydrazyl) is a stable free radical which is purple in colour. It is used to depict the ability of a compound scavenge free radical therefore measuring the antioxidant capacity of a compound [30]. A DPPH solution was prepared by dissolving 33.3 mg of DPPH powder in 100 mL 70 % methanol solution in a bottle which was covered to ensure that DPPH molecules remain intact. Various concentrations (0, 25, 50, 100, 150, 200, 400, 600, 800) µM of the Trolox standard were prepared from the Trolox stock solution to make a calibration curve.

Aliquot volumes of 150 µL of the various concentrations of the standards were further diluted ten times with 1.5 mL of distilled water in a test tube. Furthermore, 200 µL aliquots of the different concentrations were mixed with 2.5 mL of DPPH in a test tube and incubated for 30 minutes. The decrease in absorbance was read at 517 nm using a UV-Vis spectrophotometer (Evolution 204, Thermo Fisher Scientific, Wisconsin, USA).

The procedure was repeated for the test samples with the decrease in absorbance measured against a methanol blank and Trolox standard. All analyses were performed in triplicates. The DPPH radical scavenging ability was calculated in terms of percentage inhibition as follows;

$$\% \text{ inhibition} = Absorbance \text{ of } control – Absorbance \text{ of } test\ sample \Big/ Absorbance \text{ of } control$$

The Trolox equivalents were obtained through using the equation from the calibration curve (Y=mx + C) as follows;

Trolox equivalent = $(Absorbance\ of\ control - absorbance\ of\ test\ control) - C/m) \times w$

Where w represents weight of dry sample and m is the gradient from the calibration curve.

3.7.2 Evaluation of antioxidant activity using ABTS assay

ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) is a blue-green chromophore which is soluble in water [45]. ABTS was converted into a radical cation $ABTS^{\bullet+}$ by mixing prepared stock solutions of 2.45 mM of potassium persulfate and 7 mM of ABTS in distilled water. The radical cation was incubated in the dark for 16 hrs. After incubation, the radical cation was diluted with methanol to give an absorbance of 0.70 at 734 nm.

Various concentrations (0, 25, 50, 100, 150, 200, 400, 600, 800) µM of the Trolox standard were prepared from the Trolox stock solution to make a calibration curve. Various concentrations of the standards of 150 µL were further diluted ten times with 1.5 mL of distilled water in a test tube. Aliquots of 150 µL of the different concentrations were mixed with 3.0 mL of the ABTS radical cation in a test tube and incubated for 15 minutes. In the ABTS reaction, the antioxidant in the sample reacts with the ABTS radical cation which causes the intensity of the colour of the sample to reduce from blue green to colourless [45]. The decrease in colour of the sample was measured through a decrease in absorbance at 734 nm using a UV-Vis spectrophotometer (Evolution 204, Thermo Fisher Scientific, Wisconsin, USA).

The procedure was repeated for the test samples with the decreases in absorbance measured against a methanol blank and Trolox standard. All analyses were performed in triplicates. The ABTS radical scavenging ability was calculated in terms of percentage inhibition using equation 4.

The Trolox equivalents for ABTS were calculated in the same manner as Trolox equivalents for DPPH.

3.8 Identification of bioactive compounds using GC-MS

Extracts from *A. garckeana* and *P. oleracea* were filtered with a 0.21 nm filter (Millipore Sigma) to obtain clean extracts. The GC-MS procedure was adapted from Samer and Hala *et al* [46] with a few modifications. The GC-MS analysis was done with specifications of "Agilent 7890A" with the mass spectrometer detector operated at 5975 C while the computer control was set at 70 Ev. The capillary column was Hewlett Packard HP-5MS silica with the following features: 30m in length, 250 m in internal diameter and a film thickness of 0.25 m. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min and an injection volume of 1 µL with the injection temperature set at 250°C. Scanning was conducted for 18 min. The split ratio was set at 1:10. The oven temperature began to run at 50°C for 10 mins and it was set to increase at a rate of 4°C/min to 240°C for 12.5 min.

For defining the GC-MS peaks, full ion chromatograms and mass chromatograms were used, and compounds were further determined with the National Institute of Standards and Technology (NIST) 2008 mass spectral library. The percentage probability was used to identify the compounds, the percentage indicated a matching probability against a reference compound found in the mass spectral library.

3.9 Cell culture

3.9.1 Culture of HeLa cells

The cervical cancer cell line, HeLa, was used in this study. Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, Grand Island, USA), 10% Foetal Bovine Serum (FBS, Thermo Fisher Scientific, Grand Island , USA), penicillin streptomycin(Pen-Strep) (Thermo Fisher Scientific, Grand Island, USA) and L-glutamine (L-Glut) (Thermo Fisher Scientific, Grand Island, USA) were all prewarmed in a 37°C water bath. Complete culture media was prepared by mixing 440 mL of DMEM, 5 mL of FBS, 5 mL of L-Glut and 5 mL of Pen-Strep. The prepared culture media was aliquoted into two sterile 50 mL conical tubes to reduce the chances of contamination and the remaining media was stored at 2°C in a fridge. HeLa cells from liquid nitrogen storage were thawed at 37°C in a water bath . The thawed cells were pipetted into the culture media in a sterile 15 mL tube. The cells were then centrifuged (Model GS -15, Beckman Coulter, Germany) at 300 g for 3 mins. The supernatant was discarded, and the pellet was resuspended in 5 mL of culture media. HeLa cells were then cultured in T-25 flasks and incubated at 37°C, 5% CO_2 for 24 hrs. The cells were observed under an inverted microscope (Olympus IX 83, Japan, Tokyo) to check for cell attachment and growth.

3.9.2 Subculturing of HeLa cells

Upon the cells reaching 70-100% confluency, they were subcultured to ensure continuous healthy growth and survival of the cells. First, culture media, phosphate buffered saline (PBS) and trypsin-EDTA were all prewarmed at 37°C in a water bath. Old media was removed, and the cells were washed with 10 mL PBS and 1 mL trypsin was added to allow for cell detachment with gentle tapping on all corners of the T-25 flask.

To inactivate, the trypsin 9 mL of media was added. Subculturing proceeded with the transfer of cells into a 15 mL sterile tube containing 10 mL media.

The cells were centrifuged at 300 g for 3 mins. The cells were resuspended in 5 mL of culture media and separated through needling with a 5 mL syringe and 21 gauge needle. For counting the number of viable cells, 200 µL of homogenous cells were used. Counting was done by mixing 50 µL of trypan blue (Thermo Fisher Scientific, Grand Island, USA) and 50 µL of the cells and thereby counting with a hemacytometer (Gibco Thomas Fisher Scientific, Massachusetts, USA). After counting an estimated 300000 cells were cultured in a T-75 flasks (Falcon^{TM}, Thermo Fisher Scientific, Grand Island, USA) whereas other cells were seeded into 96 well plates (Falcon^{TM} , Thermo Fisher scientific, Grand Island, USA) for cell treatment. The remaining cells were frozen. The cells in T-75 (Falcon^{TM}, Thermo Fisher scientific, Grand Island, USA) flasks were passaged at a subculture ratio of 1:4 after reaching 70-100% confluency.

3.9.3 Treatment of cells

An estimated density of 5000 cells /well was seeded in a 96-well plate (Falcon^{TM}, Thermo Fisher scientific, Grand Island, USA). The cells were incubated at 37°C, 5% CO_2 for 24 hrs. Media was changed thereafter to quicken the rate of cell growth. During the beginning of cell treatment, old media was replaced with 150 µL of fresh culture media per well. Initial stock solution of extracts was diluted to 400 µg/mL and two-fold dilutions were done successively until the last concentration of 12.5 µg/mg . Cisplatin was used as a positive control and the untreated cells as negative control. The same dilutions applied to extracts were also applied to cisplatin. The treated cells were incubated for 24 hrs at 37°C, 5% CO_2.

After 24 hrs, the cells were observed using an inverted microscope (Olympus IX 83, Japan, Tokyo) to check and record for any changes in the growth patterns and morphology of the cells.

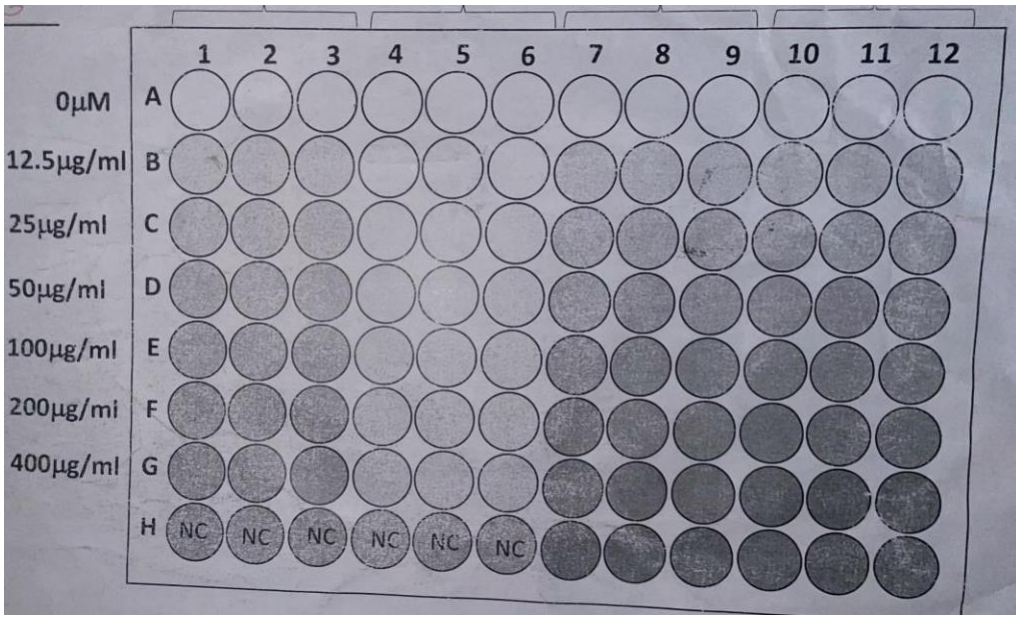


Fig. 6: Overview of the plate wells and how the dilution were carried out. Well B to G were labelled as treatment while well A was labelled as untreated HeLa cells and well H was taken as no cell control.

3.10 Determination of cell viability using MTT assay

The MTT assay was used to determine effect of the extracts on the viability of HeLa cells. The MTT assay is based on yellow tetrazolium salt being reduced to purple formazan crystals by cells which show metabolic activity through the action of NAD(P)H-dependent oxidoreductase enzyme [15]. After treatment of cells, a working MTT solution was prepared by mixing 2 mL of MTT with 18 mL culture media. A volume of 120 µL of the old media was removed and 100 µL of MTT working solution was added per well of a 96-well plate. The plate was incubated for 4 hrs at 37°C, 5% CO_2. After incubation, 80 µL of media and MTT were removed and 100 µL of DMSO was added to act as a solubilization agent. The plate was agitated on a plate shaker (MaxQ 2000, Thermo Fisher Scientific, Massachusetts, USA) for 5 mins followed by the measurement of absorbance at 540nm.

The percentage cell viability was calculated as follows;

% cell viability = ($Absorbance\ of\ treatment$/A$bsorbance\ of\ control$) x 100

The analysis was done in triplicates followed by the determination of the minimum inhibitory concentration (IC_{50}). To determine the IC_{50}, concentrations of the extracts from Fig.6 (0, 12.5, 25, 50, 100, 200, 400) µg/mL were plotted against the % cell viabilities from different concentrations and the IC_{50} was considered as the lowest concentration at which the % viability reduced to zero or almost zero.

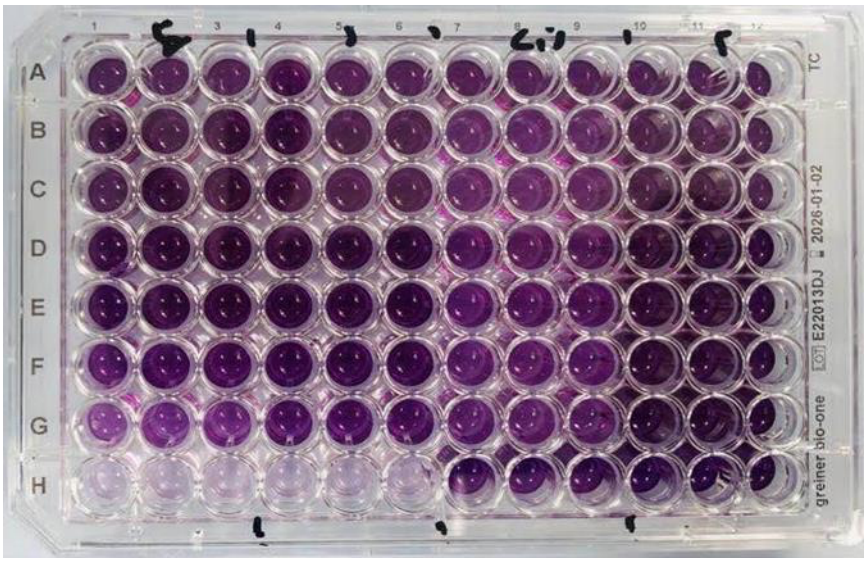


Fig. 7: Layout of a 96 well plate and appearance of MTT after a 4 hr incubation of HeLa cells. The yellow tetrazolium MTT has transformed into purple formazan crystals as seen on the plate.

3.11 Statistical analysis

Data was shown as mean ± standard deviation. For cell culture experiments the analysis was based on both standard deviation and standard error of the mean. Statistical comparisons were evaluated using one way analysis of variance (ANOVA) and Tukey kramer test for multiple comparisons (SAS-JMP software version 16). Differences were presumed significant at $P < 0.05$.

CHAPTER 4

RESULTS

4.1 Extraction yield of different extracts from both *Azanza garckeana* and *Portulaca oleracea*

Extraction of phytochemicals from leaves, stems and roots of *A. garckeana* and *P. oleracea* was done using six different solvents namely; methanol, ethanol, isopropanol, chloroform, distilled water and hexane. This was done to establish the relationship between extraction of phytochemicals and solvent polarity and to determine the efficiency of each solvent in extracting phytochemicals. The differences in means of extraction yield from different solvents were found to be statistically significant at a p-value of $P < 0.05$ as depicted by the different mean separation letters shown in Fig. 8 and appendix A. For samples denoted with the same letters, the differences in means were not statistically significant as their P-values were $P > 0.05$.

P. oleracea leaves, stems and roots distilled water crude extracts yielded 2.50 % ± 0.02, 9.16 % ± 0.05 and 5.65 % ± 0.10 whereas *A. garckeana* leaves, stems and roots distilled crude extracts yielded 5.21 % ± 0.02 , 4.22 % ± 0.04 and 4.84 % ± 0.05. Ethanol extracts of *P. oleracea* leaves, stems and roots yielded 4.32 % ± 0.02 , 3.15 % ± 0.07 and 3.90 % ± 0.01 whereas *A. garckeana* leaves, stems and roots yielded 6.09 % ± 0.03, 4.77 % ± 0.07 and 3.90 % ± 0.01. A comparison of crude extracts yields from the most polar solvents distilled water, ethanol and methanol show that distilled water yielded the highest crude extract of 9.16 % ± 0.05 from *P. oleracea* stems. This was followed by methanol which yielded a crude extract of 8.46 % ± 0.02 from *A. garckeana* leaves and 6.99 % ± 0.05 from *P. oleracea* stems respectively.

The results also show that a non-polar solvent; hexane gave the lowest yields. Hexane crude extracts of *A. garckeana* leaves, stems and roots yielded 1.13 % ± 0.06 , 0.59 % ± 0.00 and 0.51 % ± 0.00 whereas hexane extracts of *P. oleracea* leaves, stems and roots yielded of 0.60 % ± 0.03, 0.67 % ± 0.05 and 0.61 % ± 0.01.

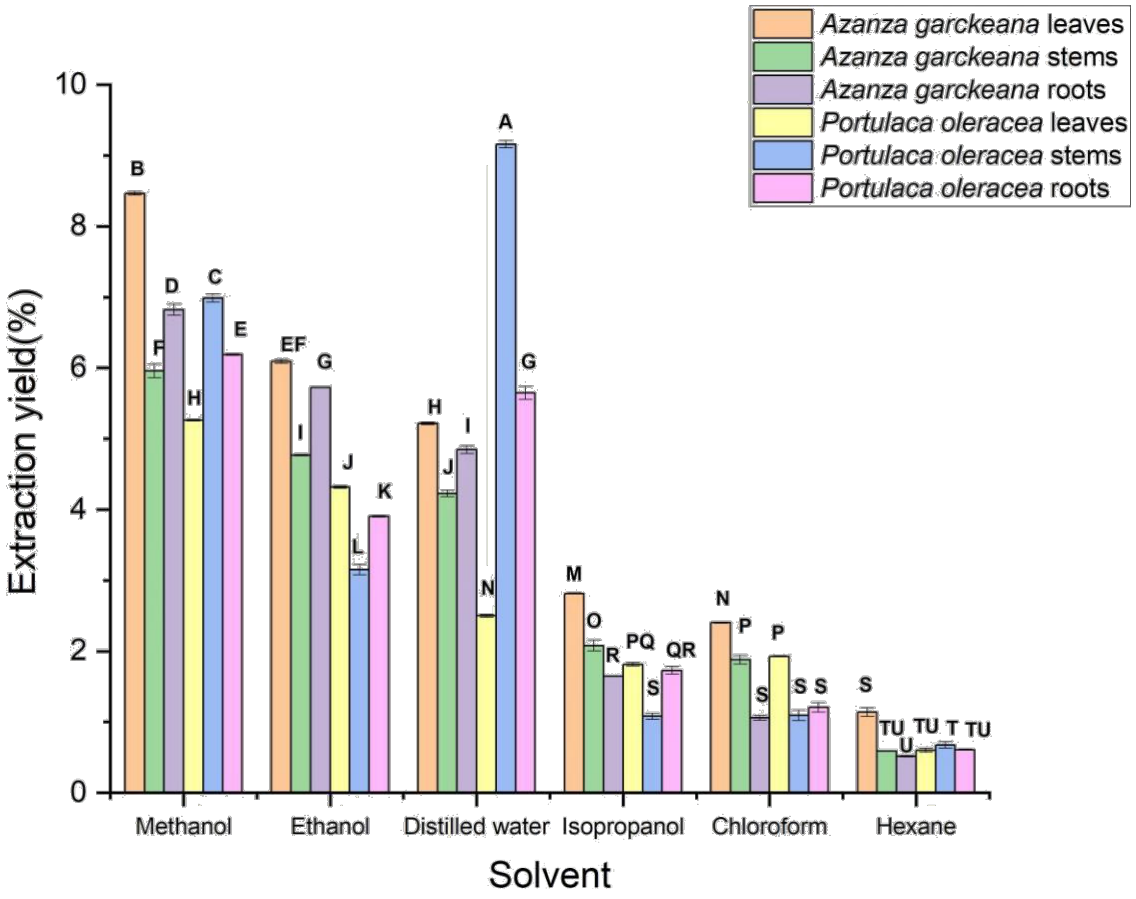


Fig. 8: Extraction yield of extracts from different solvents for both *Portulaca oleracea* and *Azanza garckeana*, (P-value < 0.05,considered statistically significant based on Tukey Kramer t-test).

All solvents yielded a certain percentage of extracts showing that all solvents were able to extract phytochemicals. A ratio of 20 g/200 mL (w/v) of plant powder to solvent was used. The polar solvents methanol, ethanol and distilled water gave significantly higher extraction yields as compared to less polar solvents, hexane as shown in Appendix A based on the threshold values which are higher than the critical value at 95% confidence level.

4.2 Phytochemical screening of leaf, stem and root extracts of *P. oleracea* and *A. garckeana*

Different phytochemicals were detected in six different extracts of *Portulaca oleracea* leaves, stems and roots (Table 1). The results indicate more phytochemicals were detected in methanolic extracts when compared to the other extracts and fewer phytochemicals were detected in hexane extracts. This indicated the *P. oleracea* was constituted by mostly polar components. Phenols were detected in most leaf and stem extracts when compared to root extracts. Overall, results show that phytochemicals were present in all the three different plant parts.

Table 1. Phytochemicals detected in different extracts of different parts of *Portulaca oleracea*

Plant	Plant part	Phytochemical	Absence/Presence of phytochemical in extract					
Portulaca oleracea	Leaves		Methanol	Ethanol	Isopropanol	Distilled water	Chloroform	Hexane
		Phenols	+	+	+	+	+	-
		Saponins	+	+	+	+	-	-
		Flavonoids	+	+	+	+	-	-
		Tannins	+	+	-	-	-	+
		Terpenoids	+	-	+	-	-	-
		Steroids	+	+	+	-	-	-
		Cardiac glycosides	+	+	+	-	-	-
	Stems	Phenols	+	+	+	+	+	-
		Saponins	+	+	-	-	-	-
		Flavonoids	+	+	+	-	-	-
		Tannins	+	-	-	+	+	-
		Terpenoids	+	+	-	+	-	-
		Steroids	+	-	-	+	-	-
		Cardiac glycosides	+	+	+	+	-	-
	Roots	Phenols	+	+	+	+	-	-
		Saponins	+	+	-	+	-	-
		Flavonoids	+	+	+	-	+	-
		Tannins	+	+	-	+	+	-
		Terpenoids	+	+	-	-	-	-
		Steroids	+	+	-	-	+	+
		Cardiac glycosides	-	-	-	-	+	-

KEY ; + indicates presence of phytochemical. – indicates absence of phytochemical.

There were different phytochemicals detected in six different extracts from *Azanza garckeana* leaves, stems and roots. Results show that phytochemicals were detected across various plant parts except for tannins in the leaves and cardiac glycosides in the roots. Many phytochemicals were found in methanolic extracts when compared to the other extraction solvents across the different plant parts. In the *Azanza garckeana* stem extracts, flavonoids were the most abundant when compared to other phytochemicals. In conclusion, the phytochemical composition of both *Azanza garckeana* and *Portulaca oleracea* was not very dissimilar.

Table 2. Phytochemicals detected in different extracts of different parts of *Azanza garckeana*

Plant	Plant part	Phytochemical	Absence/Presence of phytochemical in extract					
Azanza garckeana	Leaves		Methanol	Ethanol	Isopropanol	Distilled water	Chloroform	Hexane
		Phenols	+	+	+	-	-	-
		Saponins	+	+	-	-	-	-
		Flavonoids	+	+	+	-	+	+
		Tannins	-	-	-	-	-	-
		Terpenoids	+	+	-	-	-	+
		Steroids	+	+	-	-	+	-
		Cardiac glycosides	-	-	+	-	+	-
	Stems	Phenols	+	+	+	-	+	-
		Saponins	+	+	+	-	-	-
		Flavonoids	+	+	+	+	+	-
		Tannins	+	+	-	-	-	-
		Terpenoids	+	-	-	+	-	-
		Steroids	+	+	-	+	+	-
		Cardiac glycosides	-	+	+	-	-	-
	Roots	Phenols	+	+	-	+	-	-
		Saponins	+	-	+	+	-	-
		Flavonoids	+	+	+	-	-	-
		Tannins	+	+	-	-	-	+
		Terpenoids	+	-	+	-	+	-
		Steroids	+	-	+	-	+	-
		Cardiac glycosides	-	-	-	-	-	-

KEY;+ indicates presence of phytochemical. -indicates absence of phytochemical.

4.3 Total phenolic content of different extracts from *A. garckeana* and *P. oleracea* leaves, stems and roots

The total phenolic content (TPC) of both *A. garckeana* and *P. oleracea* leaves, stem and root extracts were determined, expressed as Trolox equivalents in mg/100 g of dry sample. The difference in means of Trolox equivalents were found to be statistically significant at $P < 0.05$. Different mean separation letters were used to differentiate the means of the total phenolic content of the different extracts whereby samples having the same separation letters were found not to be statistically significant as the P-value was at $P > 0.05$.

Results showed that *P. oleracea* leaf and stem methanolic extracts had significantly higher total phenolic content of 16.25 ± 0.48 TE mg/100 g and 16.39 ± 0.51 TE mg/100 g respectively (Fig. 9 and Appendix B). This was followed by *A. garckeana* leaf methanolic and ethanolic extracts at 14.00 ± 1.00 TE mg/100 g and 12.21 ± 0.06 TE mg/100 g respectively (Fig. 9). In addition, results indicate that leaf extracts across all solvents had a higher TPC when compared to stem and root extracts whereas root extracts had a significantly lower TPC across all solvents.

All hexane extracts had lower TPC when compared to extracts of other solvents. The most polar solvents (ethanol, methanol and distilled water) were found to have high TPC when compared to the moderately and non-polar solvents (isopropanol, chloroform and hexane).

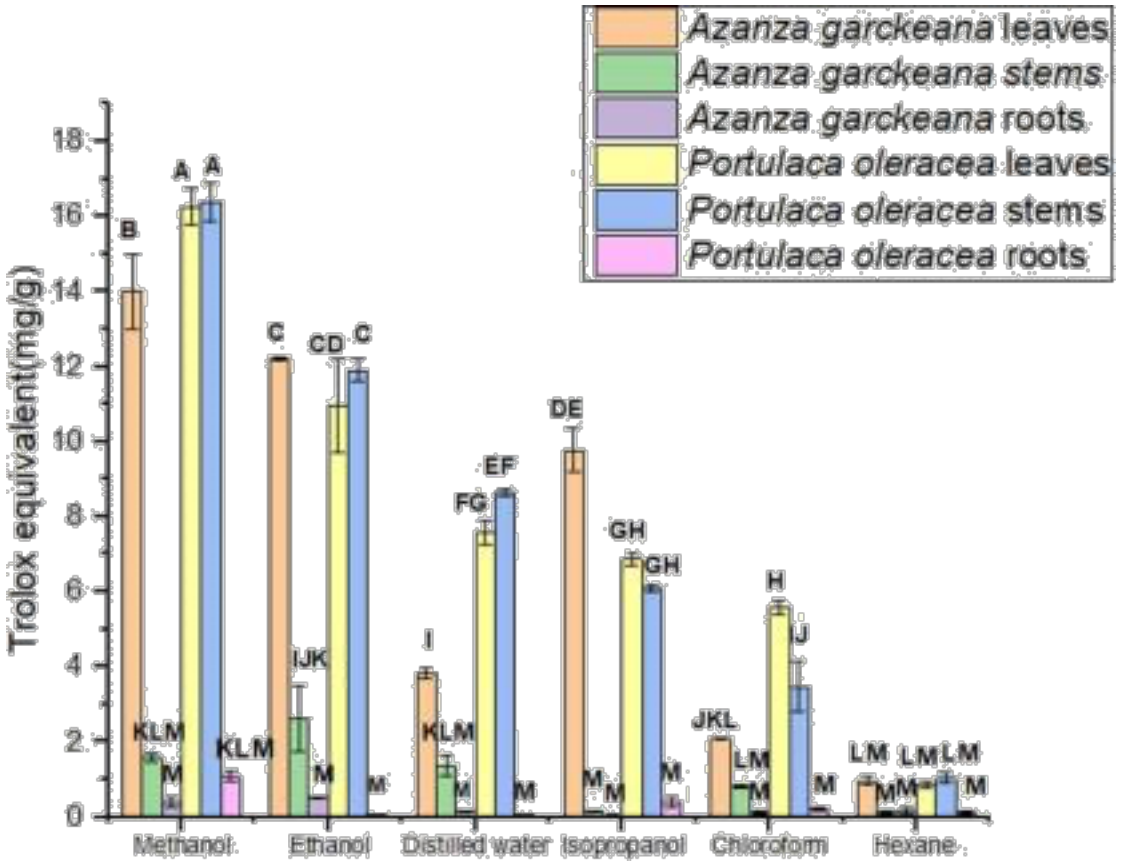


Fig. 9: Total phenolic content expressed in TE of different leaves, stems and root extracts of six different solvents in both *A. garckeana* and *P. oleracea* (P-value < 0.05,considered statistically significant based on Tukey Kramer t-test).

All extracts were found to contain a certain measure of phenolics as seen by the Trolox equivalents given. Across all solvents, root extracts had low total phenolic content as compared to leaf and stem extracts as indicated from Figure 9. There was a relationship between polarity of solvents and total phenolic content as more polar solvents had high total phenolic content and less polar solvents had low total phenolic content.

4.4 Total flavonoid content of different extracts from *A. garckeana* and *P. oleracea* leaves, stems and roots

Quercetin equivalents (QE) mg / 100 g of dry samples was used to express the different total flavonoid content of the samples. Results show that *A. garckeana* leaf methanolic and ethanolic extracts had significantly high TFC of 52.03 ± 2.44 QE mg/100 g and 55.06 ± 0.43 QE mg/100 g when compared to other extracts. Furthermore, *P. oleracea* leaf and stem methanolic extracts gave total flavonoid content of 45.81 ± 1.02 QE mg/100 g and 41.70 ± 3.69 QE mg/100 g respectively whereas *P. oleracea* leaf and stem ethanolic extracts gave total flavonoid content of 29.87 ± 2.99 QE mg/100 g and 28.09 ± 0.63 QE mg/100 g respectively as indicated in appendix c.

P. oleracea stem hexane extracts displayed significantly high TFC of 16.14 ± 0.55 QE mg/ 100 g despite the low polarity of this solvent. Extracts of *P. oleracea* and *A. garckeana* leaves displayed significantly high TFC when compared to the root extracts of these plants across all extraction solvents.

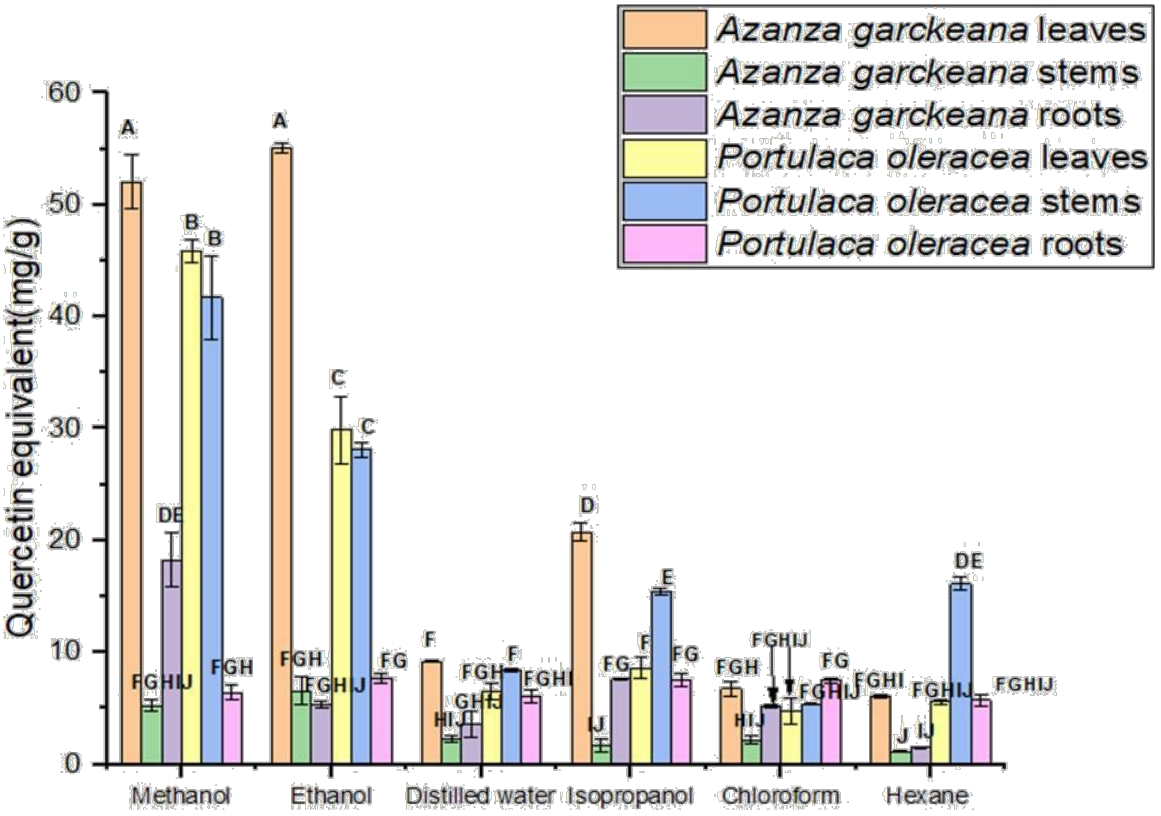


Fig. 10: Total flavonoid content expressed in QE of different leaves, stems and root extracts of six different solvents in both *A. garckeana* and *P. oleracea*.

All extracts contained a certain measure of flavonoids which was expressed in Quercetin equivalents. The mean separation letter **A** denoted the higher Quercetin equivalent of *A. garckeana* leaves methanol extracts and mean separation letter **J** denoted a lower Quercetin equivalent of *A. garckeana* leaf hexane extract. Root extract of all solvents gave low TFC as compared to leaf and stem extracts.

4.5 DPPH radical scavenging activity of different extracts from *A. garckeana* and *P. oleracea* leaves, stems and roots

The DPPH radical scavenging assay was used to determine the concentration of antioxidants (Fig. 11) and the radical scavenging activity (Fig. 12) of the different extracts from the leaves, stems and roots of both *A. garckeana* and *P. oleracea* . The antioxidant concentration was expressed as Trolox equivalents (TE Mmol/g) whereas the radical scavenging activity was expressed as a percentage (%). Differences in means were statistically significant at a P-value of $P < 0.05$ hence the use of different mean separation letters. The same mean separation letters indicated that the difference in means were not statistically significant at a P-value of $P > 0.05$ (Figs 11 and 12). *P. oleracea* root methanolic extracts displayed the highest concentration of antioxidants at 673.71 ± 23.23 Mmol/g (Fig. 11,Appendix D) and the highest radical scavenging activity of 87.69 % ± 2.43 (Fig. 12,Appendix E). It was found that *A. garckeana* leaf ethanolic extracts also had a significantly high concentration of antioxidants at 644.43 ± 24.31 Mmol/g (Fig. 11,Appendix D). Generally, across all the different plant parts, methanolic, ethanolic and chloroform extracts were found to contain a significantly high amount of antioxidants (Fig. 11,Appendix D) and therefore exhibiting significantly high radical scavenging activity (Fig. 12, Appendix E) when compared to distilled water and hexane. Across all the different plant parts, hexane extracts were found to contain a significantly low concentration of antioxidants and therefore displayed significantly reduced radical scavenging activity (Fig. 12).

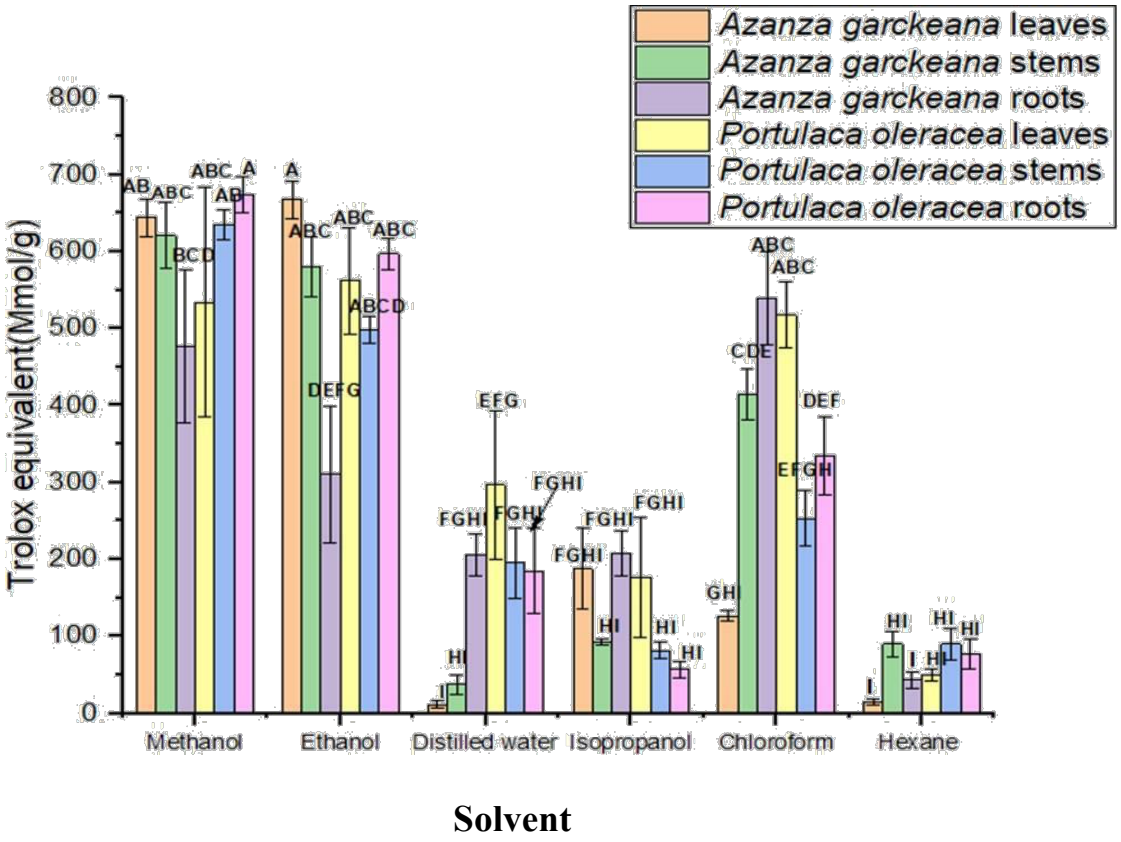


Fig. 11: Antioxidant concentration of different extracts of leaves, stems and roots in both A. *garckeana* and *P. oleracea* expressed as Trolox equivalents from DPPH assay.

All extracts were found to contain different concentrations of antioxidants as seen from Fig. 11. *P. oleracea* methanolic root and *A. garckeana* leaf methanolic extracts were found to contain high concentration of antioxidants as indicated by the mean separation letter **A**. Most hexane extracts had low concentration of antioxidants as indicated by mean separation letter **I**.

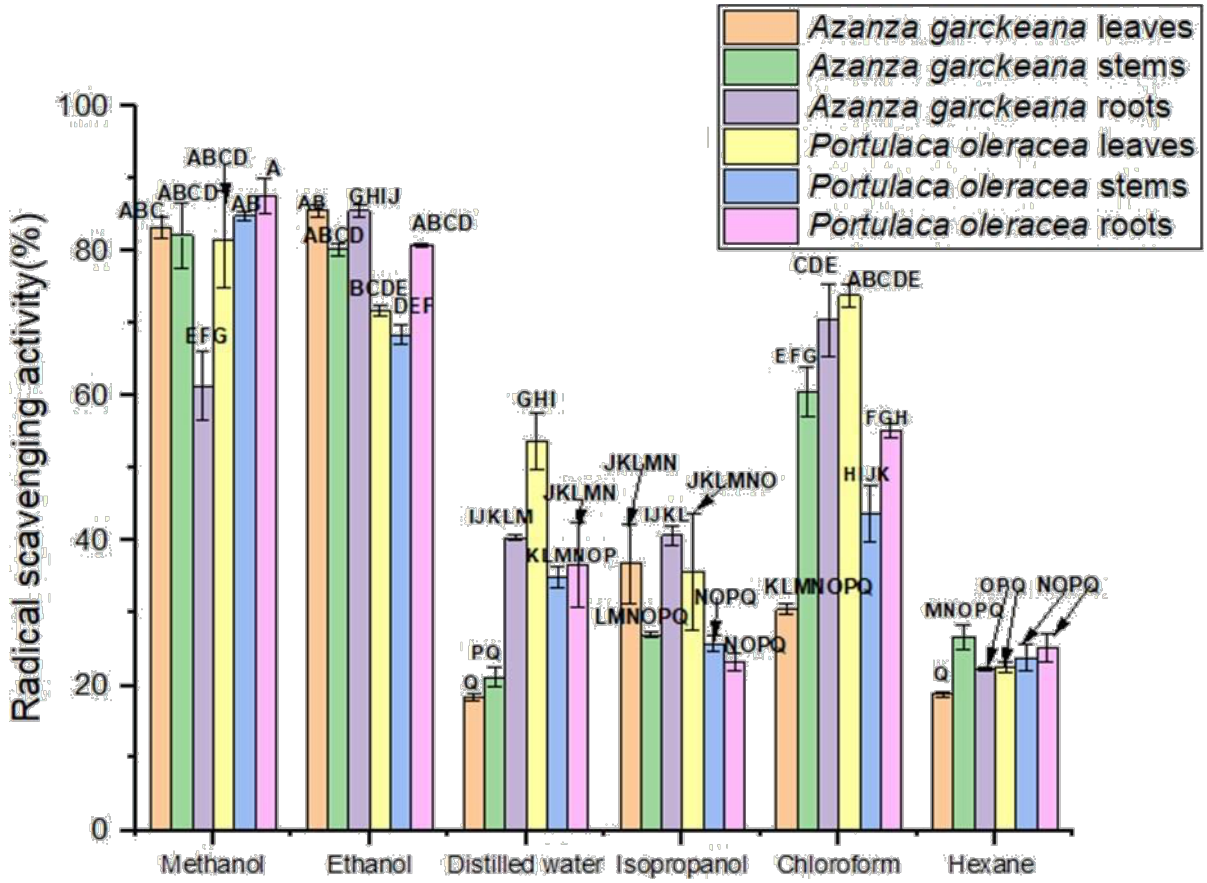


Fig. 12: DPPH radical scavenging activity (%) of different extracts of leaves, stems and roots of both *A. garckeana* and *P. oleracea* .

The DPPH radical scavenging ability was displayed across all extracts. *P. oleracea* roots methanolic extract displayed the highest radical scavenging ability as denoted by the mean separation letter **A** whereas *A. garckeana* leaf water and hexane extract displayed the lowest radical scavenging ability as indicated by mean separation letter **Q**.

4.6 ABTS radical scavenging activity of different extracts from *A. garckeana* and *P. oleracea* leaves, stems and roots

ABTS assay was also used to determine the concentration of antioxidants (Fig. 13) and the radical scavenging capacity (Fig. 14) of the different extracts from *A. garckeana* and *P. oleracea* leaves, stems and roots. The concentration of antioxidants was determined and expressed as TE Mmol/g and the radical scavenging activity was expressed as a percentage (%). *P. oleracea* root methanolic extracts were found to have a significantly high concentration of antioxidants (706.25 ± 13.25 TE Mmol/g) followed by *P. oleracea* stem methanolic extracts (705.00 ± 4.42 TE Mmol/g) (Fig. 13, Appendix F). *P. oleracea* stem chloroform extracts displayed antioxidant concentration of 687.50 ± 0.88 TE Mmol/g (Fig. 13, Appendix F) and radical scavenging activity of 97.83 % ± 1.59 (Fig. 14 , Appendix G). On the other hand, the *P. oleracea* root hexane extracts were found to have the lowest antioxidant concentration of 57.50 ± 9.72 TE Mmol/g (Fig. 13, Appendix F) and therefore the lowest radical scavenging activity of 20.25 % ± 1.16 (Fig. 14, Appendix G) when compared to other extracts.

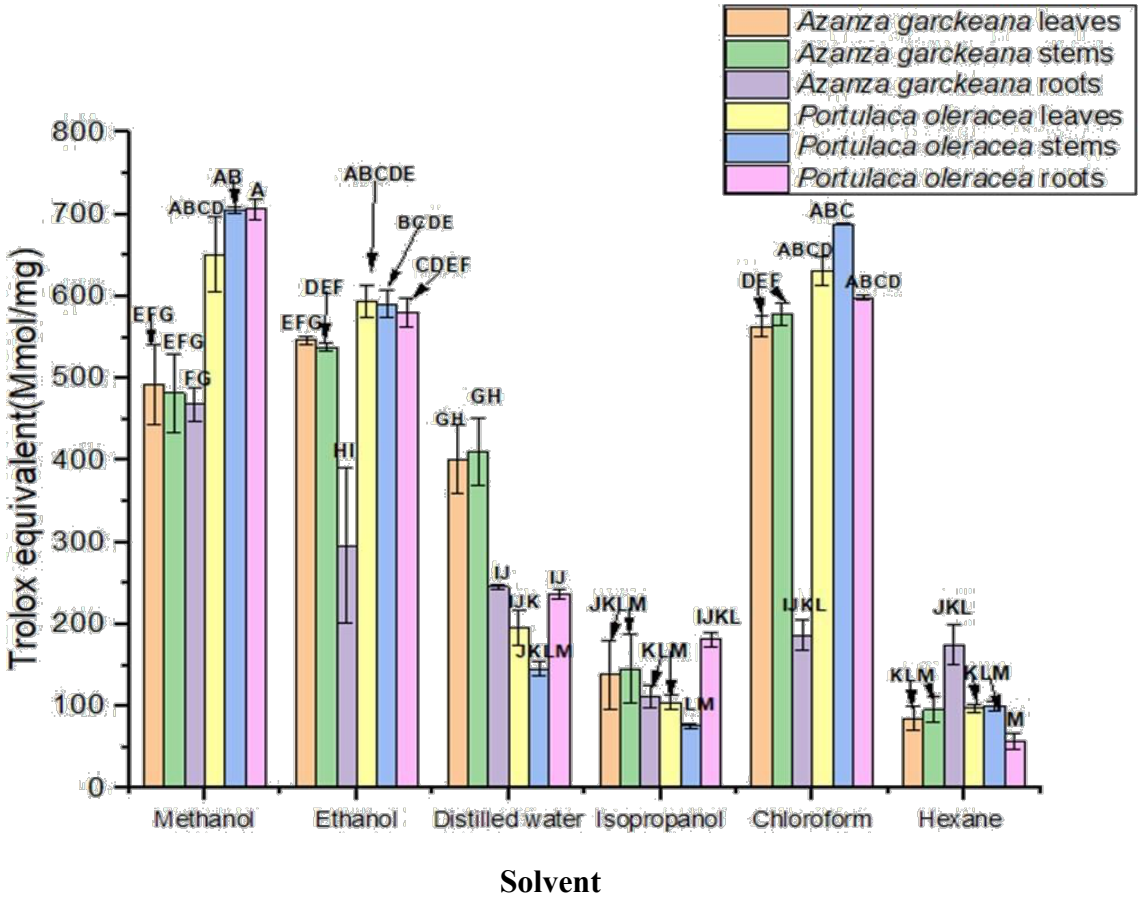


Fig. 13: Antioxidant concentration of different extracts of leaves, stems and roots in both *A. garckeana* and *P. oleracea* expressed as Trolox equivalents from ABTS assay.

All extracts were found to contain a certain measure of concentration of antioxidants measured in Trolox equivalents in Mmol/ mg of the dry sample. *P. oleracea* roots methanol extract was found to contain the highest concentration of antioxidants as denoted by mean separation letter **A** and *P. oleracea* root hexane extract was found to contain the least concentration of antioxidant as denoted by mean separation letter **M**.

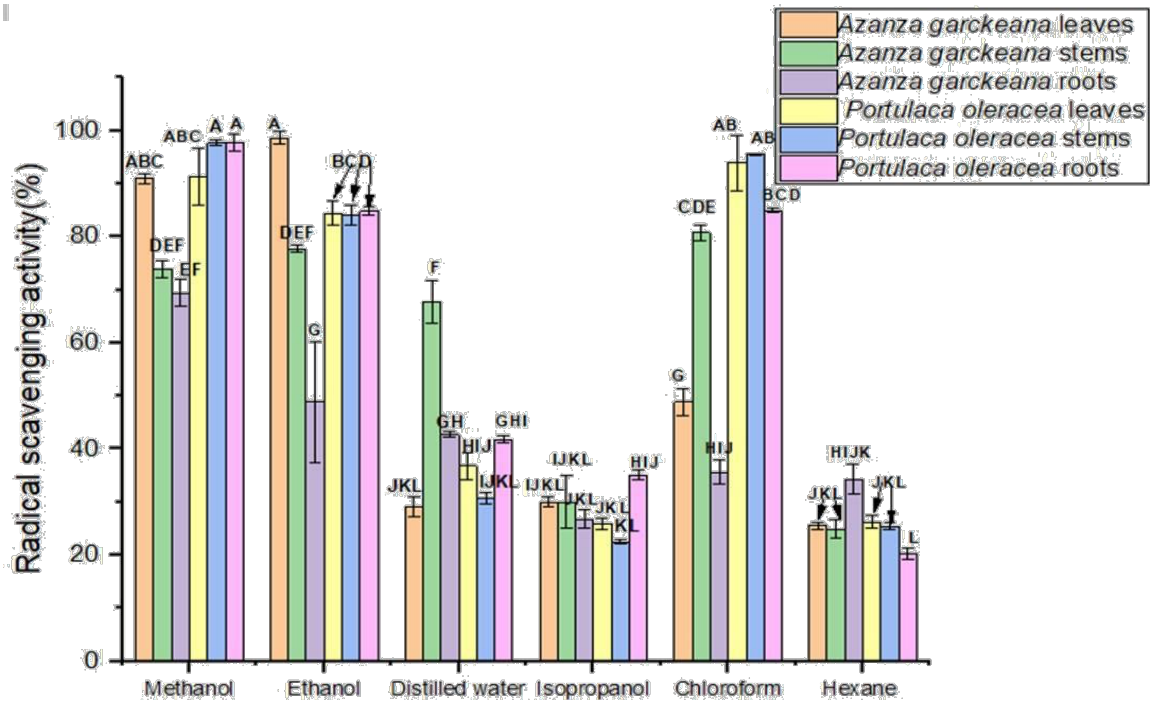


Fig. 14: Radical scavenging activity of different extracts of leaves, stems and roots of both *A. garckeana* and *P. oleracea* expressed in % from ABTS assay. All extracts displayed radical scavenging ability at different percentages.

The *P. oleracea* stem and root methanol extract and the *A. garckeana* leaf ethanol extract showed the highest radical scavenging activity as indicated by mean separation letter **A**. *P. oleracea* root hexane extract showed the lowest radical scavenging activity as seen by mean separation letter **L**.

4.7 Identification of bioactive compounds in different extracts from *P. oleracea* and *A. garckeana* using GC-MS analysis

Most compounds identified from Table 3-13 were based on probable compounds which had reported bioactivity whereas some compounds were identified based on the percentage probability. Seven compounds were identified by GC-MS with many of the compounds being antioxidants (Table 3,Appendix H1). These compounds may be responsible for the antioxidant activity of the extracts observed during DPPH and ABTS assays.

Table 3. Different bioactive compounds identified from *P. oleracea* leaf chloroform extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time (min)	% Probability	Peak area (%)	Biological activity [Reference]
6-((1,3-dihydroxypropan-2-yl)amino)-2,10-dihydroxy-12-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-12,13-dihydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione	608.55	$C_{29}H_{28}N_4O_{11}$	6.33	28.80	26.38	Anticancer [47,48]
7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	276.37	$C_{17}H_{24}O_3$	11.47	34.90	100.00	-Antioxidant [49] -Antimicrobial [49]
I-Propyl 14-methyl-pentadecanoate	298.50	$C_{19}H_{38}O_2$	11.74	10.30	19.98	Antioxidant [50]
Benzonitrile, 4-(6-butyl-2-naphthyl)-	285.38	$C_{21}H_{19}N$	14.45	23.90	7.76	-Antitumor [51] -Antioxidant [51]
Cyclononasiloxane, octadecamethyl-	667.39	$C_{18}H_{54}O_9Si_9$	16.17	60.20	2.45	-Antioxidant [52] -Antimicrobial [52]
Colchicine, N-desacetyl-N-[4-hydroxy-3,5-dimethoxycinnamoyl]-	563.59	$C_{31}H_{33}NO_9$	19.49	39.10	7.82	Anti-inflammatory [53]
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	$C_{27}H_{46}O_5$	32.76	61.30	16.59	-Antioxidant [54],Antimicrobial [54]

Four significant compounds were identified from the *P. oleracea* stem chloroform extracts (Table 4,Appendix H2) between the retention times of 19.49 and 32.76 min. Literature revealed that these compounds have anticancer and antioxidant activity. Therefore, these compounds may be responsible for the antioxidant activity of the extracts as revealed by antioxidant assays performed during this study.

Table 4. Different bioactive compounds identified from *P. oleracea* stem chloroform extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% probability	Peak area (%)	Biological activity [Reference]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	19.49	25.40	1.41	Anticancer [55,56]
Molybdenum,tetrakis[μ-(acetato-O:O')] di-, (Mo-Mo)	428.10	$C_{18}H_{12}Mo_2O_8$	24.77	29.00	12.02	Antioxidant [57] Anticancer [58]
3 Beta-chloro-5alpha-cholestane-5,6beta-diol 6-acetate	481.10	$C_{29}H_{49}ClO_3$	30.58	47.60	1.35	Antioxidant [59]
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	$C_{27}H_{46}O_5$	32.76	45.30	10.07	Antioxidant[54,60]

Six compounds (Table 5) were identified by GC-MS analysis of *A. garckeana* ethanolic extracts. Some compounds were derived from multiple different retention times such as cyclononasiloxane, octadecamethyl- which was identified at 20.34 mins, 24.85 mins and 27.61 mins retention times. Only three compounds were associated with the antioxidant nature of the extracts.

Table 5. Different bioactive compounds identified from *A. garckeana* leaf ethanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% probability	Peak area (%)	Biological activity [Reference]
3-Methylmannoside	194.18	$C_7H_{14}O_6$	8.39	10.80	8.71	-Anti-inflammatory [61] -Anti-diabetic [61] -Anti-viral [61] -Anti-bacterial [61]
3H-Furan-2-one, 3-cyclohexylidene-5-(4-hexanoyloxyphenyl)-	386.44	$C_{22}H_{26}O_6$	11.76	21.20	7.20	-Anticancer [63] -Anti-inflammatory [63] -Anti-viral[63]
Cyclononasiloxane, octadecamethyl-	667.39	$C_{18}H_{54}O_9Si_9$	16.18	24.40	2.42	-Antioxidant [52] -Antimicrobial [52]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	18.29	23.70	2.51	Anticancer [55,56]
Xanthylium, 9-[2-(ethoxycarbonyl) phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, chloride	507.06	$C_{28}H_{30}ClN_2O_3$	30.57	12.40	17.50	Antioxidant [62]
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	$C_{27}H_{46}O_5$	32.75	45.10	100.00	Antioxidant [59,60]

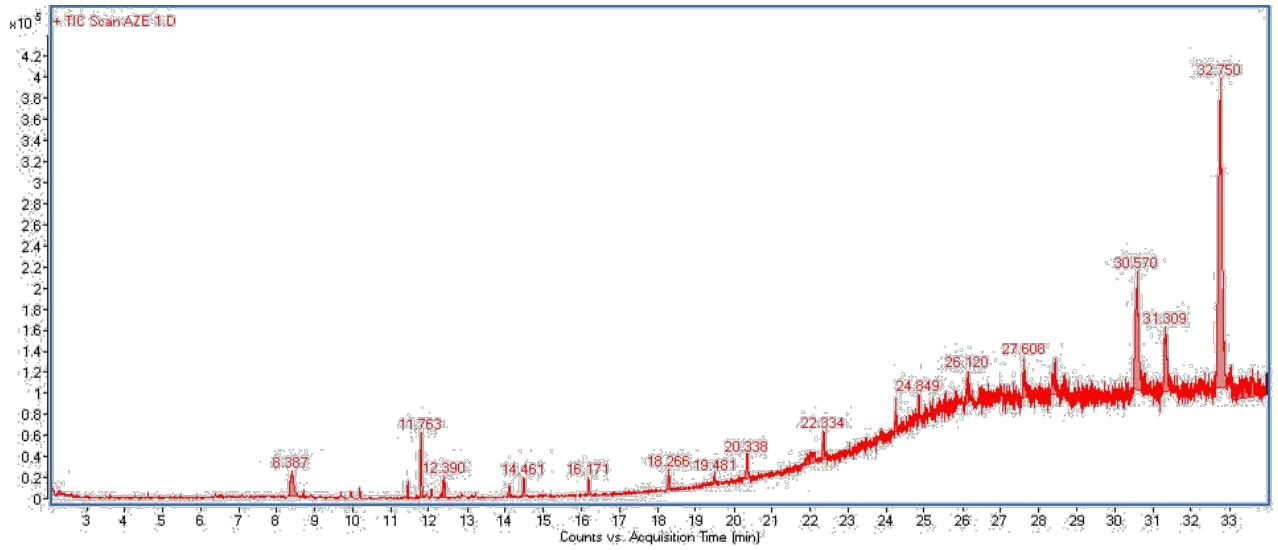


Fig. 15: A chromatogram showing the different peaks and retention times from which compounds were identified in *A. garckeana* leaf ethanolic extracts.

The chromatogram shows a total of sixteen peaks with the retention times being 8.39, 11.76 min, 12.39 min, 14.46 min, 16.17 min, 18.27 min, 19.48 min, 20.39 min, 22.33 min, 24.85 min, 26.12 min, 27.61 min, 30.57 min, 31.31 min and 32.75 min respectively. From the sixteen peaks, six important compounds were detected from the six peaks. The compounds detected were 3-Methylmannoside , 3*H*-Furan-2-one, 3-cyclohexylidene-5-(4-hexanoyloxyphenyl)- , cyclononasiloxane, octadecamethyl, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, xanthylium, 9-[2-(ethoxycarbonyl) phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, chloride and cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)- during retention times of 8.39 min, 11.76 min, 16.17 min, 18.27 min, 30.57 min and 32.75 min respectively. Peaks of retention time 12.39 min, 24.85 min and 27.61 min detected the same compound; octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- as at peak of retention time of 18.29 min. Other peaks could not be resolved and therefore compounds could not be detected.

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Six compounds were identified from the GC-MS analysis of *P. oleracea* root methanolic extracts (Table 6,Appendix H3). A wide variety of antioxidant compounds were identified, which may explain the antioxidant activity of the extracts observed during both ABTS and DPPH radical scavenging activity analysis. The identified compounds also have other biological activities such as anti-microbial and neuroprotective activities. One compound, Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-, was very abundant with 100.00% abundance showing a 100% matching probability of the compound detected with the reference compound from the mass spectral library (Table 6).

Table 6. Different bioactive compounds identified from *P. oleracea* root methanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% Probability	Peak area (%)	Biologicalactivity [Reference]
3-O-Methyl-D-glucose	194.18	$C_7H_{14}O_6$	8.49	35.60	17.31	Antioxidant [63]
Nordextromethorphan	257.37	$C_{17}H_{23}NO$	11.74	43.50	5.49	-Neuroprotective [64] -Anti-inflammatory [64]
Cyclononasiloxane, octadecamethyl-	667.39	$C_{18}H_{54}O_9Si_9$	12.05	32.30	3.63	-Antioxidant [52] -Antimicrobial [52]
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	505.09	$C_{14}H_{44}O_6Si_7$	16.14	17.90	6.55	-Antioxidant [65,[66] -Antibacterial [65,66]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	18.29	20.30	5.27	Anticancer [55,56]
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	$C_{27}H_{46}O_5$	32.73	33.50	100.00	Antioxidant [59,60]

Six different significant compounds were identified between the retention times of 11.74 and 32.76 mins during GC-MS analysis of *P. oleracea* leaf ethanolic extracts (Table 7, Appendix H4). Most compounds identified are linked to antioxidant activity whereas one compound, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, is linked to anticancer activity. This compound was obtained from several retention times explaining its abundance in the extracts (Table 7).

Table 7. Different bioactive compounds identified from *P. oleracea* leaf ethanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% Probability	Peak area (%)	Biological activity [Reference]
Nordextromethorphan	257.37	$C_{17}H_{23}NO$	11.74	25.00	8.88	-Neuroprotective [64] -Anti-inflammatory [64]
Cyclononasiloxane, octadecamethyl-	667.39	$C_{18}H_{54}O_9Si_9$	12.05	52.00	8.41	-Antioxidant [52] -Antimicrobial [52]
(6R)-5,6,7,8-Tetrahydrobiopterin	241.25	$C_9H_{15}N_5O_3$	12.39	9.97	3.57	Antioxidant [67,68]
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	505.09	$C_{14}H_{44}O_6Si_7$	19.46	35.70	6.53	-Antioxidant [65, 66] -Antibacterial [65,66]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	22.34	37.90	12.01	Anticancer [55,56]
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	$C_{27}H_{46}O_5$	32.76	59.90	100.00	Antioxidant [59,60]

GC-MS analysis identified 8 compounds from *A. garckeana* leaf methanolic extracts (Table 8, Appendix H5). Three of these compounds are associated with anticancer activity. Compounds such as Cyclononasiloxane and octadecamethyl were detected at different retention times, suggesting that the compounds are abundant in the extracts (Table 8).

The anti-inflammatory compound, 7-chloro-*N*-{3-[(diethylamino)methyl]-4[(trimethylsilyl)oxy]phenyl} *N*(trimethylsilyl)quinolin-4-amine, was found to cover the greatest peak area when compared to the other compounds. One detected compound, cyclobutane, 1*R*,3*E*-*bis*(4-methoxy-2-oxo- 2*H*-pyran-6-yl)-2*Z*,4*E*-*bis*(4-ethylphenyl)-, is not associated with the antioxidant or anticancer activity but rather with antifungal activity (Table 8).

Table 8. Different bioactive compounds identified from *A. garckeana* leaf methanolic extract

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% probability	Peak area (%)	Biological activity [Reference]
3-O-Methyl-D-glucose	194.18	$C_7H_{14}O_6$	8.36	38.80	19.03	Antioxidant [63]
10-Acetoxy-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester	342.38	$C_{20}H_{22}O_5$	9.93	15.20	4.10	Anticancer [69]
Cyclobutane, 1R,3E-bis(4-methoxy-2-oxo-2H-pyran-6-yl)-2Z,4E-bis(4-ethylphenyl)-	512.59	$C_{32}H_{32}O_6$	11.74	21.80	15.17	Antifungal [70]
3(2H)-Benzofuranone, 6-methoxy 2-[(3-methoxyphenyl) methylene]-, (E)-	289.29	$C_{17}H_{14}O_4$	12.42	23.10	12.78	Anti-tumor [71]
7-Chloro-N-{3-[(diethylamino)methyl]-4-[(trimethylsilyl)oxy] phenyl}-N-(trimethylsilyl)quinolin-4-amine	500.22	$C_{26}H_{38}ClN_3OSi_2$	16.14	13.60	35.34	Anti-inflammatory [72,73]
5H-Cyclohepta[b]pyridine-3-carbonitrile, 6,7,8,9-tetrahydro-2-amino-4-(4-fluorophenyl)-	281.33	$C_{17}H_{16}FN_3$	17.37	4.90	5.84	Antimycobacterial [74]
Cyclononasiloxane, octadecamethyl-	667.39	$C_{18}H_{54}O_9Si_9$	18.26	25.20	3.85	-Antioxidant [52] -Antimicrobial [52]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	19.49	15.30	6.53	Anticancer [55,56]

Nine different compounds were detected by GC-MS analysis of *P. oleracea* leaf methanolic extracts (Table 9,Appendix H6). Detection of the compounds was between the retention times of 6.74 and 32.79 min. The identified compounds have diverse biological activities from anticancer, antioxidant, antidiabetic, anti-inflammatory, anti-tumor, anti-HIV and anti-microbial activities [52,55-56,63,69,70-74]. Most compounds were reported to exhibit antioxidant and anticancer activities (Table 9).

Table 9. Different bioactive compounds identified from *P. oleracea* leaf methanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% Probability	Peak area (%)	Biological activity [Reference]
4-Ethyl-2*E*-(hydroxyimino)-5-nitro-3*E*-hexenamide	215.21	$C_8H_{13}N_3O_4$	6.74	38.00	1.12	-Anticancer [75] -Anti-HIV [75] -Anti-bacterial [75]
Cyclooctasiloxane, hexadecamethyl-	593.23	$C_{16}H_{48}O_8Si_8$	8.39	48.90	27.21	-Antioxidant [76] -Antimicrobial [76]
10-Acetoxy-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester	342.38	$C_{20}H_{22}O_5$	9.93	18.30	2.83	Anticancer [69]
Cyclononasiloxane, octadecamethyl	667.38	$C_{18}H_{54}O_9Si_9$	10.14	58.50	7.56	-Antimicrobial [77] -Anticancer [77]
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292.41	$C_{18}H_{28}O_3$	11.65	66.90	3.50	-Antimicrobial [78] -Antioxidant [78]
1-Methyl-10,18-bisnorabieta-8,11,13-triene	256.42	$C_{19}H_{28}$	12.36	7.65	7.64	Anticancer, Antidiabetic, Antimicrobial, Anti-inflammatory [79]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	24.28	55.60	7.690	Anticancer [55,56]
Cyclopropa[3',4'] benz[1',2':4,5]azuleno[1,8a-d]-1,3-dioxole-5b,7,7a-triol, 3a,5a,6,7,8,8a,8b,11-octahydro-10-(hydroxymethyl	490.58	$C_{27}H_{38}O_8$	31.32	22.90	16.20	Antioxidant, Anti-tumor, Anti-inflammatory and Anti-diabetic [80]
Podofilox	414.40	$C_{22}H_{22}O_8$	32.79	47.20	12.90	Anticancer [81,82]

Seven compounds were identified by GC-MS between retention times of 6.49 min and 32.73 mins from *P. oleracea* stem methanolic extracts (Table 10, Appendix H7). Most of the identified compounds have antioxidant and anticancer activities.

Table 10. Different bioactive compounds identified from *P. oleracea* stem methanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% Probability	Peak area (%)	Biological activity [Reference]
Salmeterol	415.56	$C_{25}H_{37}NO_4$	6.49	33.80	11.91	Anti-inflammatory [83]
Cyclooctasiloxane, hexadecamethyl-	593.23	$C_{16}H_{48}O_8Si_8$	8.39	59.30	11.68	Antioxidant, Antimicrobial [76]
Cyclononasiloxane, octadecamethyl	667.38	$C_{18}H_{54}O_9Si_9$	10.14	37.10	12.09	Antimicrobial, Anticancer [77]
3H-Furan-2-one, 3-cyclohexylidene-5-(4-hexanoyloxyphenyl)-	386.44	$C_{22}H_{26}O_6$	11.74	20.40	15.59	Anti-cancer, Anti-inflammatory, Anti-viral [84]
: Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	18.29	20.70	9.97	Anticancer [55,58]
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	505.09	$C_{14}H_{44}O_6Si_7$	21.45	37.40	18.25	Antioxidant, Antibacterial [65,66]
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	$C_{27}H_{46}O_5$	32.73	57.90	4.49	Antioxidant [59,60]

During the GC-MS analysis of compounds from *P. oleracea* stem ethanolic extracts, 8 compounds were identified between the retention times of 4.58 min and 31.38 min (Table 11, Appendix H8). Compounds identified were found to exhibit mostly antioxidant and anticancer activities with a few displaying antimicrobial activity (Table 11).

Table 11. Different bioactive compounds identified from *P. oleracea* stem ethanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% Probability	Peak area (%)	Biological activity [Reference]
Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$	4.58	13.10	8.95	Antioxidant, Antimicrobial [76]
Cycloheptasiloxane, tetradecamethyl-	519.08	$C_{14}H_{42}O_7Si_7$	6.49	35.40	7.37	Antioxidant, Antimicrobial [76]
3-O-Methyl-D-glucose	194.18	$C_7H_{14}O_6$	8.70	48.60	44.20	Antioxidant [63]
Acetamide, 2-(1,2,3,4-tetrahydro-3,3-dimethylisoquinolin-1-ylideno)-N-(2-chlorophenyl)-	326.82	$C_{19}H_{19}ClN_2O$	9.90	9.34	8.51	Anti-inflammatory, Anti-neoplastic, Antioxidant [85]
Aminoglutethimide	232.28	$C_{13}H_{16}N_2O_2$	11.41	7.57	1.94	Anti-cancer [86]
Cyclononasiloxane, octadecamethyl	667.38	$C_{18}H_{54}O_9Si_9$	12.05	53.80	5.91	Antimicrobial, Anticancer [77]
1-Methyl-10,18-bisnorabieta-8,11,13-triene	256.42	$C_{19}H_{28}$	12.39	10.60	4.09	Anticancer, Antidiabetic, Antimicrobial, Anti-inflammatory [79]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	31.38	21.30	4.85	Anticancer [55,56]

Eight compounds were detected by GC-MS analysis of *A. garckeana* root methanolic extracts (Table 12, Appendix H9). The compounds were identified between retention times of 4.61 min and 32.76 mins. Most of the identified compounds have antioxidant and anticancer activities[55-56,63,76-77,87-95].

One compound, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, was detected at a peak area of 100% which suggests that it was abundant in the extracts (Table 12). Most of the identified compounds seem to be related as they are methyl derivatives. The compound 2(1*H*)-Pyrimidinone, 5-chloro-4,6-diphenyl- has cytotoxic activity [94,95] (Table 12).

Table 12. Different bioactive compounds identified from *A. garckeana* root methanolic extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% probability	Peak area (%)	Biological activity [Reference]
Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$	4.61	94.40	15.14	Antioxidant, Antimicrobial [76]
Cyclooctasiloxane, hexadecamethyl-	593.23	$C_{16}H_{48}O_8Si_8$	8.39	51.80	13.95	Antioxidant, Antitumor, Antifungal, Antibacterial [87,88]
Cyclononasiloxane, octadecamethyl-	667.39	$C_{18}H_{54}O_9Si_9$	10.14	50.00	14.66	Anti-hypertensive, Anti-bacterial [76,77],[89]
Benzoic acid, 4-amino-2-hydroxy-, tris(trimethylsilyl) deriv.	631.09	$C_{16}H_{31}NO_3Si_3$	12.15	82.70	11.70	Anti-inflammatory, Anti-fungal [90,91]
Tetracosamethyl-cyclododecasiloxane	889.84	$C_{24}H_{72}O_{12}Si_{12}$;	16.17	43.60	15.77	Antioxidant, Anti-bacterial [92,93]
2(1H)-Pyrimidinone, 5-chloro-4,6-diphenyl-	282.72	$C_{16}H_{11}ClN_2O$	17.33	14.70	60.57	Cytotoxic , Anti-bacterial, Anti-inflammatory, Anti-malarial, Anti-viral [94,95]
Cyclononasiloxane, octadecamethyl	667.38	$C_{18}H_{54}O_9Si_9$	18.26	67.20	13.48	Antimicrobial, Anticancer [77]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	32.76	100.00	11.99	Anticancer [55,56]

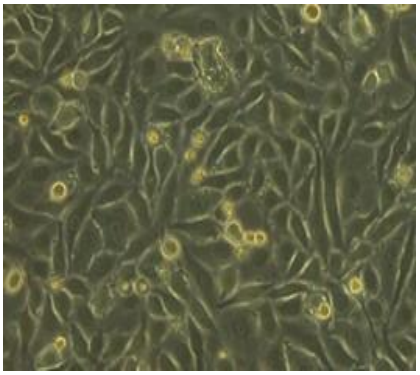
There were six different compounds identified from *A. garckeana* stem methanolic extracts between retention times of 8.43 min and 22.04 min (Table 13, Appendix H10). When compared to other extracts, the detected compounds were less, and the first compound was detected late as seen from the retention time. All the identified compounds either displayed antioxidant, anti- inflammatory, anti-microbial or anticancer activity [55-56,63,77,96-98]. Most of the compounds show antioxidant and anticancer activities (Table 13).

Table 13. Different bioactive compounds identified from *A. garckeana* stem methanolic extracts

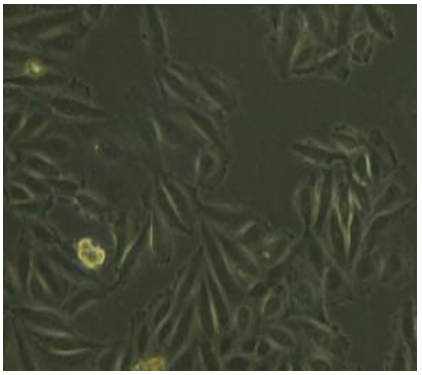
Name of compound	Molecular weight (g/mol)	Formula of compound	Retention time(min)	% Probability	Peak area (%)	Biological activity [Reference]
3-O-Methyl-D-glucose	194.18	$C_7H_{14}O_6$	8.43	44.70	23.31	Antioxidant [63]
1-Methyl-10,18-bisnorabieta-8,11,13-triene	256.43	$C_{19}H_{28}$	12.39	8.43	2.00	Antioxidant, Anti-microbial, Anti-inflammatory [96]
Benzoic acid, 4-amino-2-hydroxy-, tris(trimethylsilyl) deriv	369.68	$C_{16}H_{31}NO_3Si_3$	16.14	13.20	5.33	Antioxidant [97]
Cyclononasiloxane, octadecamethyl	667.38	$C_{18}H_{54}O_9Si_9$	18.23	31.60	7.20	Antimicrobial, Anticancer [77]
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	$C_{16}H_{50}O_7Si_8$	19.52	30.00	4.51	Anticancer [55,56]
4-Pyrimidinecarboxylic acid, 2,6-bis[(tert-butyldimethylsilyl) oxy]-, tert-butyldimethylsilyl ester	498.88	$C_{23}H_{46}N_2O_4Si_3$	22.04	57.80	6.82	Antioxidant [98]

4.8 Morphology of HeLa cells

Before treatment with the plant extracts, HeLa cells appeared uniform in size and glossy (Fig. 16 a and b). The cells also appeared to be proliferating at a fast rate as the cells took 48 hrs to reach 80 % confluency. The cells were passaged after those 48 hrs twice in two weeks in the absence of growth factors and presence of Pen-strep antibiotics. Moreover, the cells were attached to the culture surface truly confirming that they are adherent cells. The cells were polygonal with fibrous shape and compact. They were spread out on the whole surface at an estimated confluency of 80 % (Fig. 16 a and b).



a.

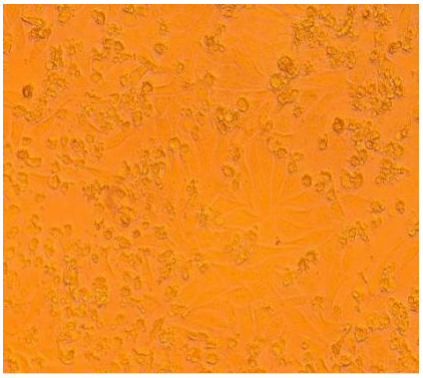


b.

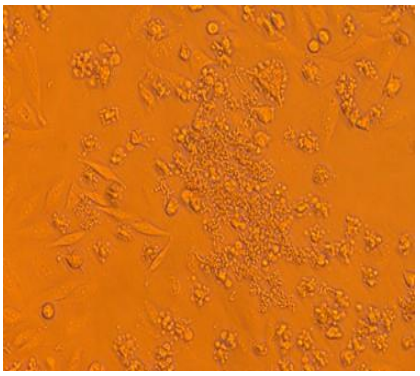
Fig. 16: Morphology of HeLa cells (P20) before treatment with *A. garckeana* and *P. oleracea* extracts at a; ×40 magnification and b; ×5 magnification captured with an inverted microscope (Olympus IX 83, Japan, Tokyo). The cells were at 80% confluency and had firmly attached to the cell culture plate surface. HeLa cells were also spread out and appeared fibrous.

4.8.1. Effects of *A. garckeana* leaf methanolic extracts on the morphology of HeLa cells

After treatment for 24 hrs with the following concentrations; 25, 50, 100, 200 and 400 µg/mL of *A. garckeana* leaf methanolic extract, HeLa cells were still attached to the surface under exposure of 25 µg/mL of *A. garckeana* leaf methanolic extracts . Media was changed once after 12 hrs of incubation and no antibiotics were used during the incubation process. The cells were no longer compact (Fig. 17 a and b). After the cells were treated with a higher concentration of *A. garckeana* leaf methanolic extracts (200 µg/mL), they appeared to be rounding up and detached from the cell culture dish, an indication of possible disturbance in cell proliferation . Dead cells were also observed (Fig. 17 b).



a.



b.

Fig. 17: Morphology of HeLa cells (P20) after 24 hr incubation with *A. garckeana* leaves methanolic extract (×40) at different concentrations (a; concentration of extract at 25 µg/ml b; concentration of extract at 200 µg/mL). The morphology was observed with an inverted microscope (Olympus IX 83, Japan, Tokyo). HeLa cells were at an estimated confluency of 30%.

4.8.2. Effects of *A. garckeana* leaf ethanolic extracts on the morphology of HeLa cells

HeLa cells were treated and incubated for 24 hrs with the following concentrations; 12.5, 25, 50, 100, 200 and 400 µg/mL. Throughout the incubation period, no antibiotics was applied onto the cells and the media was changed once after 12 hrs. The morphology was observed every after 12 hrs and observations were noted. HeLa cells were rounding up and detached from the cell culture surface after exposure to the *A. garckeana* leaf ethanolic extracts for 24 hrs (Fig. 18 a). There was a lot of cell death as seen by the many aggregates of dead cells which suggests cytotoxicity of the extracts towards the cells at a concentration of 400 µg/mL (Fig. 18 b).

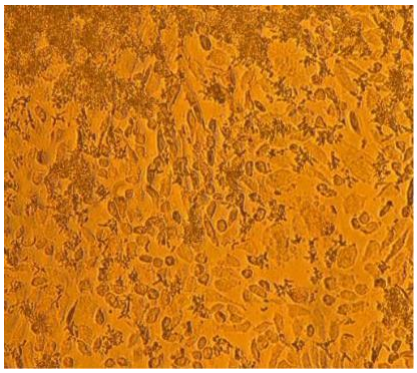


a. b.

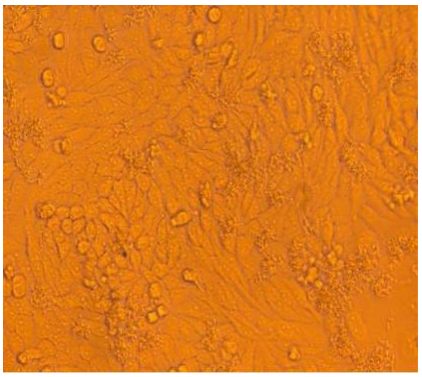
Fig. 18: Morphology of HeLa cells (P20) after 24 hr incubation with *A. garckeana* leaves ethanolic extract (×40) at different concentration (a; concentration of extract at 100 µg/ml b; concentration of extract at 400 µg/mL). The morphology of the cells was observed with an inverted microscope (Olympus IX 83, Japan, Tokyo).Confluency of the cells was estimated to be at 20 %.

4.8.3. Effects of *P. oleracea* leaf methanolic extracts on the morphology of HeLa cells

The cells were treated with the following concentrations of the extract; 12.5, 25, 50, 100, 200 and 400 µg/mL and exposed to the different concentrations of the extracts for 24 hrs. After treatment with *P. oleracea* leaf methanolic extracts, HeLa cells shrunk and detached from the cell culture surface (Fig. 19 a). The cells lost their fibrous polygonal shape and were seen floating in media (Fig. 19 b). The cells lost their compactness and no longer covered a large portion of the cell culture surface.



a.



b.

Fig. 19: Morphology of HeLa cells (P20) after 24 hr incubation with *P. oleracea* leaves methanolic extract (×40) at different concentrations (a; concentration of extract at 100 µg/ml b; concentration of extract at 400 µg/mL) captured under light inverted microscope (Olympus IX 83, Japan, Tokyo). Gaps were seen between the cells and clumps of dead cells as shown by the dark brown structures. Shiny structures show HeLa cells were still attached to the plate surface.

4.8.4. Effects of *P. oleracea* stem methanolic extracts on the morphology of HeLa cells

HeLa cells were exposed for 24 hrs under the following concentrations of the extracts 12.5, 25, 50, 100, 200 and 400 µg/mL .After treatment with *P. oleracea* stem methanolic extracts, HeLa cells were still attached to the cell culture surface (Fig. 20 a). However, cell death was seen as indicated by small aggregates of dead cells (Fig. 20 b).

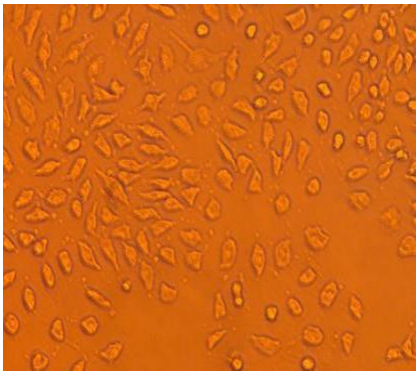


a. b.

Fig. 20: Morphology of HeLa cells (P20) after 24 hr incubation with *P.oleracea* stems methanolic extract (×40) at different concentration (a; concentration of extract at 100 µg/ml b; concentration of extract at 400 µg/mL). The morphology of the cells was captured with a light inverted microscope (Olympus IX 83, Japan, Tokyo). The shiny structures depict HeLa cells which were still attached, and the brown clumps show cells which were dead and were no longer attached to the surface of the plate.

4.8.5. Effects of cisplatin as a positive control on the morphology of HeLa cells

HeLa cells were treated with the following concentrations of cisplatin 12.5, 25, 50, 100, 200 and 400 µg/mL for 24 hrs . Cisplatin was seen to exert its effect on morphology of HeLa cells from a low concentration of 50 µg/mL (Fig. 21 a).The cells were sparse and detached from the cell culture surface. Moreover, the cells have lost their polygonal, fibrous shape (Fig. 21 a). Aggregates of dead cells were observed, a possible indication of the cytotoxicity of cisplatin towards the cells (Fig. 21 b).



a.



b.

Fig. 21: Morphology of HeLa cells (P20) after 24 hr incubation with cisplatin (×40) at different concentration (a; concentration of cisplatin at 50µg/ml b; concentration of cisplatin at 400 µg/mL) captured under a light inverted microscope (Olympus IX 83, Japan, Tokyo). The shiny structures indicate HeLa cells which are losing their attachment ability as seen from the rounding up of the cells. The brown clumps indicate cells which were dead.

4.8.6. Effects of *A. garckeana* and *P. oleracea* extracts on the viability of HeLa cells

MTT assay was used to determine the viability of HeLa cells after treatment with *A. garckeana* leaves methanolic extracts, *A. garckeana* leaf ethanolic extracts, *P. oleracea* leaves methanolic extracts and *P. oleracea* stem methanolic extracts. The anticancer drug cisplatin was used as a positive control. The results were found to be statistically significant at a p-value of $P < 0.05$. Cell viability was affected in a dose-dependent manner as the greater the concentration of the extracts the less the cell viability as seen across all extracts and cisplatin (Fig. 22). *A. garckeana* leaf ethanolic extracts reduced cell viability by 92.00 % from 97.00 % to 5.00 % at 400 µg/ml extract concentration. This indicates potential anti-proliferative activity of these extracts. *P. oleracea* leaf methanolic extracts also exhibited a significant effect on cell viability as viability decreased by a margin of 89.10 % from 96.60 % to 7.50 % at 400 µg/ml extract concentration (Fig. 22). *A. garckeana* leaf methanolic extracts reduced cell viability by 91.00 % from 95.00 % to 4.00 %. Lastly, *P. oleracea* stem methanolic extracts reduced cell viability by 69.00 % from 99.00 % to 30.00 % at 400 µg/ml extract concentration (Fig. 22). Cisplatin significantly reduced cell viability from 95.00 % to 12.00 %, a confirmation of its cytotoxic effect on cells as an anticancer drug.

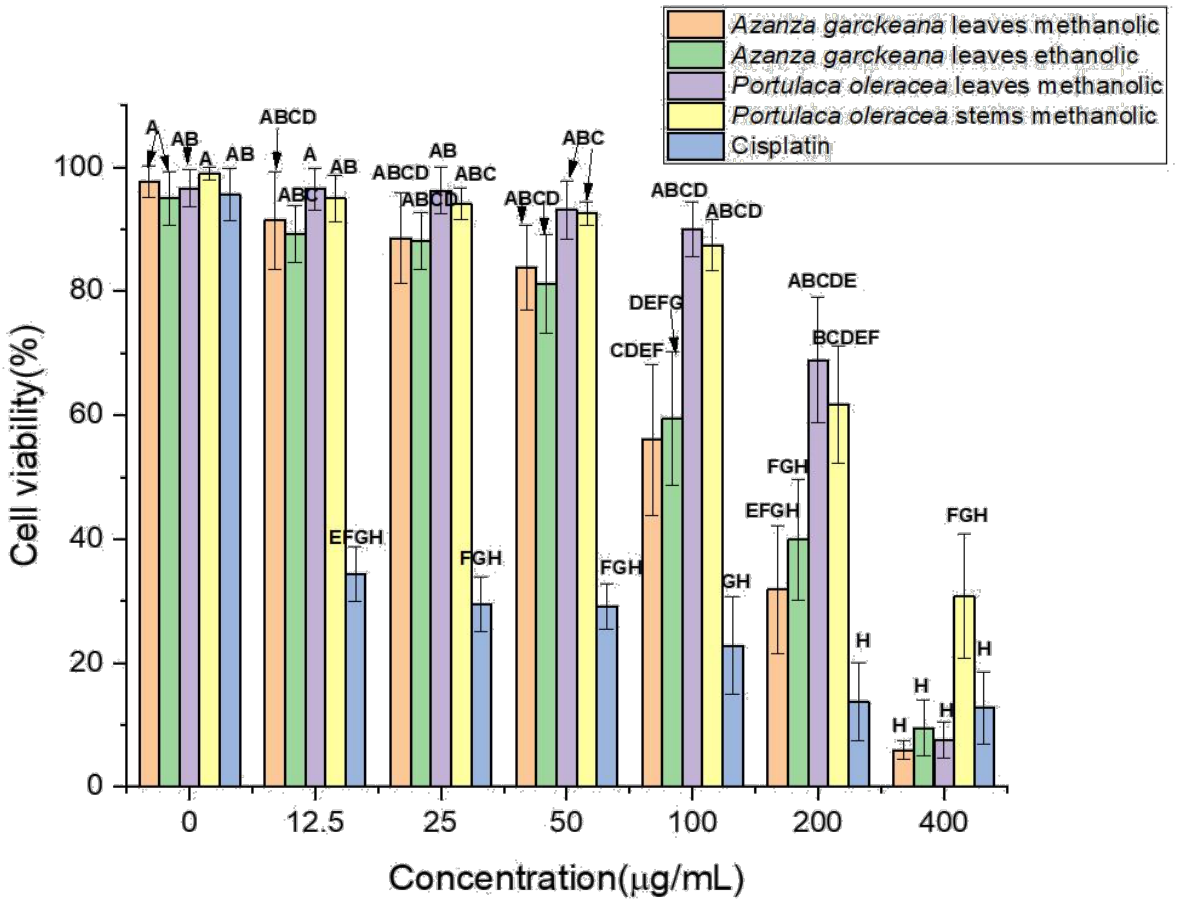


Fig. 22: Cell viability of HeLa cells (P20, n=3) determined by MTT assay after 24 hr exposure of *A. garckeana* and *P. oleracea* extracts at different concentrations ($P < 0.05$ considered statistically significant based on Tukey Kramer t-test).

The cell viability significantly decreased across all extracts after exposure of the HeLa cells for 24 hrs with the extracts at different concentrations. HeLa cells showed a significantly lower cell viability after exposure to a high concentration of 400 µg/mL of the four extracts . Cisplatin as a positive control caused a significant decrease in cell viability across all concentrations.

The IC_{50} values of the four used extracts and cisplatin were determined. Results were found to be statistically significant at $P < 0.05$ hence the use of different mean separation letters. The IC_{50} value indicates the potency of an extract or drug and level of its cytotoxic activity. This value is the concentration required to reduce proliferation of cells by 50.00 %. Therefore, the lower the IC_{50} value, the greater the cytotoxic effect of the extract. *P. oleracea* leaf methanolic extracts displayed the highest IC_{50} value (329.31 µg/mL ± 54.69), indicating the least cytotoxic effect of these extracts on HeLa cells when compared to other extracts. *A. garckeana* leaf methanolic extracts displayed the lowest IC_{50} value (176.44 µg/mL ± 25.49) amongst all the extracts, an indication of the great cytotoxicity of these extracts on HeLa cells. Cisplatin, as a positive control, displayed the lowest IC_{50} value (22.78 µg/mL ± 10.37) when compared to the extracts. This is a confirmation of the cytotoxic activity of cisplatin against cancer cells.

Table 14. Half maximal inhibitory concentrations (IC_{50}) of *A. garckeana* and *P. oleracea* extracts and cisplatin on HeLa cells after cell viability assay

Sample	Extract	IC_{50} (µg/mL)
A. garckeana leaves	Methanolic	176.44 ± 25.49 (D)
A. garckeana leaves	Ethanolic	190.47 ± 46.10 (C)
P. oleracea leaves	Methanolic	329.31 ± 54.69 (A)
P. oleracea stems	Methanolic	242.09 ± 23.60 (B)
Cisplatin	Anti-cancer drug(cisplatin)	22.78 ± 10.37 (E)

CHAPTER 5

DISCUSSION

In this study, extraction yield indicated that the extraction technique of maceration was not very effective in extracting higher quantities of phytochemicals as all solvents gave extraction yields of less than 10 % as seen from results displayed in fig. 8 and appendix A. This correlates with results from a previous study which showed that maceration can be attributed to low efficiency [42]. In one study where flavonoids were extracted from *Cajan* leaves by maceration, the extraction yield of flavonoids was found to be significantly low at 5.32 % ± 2.12 [42,99]. Factors that may have contributed to differences in extraction yield of the different solvents are solvent polarity [39], material to solvent ratio, temperature of extraction, the duration of extraction, pressure applied during extraction and pH of the solvent [100-102]. It has been reported that methanol is the most effective solvent during extraction because of its ability to impede polyphenol oxidase to oxidize phenolics [44].

From the current study, methanol and distilled water was the most efficient extraction solvent as it gave the highest extraction yields. Other studies have established a link between solvent polarity and extraction yield. It has been reported that more polar solvents result in greater extraction yield when compared to non-polar solvents. However, high polar solvents are not always effective based on extraction yield and total phenolic and flavonoid content. This is the case with distilled water which was found to result in high extraction yield but low total flavonoid and phenolic content [39,103].Other factors such as temperature can affect extraction efficiency.

It has been reported that high temperatures degrade heat sensitive compounds [39,44]. In this study, however, extraction was conducted at 37°C to preserve all heat sensitive phytochemicals. Furthermore, the material to solvent ratio which refers to the proportion of the mass of plant dry powder to the volume of liquid solvent used in extraction and the duration of extraction cannot be attributed to the low extraction yields because the duration of extraction was 72 hrs and the volumes of solvents used were significantly higher thereby allowing the interaction of phytochemicals and solvents to ensure maximal extraction.

Studies have reported that the total phenolic content of medicinal plants can be dependent on the mode of processing, part of plant from which phytochemicals are extracted from and the growth stage of plant [41,29,104]. One study reported that *P. oleracea* leaf methanolic extracts had a total phenolic content (TPC) of 1380.00 mg/100 g dry weight which drastically reduced to 1140.00 mg/100 g dry weight after the steaming processing plant [29,41,104]. Another study highlighted that the TPC of *P. oleracea* methanolic extracts of leaves improved from 127.00 to 478.00 mg /100 g of dry sample plant [29,41,104]. In this study, *P. oleracea* leaf and stem methanolic extracts displayed significantly high total phenolic content of 16.25 ± 0.48 TE mg/100 g and 16.39 ± 0.51 TE mg/100 g respectively as indicated in Fig. 9 and appendix B and it is worth noting that the TPC was the same between these extracts. However, the variation of the TPC content from results and of other studies may be attributed to differences in processing, extraction techniques and the age and growth stage of plant as pointed out by studies.

One study reported that the amount of phenols in both leaf and stem methanolic extracts of *P . oleracea* during flowering was 698.60 mg GAE/100 g and 70.90 mg GAE/100 g dry weight respectively. However, at the vegetative stage, the amount of total phenols of the same leaf extracts was 506.60 GAE mg/100 g dry weight. This confirms a link between phenolic content and the growth stage of plants [105]. The high TPC in methanolic extracts could also be attributed to the greater polarity and improved solubility of phenolic compounds found in *P. oleracea* [105].

Studies on total flavonoid content (TFC) have shown that the TFC is influenced by factors such as the part of the plant and the method of extraction. According to one study, TFC in *P. oleracea* methanolic leaf extracts was between 25.21 and 810.40 mg GAE/100 g. This variation was due to microwave and fermentation treatment [106]. Results in the current study as observed from fig. 10 indicate that *P. oleracea* leaf and stem methanolic and ethanolic extracts had a composition of flavonoids of 45.81 ± 1.02 QE mg/100 g, 41.70 ± 3.69 QE mg/100 g, 29.87 ± 2.99 QE mg/ 100 g and 28.09 ± 0.63 QE mg/100 g respectively which is in close range with values from previous studies. Another study indicated that *P. oleracea* ethyl acetate and methanolic extracts had TFC between 11.56 and 14.74 µg QE/g [107]. Another study reported that from *P. oleracea* aerial parts, numerous flavonoids were obtained from quercetin, epicatechin and kaempferol [104].

A study conducted on the pharmacological activity of *A. garckeana* pulp revealed that methanol extracts had a total phenolic and flavonoid composition of 260.80 ± 2.23 mg/100 g and 10.28 ± 1.29 mg/100 g respectively [108] . In comparison, the current study reported total phenolic and flavonoid content obtained from *A. garckeana* leaf methanolic extracts at 14.00 ± 1.00 TE mg/100 g and 52.04 ± 2.44 QE mg/100 g respectively as indicated from fig. 9 and 10. Another study evaluated the phenolic composition of *A. garckeana* fruits and reported TPC of 34.32 ± 2.34 mg/100 g [19]. Another study determined the phytochemical composition of *A. garckeana* leaf hexane, ethanol and chloroform extracts. Most phytochemicals were undetected in hexane extracts except for steroids [109]. This is in line with the results obtained from the phytochemical screening of extracts in this study as indicated in Table 1 and 2. A wide range of phytochemicals were detected in ethanol extracts [109]. This was attributed to variations of polarity of phytochemicals and solvents used during extraction [110].

It is worth noting that leaf extracts had a significantly higher total phenolic and flavonoid content in both *P. oleracea* and *A. garckeana*. According to studies light induction may alleviate the production of phenols more especially flavanols in chloroplasts and cytoplasm [44].

It is paramount for both the ABTS and DPPH assays to be used when evaluating antioxidant activity of extracts as a greater comprehensive evaluation of antioxidant activity of the extracts is provided since the assays measure different components of antioxidant activity [30,39]. GC-MS results indicated in Table 3-13 in the current study indicate the presence of compounds which have antioxidant activity. The high antioxidant activity seen in some of the extracts from both *P. oleracea* and *A. garckeana* extracts may be due to the antioxidant compounds present in extracts which participate in hydrogen electron transfer (HAT), single electron transfer (SET) and chelation of transition metals reactions [111]. These mechanisms of the three antioxidant reactions elucidated work by opposing oxidative stress and therefore bringing about antioxidant activity of the extracts. Some of the compounds that were detected from extracts with high antioxidant activity include cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)- and 3-O-Methyl-D-glucose. These compounds display antioxidant action through the presence of hydroxyl groups which modulate this activity [112]. It has been reported that phenols exhibit antioxidant activity through the presence of hydroxyl groups connected to aromatic hydrocarbon rings which give them liberty to be antioxidants by the donation of hydrogen atoms or electrons to enable free radicals to be neutralized [112].

On the other hand, polar compounds are not detected by the GC-MS and to detect compounds from *P. oleracea* and *A. garckeana* methanol and ethanol extracts as indicated in Table 5-13 derivatizing agents such as silicon agents to convert polar compounds into more volatile, less polar compounds. It has been reported that silicon agents cause silylation which replaces active hydrogens with trimethylsilyl groups increasing volatility [46].

Results from the current study correlate with previous investigations which reported the antioxidant activity of *P. oleracea* leaf and stem methanolic extracts. These extracts have been reported to display antioxidant activity by blocking the action of xanthine oxidase which is associated with producing reactive oxygen species (ROS) [46].

In the current study, high antioxidant activity as indicated in results presented from Fig. 11-14 was observed in most methanol extracts and GC-MS analysis as presented from Table 3-13 indicated the presence of many antioxidant compounds in methanolic extracts of both *P. oleracea* and *A. garckeana*. These findings correlate with findings of another study which highlighted that *P. oleracea* varieties display a significantly high antioxidant activity because polar solvents are highly effective in extracting antioxidants [44].

Another factor that can influence the antioxidant activity of extracts is the structure and type of phenolic compounds present in the extracts, particularly the number and configuration of the hydroxyl group and glycosylation. The more the number of hydroxyls the greater the antioxidants [44]. Indeed, in the current study, most extracts had compounds containing many hydroxyl groups which may be responsible for their antioxidant activity.

Within this study, hexane extracts displayed low antioxidant activity despite high total flavonoid composition. This may be attributed to the structure of the flavonoids without hydroxyl groups which leads to reduced antioxidant activity as reported previously reported [44].

The viability of HeLa cells from the current study was affected in a dose dependent manner as indicated in Fig. 22. This correlates with findings from previous study which indicated that *P. oleracea* extracts bring about anti-proliferative effects in a dose dependent fashion [32]. An additional study which highlighted the performance of *P. oleracea* extracts indicated that the IC_{50} of *P. oleracea* leaf methanolic and ethanolic extracts was 237.00 µg/mL, 155.00 µg/mL and 137.00 µg/mL after incubation with extract for 24 hrs, 48 hrs and 72 hrs in HeLa cells respectively [33].

Moreover, from the current investigation ,the IC_{50} values of *P. oleracea* methanolic leaf and stem extracts were 329.31 ± 54.69 µg/mL and 242.09 ± 23.60 µg/mL respectively after 24 hr incubation of HeLa cells as indicated in Table 14. On the other hand, from a reported study the IC_{50} value of the *A. garckeana* fruit pulp chloroform extract was found to be 767.50 µg/mL [13] .

This value was higher as compared to the value obtained from the results of IC_{50} values of 176.44 ± 25.49 µg/mL and 190.47 ± 46.10 µg/mL of *A. garckeana* leaves methanolic and ethanolic extract respectively after 24 hr. incubation of extracts in HeLa cells as indicated in table 14. This suggests that *A. garckeana* extracts from leaves have great anti-proliferative activity towards HeLa cells when compared to extracts from fruit pulp.

GC-MS results indicated from Table 3-13 from the current study are in support of the MTT assay results from Fig. 22. Some compounds identified by the GC-MS were, 10-Acetoxy-1,4a- dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester, octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- and 3(2*H*)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (*E*)-. The anti-proliferative activity displayed by extracts from *A. garckeana* may be due to the presence of anticancer compounds such as 10- acetoxy-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester are diterpenoids which demonstrate their anticancer effect by cell cycle arrest, initiating apoptosis and preventing the growth of tumours [113]. Diterpenoids bring about cell cycle arrest at the G1 and G2 M-phases, consequently blocking tumour growth and metastasis [113]. Moreover, diterpenoids exhibit their anti-proliferative activity by causing the reversal of multi drug resistance which, therefore, curbs chemoresistance and ensures that cancer cells respond swiftly to chemotherapy drugs [113,114].

Antioxidant compounds detected in both *P. oleracea* and *A. garckeana* are also attributed to the anti-proliferative activity of the extracts against HeLa cells . Studies have indicated that polyphenols block the early onset of cervical cancer by causing a regression of the proliferation of cancer cells, hindering DNA damage, lowering immunosuppression and causing the stabilization of p53 [9].

The stabilization of p53 mechanism contributes to anticancer activity as the oxidation-reduction reactions are catalysed which makes ROS to activate the release of mitochondrial cytochrome C [115].In addition apoptosis is initiated through the overexpression of the Bax. The Bax is then relayed to the mitochondrial outer membrane which stimulates the mitochondria to release cytochrome C along with other effector factors, this triggers the mitochondrial regulation pathway of apoptosis [33].

It has been reported that the application of *P. oleracea* extracts on HeLa cells induces reduction in the activity of antioxidant enzymes such as SOD, CAT and GSH-Px, rendering these enzymes ineffective in protecting HeLa cells from oxidative damage which can lead to apoptosis [33].

Podofilox, an anticancer compound that was detected from *P. oleracea* methanolic extracts, exerts its anticancer activity by alleviating cell cycle arrest at the G0/G1 phase which restrains the proliferation of cells and colony formation [82]. This compound also exerts its anticancer effect by disturbing microtubules, slowing down cell cycle progression at the G1 phase and binding to DNA [81,82]. *A. garckeana* and *P. oleracea* extracts may induce their anti-proliferative effect through the presence of flavonoids, which may prevent the formation of cyclooxygenase-2, which halts inflammation [32].

Another compound that was identified in this study, cyclononasiloxane, octadecamethyl-, exerts its anticancer activity by promoting apoptosis, preventing cell growth and altering DNA damage/repair mechanisms [116,117]. Alkaloids demonstrate anticancer activity by causing DNA damage, activating caspase, altering signalling pathways such as the mitogen activated protein kinase (MAPK) and the nuclear factor kappa B (NF-$_{k}$B) pathways and restraining the p-glycoprotein ABCB1.

Additionally, alkaloids temper with the MAPK pathway which stops uncontrollable cell divisions and thereby preventing malignancies to be initiated. The MAPK pathway is associated with signalling extracellularly to maintain growth, proliferation, differentiation, cell migration and apoptosis [118,119]. Signalling starts with the binding of a ligand to a tyrosine kinase receptor, stimulating the action of the Ras protein by phosphorylation [119]. A formed complex will then connect with effector proteins such as B-Raf to stimulate MAP kinase kinase 1 (MEK 1) and 2 [119]. These enzymes cause a cascade of events in which the extracellular signal regulated kinase-ERK 1 and 2 are activated, stimulating various transcriptional factors of the AP-1 family and these agents transport themselves to the nucleus and switches on genes encoding growth factors and proteins in cell proliferation [119]. Alkaloids, by interfering with the MAPK pathway, disrupts cell proliferation, exerting an anti-proliferative effect on HeLa cells.

Anticancer effects by alkaloids target the NF-κB pathway which is associated with switching on genes influencing cell survival and proliferation. Therefore, by targeting this pathway, alkaloids lead to apoptosis which subdues tumorigenesis [118]. Alkaloids also exert anticancer effects by interfering with the action of the p-glycoprotein, ABCB1, which is a member of the ATP binding cassette (ABC) proteins and is associated with multidrug resistance [118]. Lastly, the interaction of alkaloids with the p-glycoprotein causes the ABC protein to be downregulated, increasing the alertness of HeLa cells toward any cytotoxic agent [118].

CHAPTER 6

CONCLUSION

In conclusion, the current study has shown that *P. oleracea* and *A .garckeana* extracts display a certain measure of antioxidant activity with *P. oleracea* methanolic and chloroform extracts exerting greater antioxidant activity. This may be due to antioxidant compounds that were detected in extracts from both plants. *A. garckeana* methanolic extracts were found to display greater antioxidant activity when compared to other extracts. Methanol was found to be a more suitable solvent for extracting phytochemicals as it was able to extract a wide range of phytochemicals both polar and non-polar when compared to other extracts. Methanol was also found to be suitable for extracting antioxidant compounds whereas hexane was found to be a poor extraction solvent.

Leaf extracts of both *A. garckeana* and *P. oleracea* were found to have greater concentrations of flavonoids and phenolics when compared to the root extracts of these plants. There was no relationship between total flavonoid and phenolic content and antioxidant activity as extracts with low total phenolic and flavonoid composition displayed good antioxidant activity. There was a relationship between radical scavenging activity and antioxidant concentration because as the concentration of antioxidants increased, so did the radical scavenging activity of the compounds and vice versa. It is recommended that in future studies such as the current one, phytochemicals from *P. oleracea* whole plant and *A . garckeana* leaves, stems and roots could be combined to evaluate any singlestick or synergistic effects on their antioxidant activity.

Both *P. oleracea* and *A. garckeana* exerted anti-proliferative activity towards HeLa cells. This may be due to both anticancer and antioxidant compounds; 10-Acetoxy-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester , Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, 3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-, Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-, 3-O-Methyl-d-glucose, Cyclononasiloxane and octadecamethyl present in the extracts. The current study found out that the viability of HeLa cells was affected in a concentration dependent mode after 24 hr incubation of the HeLa cells with the extracts.

A. garckeana leaf extracts were found to exert more anti-proliferative activity than *P. Oleracea* extracts. These extracts exert anticancer activity by inducing apoptosis, causing DNA damage, increasing oxidative stress in HeLa cells which leads to oxidative damage, interfering with cell cycle progression and interacting with pathways such as MAPK and NF-$_{k}$B pathways. It is recommended that in future similar studies, the incubation of HeLa cells after treatment with plant extracts be done at different time intervals. Additionally, gene expression analysis should be undertaken in future to determine the effect of the extracts on the expression of different genes associated with cervical cancer. It is also recommended that assays such as Annexin V, flow cytometry, cytochrome c and western blot be conducted to be able to accurately elucidate mechanisms by which phytochemicals exhibit an antiproliferative activity towards HeLa cells. Lastly it is recommended that the extracts be tested on normal cell lines to see if the extracts are cytotoxic towards normal cells and are safe for administration as anticancer agents.

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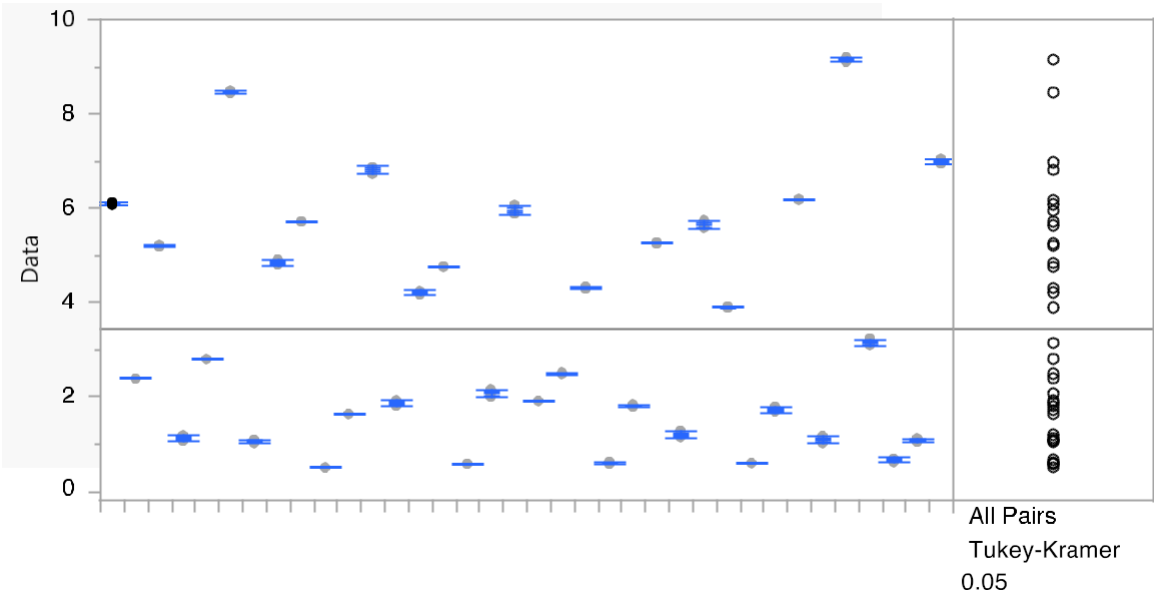
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Appendix A-Extraction yield of different solvents

Oneway Analysis of concentration by treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZLE	3	6.0933333	0.0259294	0.0149703	6.0289212	6.1577455
AZLC	3	2.4026667	0.0106927	0.0061734	2.3761046	2.4292287
AZLD	3	5.2163333	0.0195021	0.0112596	5.1678873	5.2647793
AZLH	3	1.1393333	0.0626844	0.0361909	0.9836167	1.29505
AZLI	3	2.8146667	0.0095044	0.0054874	2.7910565	2.8382769
AZLM	3	8.4646667	0.0244199	0.0140989	8.4040042	8.5253292
AZRC	3	1.0606667	0.0336056	0.0194022	0.9771858	1.1441475
AZRD	3	4.8476667	0.0540956	0.0312321	4.7132858	4.9820476
AZRE	3	5.7246667	0.0065064	0.0037565	5.7085039	5.7408295
AZRH	3	0.5166667	0.0097125	0.0056075	0.4925394	0.5407939
AZRI	3	1.645	0.006	0.0034641	1.6300952	1.6599048
AZRM	3	6.822	0.0806164	0.0465439	6.6217378	7.0222622
AZSC	3	1.8813333	0.061655	0.0355965	1.7281739	2.0344927
AZSD	3	4.2246667	0.0449704	0.0259636	4.1129541	4.3363792
AZSE	3	4.7686667	0.0109697	0.0063333	4.7414165	4.7959168
AZSH	3	0.589	0.0065574	0.0037859	0.5727104	0.6052896
AZSI	3	2.08	0.0754983	0.043589	1.8924517	2.2675483
AZSM	3	5.958	0.097509	0.0562968	5.7157743	6.2002257
POLC	3	1.9263333	0.0102632	0.0059255	1.9008381	1.9518285
POLD	3	2.4986667	0.0197569	0.0114066	2.4495879	2.5477454
POLE	3	4.318	0.0165227	0.0095394	4.2769553	4.3590447
POLH	3	0.6006667	0.0258908	0.0149481	0.5363504	0.664983
POLI	3	1.8126667	0.0244199	0.0140989	1.7520042	1.8733292
POLM	3	5.2623333	0.0120554	0.0069602	5.232386	5.2922807
PORC	3	1.2053333	0.0679877	0.0392527	1.0364424	1.3742243

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
PORD	3	5.648	0.0895991	0.0517301	5.4254235	5.8705765
PORE	3	3.904	0.01253	0.0072342	3.8728738	3.9351262
PORH	3	0.6090333	0.0104835	0.0060526	0.5829909	0.6350757
PORI	3	1.728	0.0539259	0.0311341	1.5940407	1.8619593
PORM	3	6.187	0.0115326	0.0066583	6.1583515	6.2156485
POSC	3	1.0953333	0.0735278	0.0424513	0.9126802	1.2779864
POSD	3	9.159	0.0502096	0.0289885	9.0342725	9.2837275
POSE	3	3.151	0.0720902	0.0416213	2.971918	3.330082
POSH	3	0.6723667	0.0457286	0.0264014	0.5587706	0.7859628
POSI	3	1.08	0.0408534	0.0235867	0.9785145	1.1814855
POSM	3	6.987	0.0549454	0.0317228	6.850508	7.123492

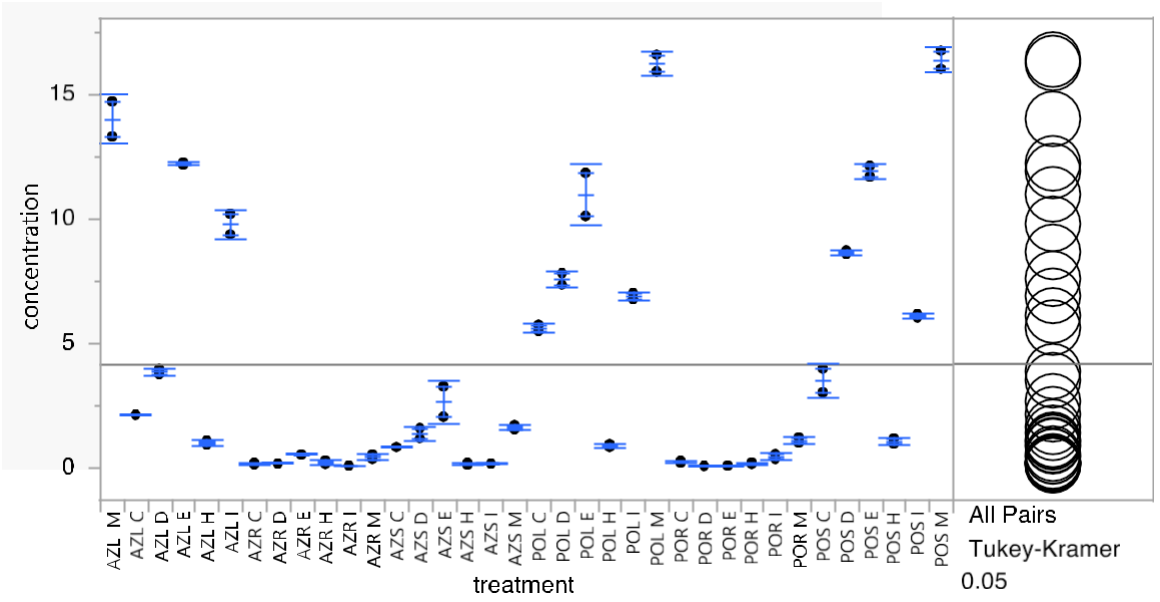
Connecting Letters Report

Level		Mean
POSD	A	9.1590000
AZLM	B	8.4646667
POSM	C	6.9870000
AZRM	D	6.8220000
PORM	E	6.1870000
AZLE	E F	6.0933333
AZSM	F	5.9580000
AZRE	G	5.7246667
PORD	G	5.6480000
POLM	H	5.2623333
AZLD	H	5.2163333
AZRD	I	4.8476667
AZSE	I	4.7686667
POLE	J	4.3180000
AZSD	J	4.2246667
PORE	K	3.9040000
POSE	L	3.1510000
AZLI	M	2.8146667
POLD	N	2.4986667
AZLC	N	2.4026667
AZSI	O	2.0800000
POLC	P	1.9263333
AZSC	P	1.8813333
POLI	P Q	1.8126667
PORI	Q R	1.7280000
AZRI	R	1.6450000
PORC	S	1.2053333
AZLH	S	1.1393333
POSC	S	1.0953333
POSI	S	1.0800000
AZRC	S	1.0606667
POSH	T	0.6723667
PORH	T U	0.6090333
POLH	T U	0.6006667
AZSH	T U	0.5890000
AZRH	U	0.5166667

Levels not connected by same letter are significantly different.

Appendix B-Total Phenolic content

Oneway Analysis of concentration by treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZL M	2	13.996	1.0040916	0.71	4.9745946	23.017405
AZL C	2	2.096	0.0141421	0.01	1.968938	2.223062
AZL D	2	3.8255	0.1421285	0.1005	2.5485264	5.1024736
AZL E	2	12.206	0.0565685	0.04	11.697752	12.714248
AZL H	2	0.966	0.1131371	0.08	-0.050496	1.9824964
AZL I	2	9.766	0.5939697	0.42	4.429394	15.102606
AZR C	2	0.1195	0.0374767	0.0265	-0.217214	0.4562144
AZR D	2	0.146	0.0282843	0.02	-0.108124	0.4001241
AZR E	2	0.5085	0.0247487	0.0175	0.2861414	0.7308586
AZR H	2	0.176	0.0989949	0.07	-0.713434	1.0654343
AZR I	2	0.0415	0.006364	0.0045	-0.015678	0.0986779
AZR M	2	0.396	0.1272792	0.09	-0.747558	1.5395584
AZS C	2	0.806	0.0282843	0.02	0.5518759	1.0601241
AZS D	2	1.346	0.2828427	0.2	-1.195241	3.8872409
AZS E	2	2.616	0.8626703	0.61	-5.134785	10.366785
AZS H	2	0.116	0.0424264	0.03	-0.265186	0.4971861
AZS I	2	0.146	0.0282843	0.02	-0.108124	0.4001241
AZS M	2	1.586	0.1131371	0.08	0.5695036	2.6024964
POL C	2	5.586	0.1697056	0.12	4.0612554	7.1107446
POL D	2	7.556	0.3252691	0.23	4.6335729	10.478427
POL E	2	10.956	1.2303658	0.87	-0.098398	22.010398
POL H	2	0.846	0.0848528	0.06	0.0836277	1.6083723
POL I	2	6.866	0.1697056	0.12	5.3412554	8.3907446
POL M	2	16.246	0.4808326	0.34	11.92589	20.56611
POR C	2	0.196	0.0424264	0.03	-0.185186	0.5771861
POR D	2	0.046	0.0282843	0.02	-0.208124	0.3001241

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POR E	2	0.056	0.0141421	0.01	-0.071062	0.183062
POR H	2	0.1325	0.0374767	0.0265	-0.204214	0.4692144
POR I	2	0.406	0.1414214	0.1	-0.86462	1.6766205
POR M	2	1.066	0.1414214	0.1	-0.20462	2.3366205
POS C	2	3.466	0.6788225	0.48	-2.632978	9.5649783
POS D	2	8.636	0.0989949	0.07	7.7465657	9.5254343
POS E	2	11.896	0.2969848	0.21	9.227697	14.564303
POS H	2	1.026	0.1414214	0.1	-0.24462	2.2966205
POS I	2	6.076	0.0989949	0.07	5.1865657	6.9654343
POS M	2	16.386	0.5091169	0.36	11.811766	20.960234

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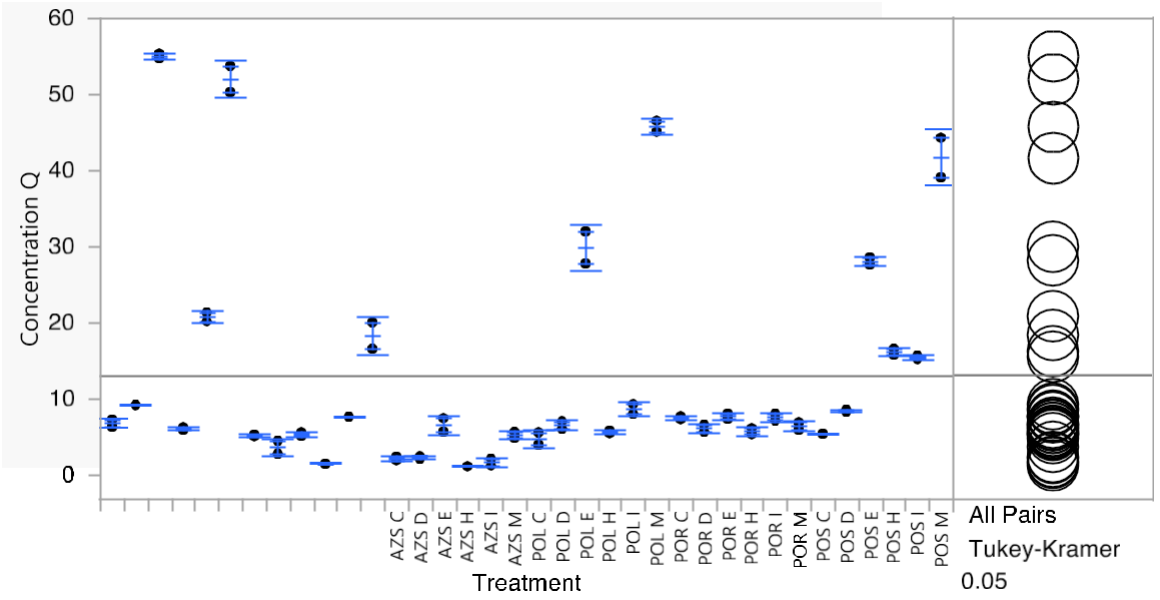
Connecting Letters Report

Level	Letters	Mean
POS M	A	16.386000
POL M	A	16.246000
AZL M	B	13.996000
AZL E	C	12.206000
POS E	C	11.896000
POL E	C D	10.956000
AZL I	D E	9.766000
POS D	E F	8.636000
POL D	F G	7.556000
POL I	G H	6.866000
POS I	G H	6.076000
POL C	H	5.586000
AZL D	I	3.825500
POS C	I J	3.466000
AZS E	I J K	2.616000
AZL C	J K L	2.096000
AZS M	K L M	1.586000
AZS D	K L M	1.346000
POR M	K L M	1.066000
POS H	L M	1.026000
AZL H	L M	0.966000
POL H	L M	0.846000
AZS C	L M	0.806000
AZR E	M	0.508500
POR I	M	0.406000
AZR M	M	0.396000
POR C	M	0.196000
AZR H	M	0.176000
AZR D	M	0.146000
AZS I	M	0.146000
POR H	M	0.132500
AZR C	M	0.119500
AZS H	M	0.116000
POR E	M	0.056000
POR D	M	0.046000
AZR I	M	0.041500

Levels not connected by same letter are significantly different.

Appendix C-Total flavonoid content

Oneway Analysis of Concentration Q By Treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZL C	2	6.7555	0.6286179	0.4445	1.107592	12.403408
AZL D	2	9.1845	0.0572756	0.0405	8.6698987	9.6991013
AZL E	2	55.0615	0.4320422	0.3055	51.179754	58.943246
AZL H	2	6.061	0.1965757	0.139	4.2948375	7.8271625
AZL I	2	20.747	0.7976164	0.564	13.580701	27.913299
AZL M	2	52.0335	2.4359829	1.7225	30.147062	73.919938
AZR C	2	5.1445	0.1576848	0.1115	3.7277582	6.5612418
AZR D	2	3.589	1.1780399	0.833	-6.995269	14.173269
AZR E	2	5.311	0.3139554	0.222	2.4902225	8.1317775
AZR H	2	1.4775	0.0784889	0.0555	0.7723056	2.1826944
AZR I	2	7.6165	0.0388909	0.0275	7.2670794	7.9659206
AZR M	2	18.283	2.4748737	1.75	-3.952858	40.518858
AZS C	2	2.117	0.3535534	0.25	-1.059551	5.2935512
AZS D	2	2.2555	0.2354666	0.1665	0.1399169	4.3710831
AZS E	2	6.533	1.2572359	0.889	-4.762816	17.828816
AZS H	2	1.1445	0.0784889	0.0555	0.4393056	1.8496944
AZS I	2	1.6445	0.6286179	0.4445	-4.003408	7.292408
AZS M	2	5.228	0.5105311	0.361	0.6410601	9.8149399
POL C	2	4.7275	1.139149	0.8055	-5.507348	14.962348
POL D	2	6.5055	0.6682159	0.4725	0.5018183	12.509182
POL E	2	29.867	2.9854048	2.111	3.0442018	56.689798
POL H	2	5.589	0.2361737	0.167	3.4670638	7.7109362
POL I	2	8.617	0.9036825	0.639	0.4977352	16.736265
POL M	2	45.811	1.0210622	0.722	36.63712	54.98488
POR C	2	7.459	0.2616295	0.185	5.1083521	9.8096479

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POR D	2	6.0885	0.6286179	0.4445	0.440592	11.736408
POR E	2	7.6725	0.4320422	0.3055	3.7907545	11.554246
POR H	2	5.709	0.562857	0.398	0.6519305	10.766069
POR I	3	7.552	0.602455	0.426	2.1391568	12.964843
POR M	2	6.3945	0.6682159	0.4725	0.3908183	12.398182
POS C	2	5.3945	0.0388909	0.0275	5.0450794	5.7439206
POS D	2	8.3945	0.1958686	0.1385	6.6346906	10.154309
POS E	2	28.0885	0.6286179	0.4445	22.440592	33.736408
POS H	2	16.1445	0.549422	0.3885	11.208139	21.080861
POS I	2	15.422	0.3139554	0.222	12.601223	18.242777
POS M	2	41.7	3.6925116	2.611	8.5240994	74.875901

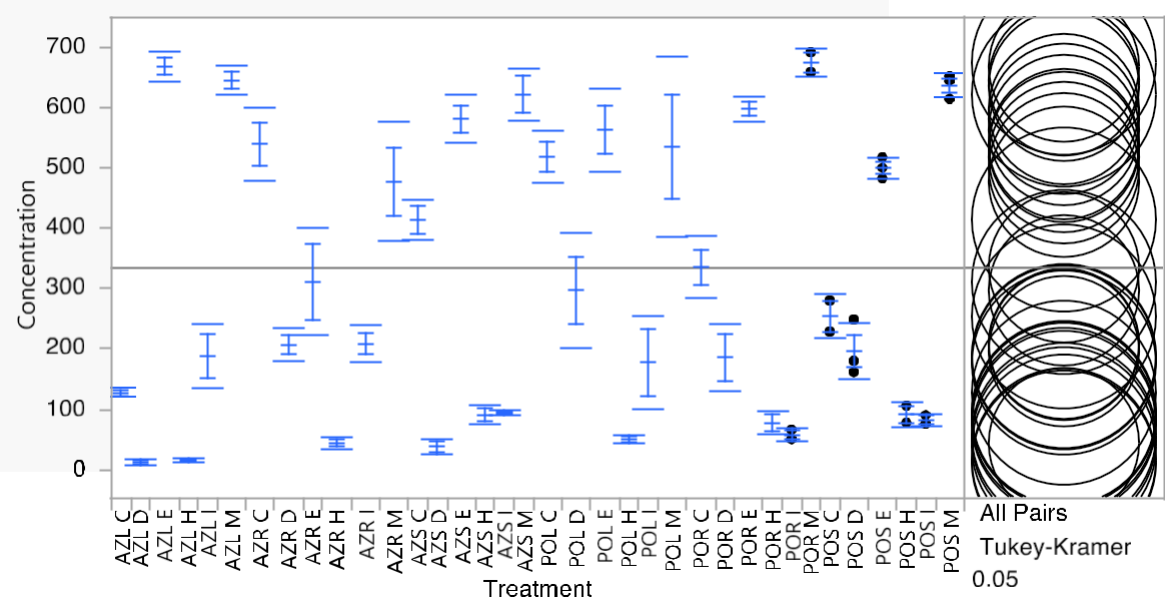
Connecting Letters Report

Level										Mean
AZL E	A									55.061500
AZL M	A									52.033500
POL M		B								45.811000
POS M		B								41.700000
POL E			C							29.867000
POS E			C							28.088500
AZL I				D						20.747000
AZR M				D	E					18.283000
POS H				D	E					16.144500
POS I					E					15.422000
AZL D						F				9.184500
POL I						F				8.617000
POS D						F				8.394500
POR E						F	G			7.672500
AZR I						F	G			7.616500
POR I						F	G			7.552000
POR C						F	G			7.459000
AZL C						F	G	H		6.755500
AZS E						F	G	H		6.533000
POL D						F	G	H		6.505500
POR M						F	G	H		6.394500
POR D						F	G	H	I	6.088500
AZL H						F	G	H	I	6.061000
POR H						F	G	H	I	J 5.709000
POL H						F	G	H	I	J 5.589000
POS C						F	G	H	I	J 5.394500
AZR E						F	G	H	I	J 5.311000
AZS M						F	G	H	I	J 5.228000
AZR C						F	G	H	I	J 5.144500
POL C						F	G	H	I	J 4.727500
AZR D							G	H	I	J 3.589000
AZS D								H	I	J 2.255500
AZS C								H	I	J 2.117000
AZS I									I	J 1.644500
AZR H									I	J 1.477500
AZS H										J 1.144500

Levels not connected by same letter are significantly different.

Appendix D-DPPH Trolox equivalents

Oneway Analysis of Concentration by Treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZL C	2	126.57143	7.0710678	5	63.040405	190.10245
AZL D	2	10.857143	5.0507627	3.5714286	-34.52216	56.236445
AZL E	3	667.28571	24.369603	14.069797	606.74826	727.82316
AZL H	2	14.428571	4.0406102	2.8571429	-21.87487	50.732014
AZL I	2	187.28571	52.527932	37.142857	-284.659	659.23046
AZL M	3	644.42857	24.411439	14.093951	583.78719	705.06995
AZR C	3	538.71429	60.827625	35.118846	387.61009	689.81848
AZR D	3	205.85714	27.367492	15.800629	137.87253	273.84176
AZR E	2	310.14286	88.893424	62.857143	-488.5329	1108.8186
AZR H	3	43	10	5.7735027	18.158623	67.841377
AZR I	3	207.7619	30.113165	17.385844	132.95666	282.56715
AZR M	3	476.33333	98.977768	57.144841	230.45893	722.20774
AZS C	2	413.71429	33.335034	23.571429	114.21089	713.21768
AZS D	2	37.285714	12.121831	8.5714286	-71.62461	146.19604
AZS E	3	580.61905	39.183851	22.622807	483.28097	677.95713
AZS H	2	90.142857	16.162441	11.428571	-55.07091	235.35663
AZS I	2	93	4.0406102	2.8571429	56.696558	129.30344
AZS M	2	620.85714	43.436559	30.714286	230.59514	1011.1191
POL C	3	517.28571	43.236417	24.962557	409.8805	624.69093
POL D	3	296.33333	95.685852	55.244253	58.636499	534.03017
POL E	3	562.04762	69.050903	39.866557	390.51567	733.57957
POL H	2	49.428571	7.0710678	5	-14.10245	112.9596
POL I	2	176.57143	77.781746	55	-522.2698	875.41269
POL M	3	533.95238	149.04686	86.052244	163.69946	904.2053
POR C	3	334.42857	51.130086	29.519969	207.4144	461.44275
POR D	2	185.14286	55.55839	39.285714	-314.0295	684.31519

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POR E	3	596.80952	20.66908	11.933299	545.46468	648.15437
POR H	2	76.571429	19.192898	13.571429	-95.86992	249.01278
POR I	2	56.571429	11.111678	7.8571429	-43.26304	156.40589
POR M	2	673.71429	23.233508	16.428571	464.96949	882.45908
POS C	2	253	36.365492	25.714286	-73.73098	579.73098
POS D	3	195.38095	45.899921	26.500332	81.359228	309.40268
POS E	3	498.71429	17.142857	9.8974332	456.12907	541.2995
POS H	2	90.142857	20.203051	14.285714	-91.37435	271.66007
POS I	2	81.571429	10.101525	7.1428571	-9.187177	172.33003
POS M	3	635.38095	19.708764	11.37886	586.42167	684.34024

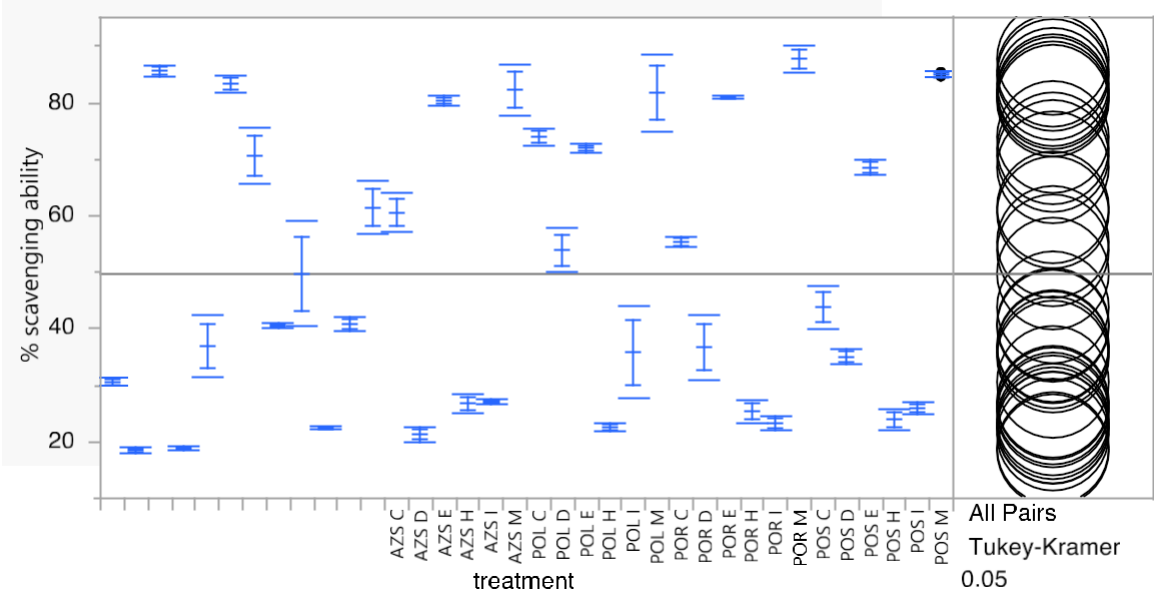
Connecting Letters Report

Level									Mean
POR M	A								673.71429
AZL E	A								667.28571
AZL M	A	B							644.42857
POS M	A	B							635.38095
AZS M	A	B	C						620.85714
POR E	A	B	C						596.80952
AZS E	A	B	C						580.61905
POL E	A	B	C						562.04762
AZR C	A	B	C						538.71429
POL M	A	B	C						533.95238
POL C	A	B	C						517.28571
POS E	A	B	C	D					498.71429
AZR M		B	C	D					476.33333
AZS C			C	D	E				413.71429
POR C				D	E	F			334.42857
AZR E				D	E	F	G		310.14286
POL D					E	F	G		296.33333
POS C					E	F	G	H	253.00000
AZR I						F	G	H	I	207.76190
AZR D						F	G	H	I	205.85714
POS D						F	G	H	I	195.38095
AZL I						F	G	H	I	187.28571
POR D						F	G	H	I	185.14286
POL I						F	G	H	I	176.57143
AZL C							G	H	I	126.57143
AZS I								H	I	93.00000
AZS H								H	I	90.14286
POS H								H	I	90.14286
POS I								H	I	81.57143
POR H								H	I	76.57143
POR I								H	I	56.57143
POL H								H	I	49.42857
AZR H									I	43.00000
AZS D								H	I	37.28571
AZL H									I	14.42857
AZL D									I	10.85714

Levels not connected by same letter are significantly different.

Appendix E-DPPH radical scavenging activity

Oneway Analysis of % scavenging ability by treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZL C	2	30.522388	0.7387683	0.5223881	23.884818	37.159958
AZL D	2	18.432836	0.5276916	0.3731343	13.691715	23.173957
AZL E	2	85.597015	0.9498449	0.6716418	77.062997	94.131033
AZL H	2	18.80597	0.4221533	0.2985075	15.013073	22.598867
AZL I	2	36.865672	5.4879929	3.880597	-12.44199	86.173332
AZL M	2	83.283582	1.4775366	1.0447761	70.008443	96.558721
AZR C	2	70.522388	4.9603013	3.5074627	25.955849	115.08893
AZR D	2	40.447761	0.4221533	0.2985075	36.654864	44.240658
AZR E	2	49.701493	9.2873726	6.5671642	-33.74224	133.14523
AZR H	2	22.38806	0.2110766	0.1492537	20.491611	24.284508
AZR I	2	40.746269	1.2664599	0.8955224	29.367578	52.124959
AZR M	2	61.41791	4.7492246	3.358209	18.74782	104.088
AZS C	2	60.522388	3.4827647	2.4626866	29.230988	91.813788
AZS D	2	21.19403	1.2664599	0.8955224	9.815339	32.572721
AZS E	2	80.298507	0.8443066	0.5970149	72.712714	87.884301
AZS H	2	26.716418	1.6886132	1.1940298	11.54483	41.888006
AZS I	2	27.014925	0.4221533	0.2985075	23.222028	30.807822
AZS M	2	82.164179	4.538148	3.2089552	41.390537	122.93782
POL C	2	73.880597	1.4775366	1.0447761	60.605458	87.155736
POL D	2	53.80597	3.904918	2.761194	18.721673	88.890267
POL E	2	71.865672	0.7387683	0.5223881	65.228102	78.503241
POL H	2	22.462687	0.7387683	0.5223881	15.825117	29.100256
POL I	2	35.746269	8.1264511	5.7462687	-37.267	108.75953
POL M	2	81.641791	6.7544528	4.7761194	20.95544	142.32814
POR C	2	55.298507	0.9498449	0.6716418	46.764489	63.832526
POR D	2	36.641791	5.8046079	4.1044776	-15.51054	88.794124
POR E	2	80.895522	0.2110766	0.1492537	78.999074	82.791971
POR H	2	25.298507	2.0052282	1.4179105	7.282247	43.314768

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POR I	2	23.208955	1.1609216	0.8208955	12.778489	33.639422
POR M	2	87.686567	2.4273815	1.7164179	65.87741	109.49572
POS C	2	43.731343	3.7993797	2.6865672	9.5952708	77.867416
POS D	2	35	1.3719982	0.9701492	22.673085	47.326915
POS E	2	68.507463	1.2664599	0.8955224	57.128772	79.886153
POS H	2	23.880597	1.8996899	1.3432836	6.8125608	40.948633
POS I	2	25.820896	1.0553833	0.7462687	16.338653	35.303138
POS M	2	84.850746	0.5276916	0.3731343	80.109625	89.591867

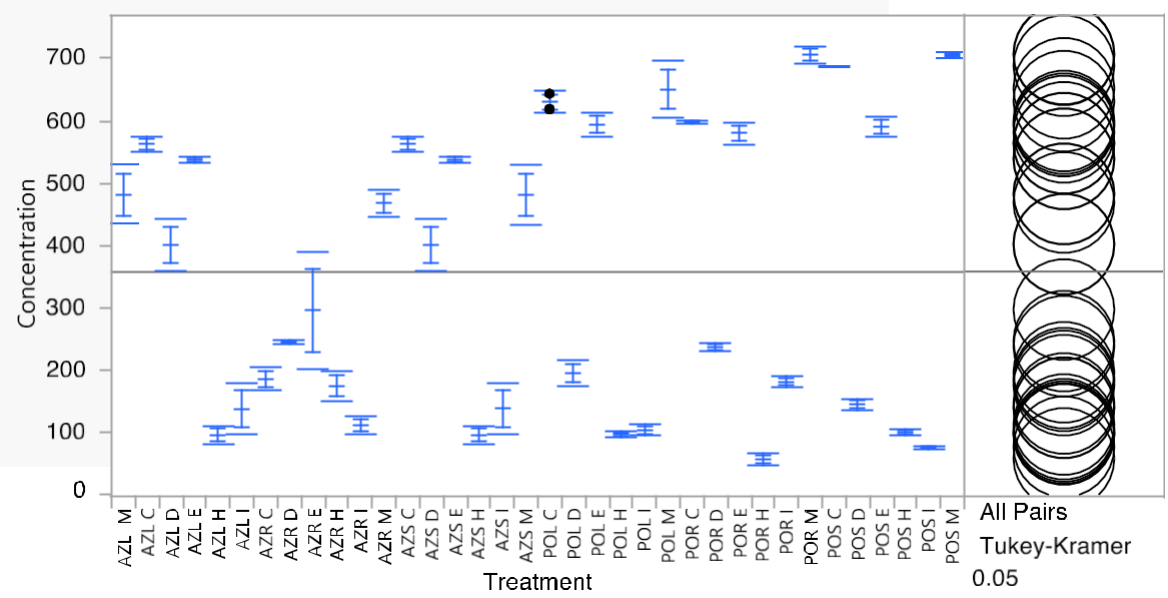
Connecting Letters Report

Level																		Mean
POR M	A																	87.686567
AZL E	A	B																85.597015
POS M	A	B																84.850746
AZL M	A	B	C															83.283582
AZS M	A	B	C	D														82.164179
POL M	A	B	C	D														81.641791
POR E	A	B	C	D														80.895522
AZS E	A	B	C	D														80.298507
POL C	A	B	C	D	E													73.880597
POL E		B	C	D	E													71.865672
AZR C			C	D	E													70.522388
POS E				D	E	F												68.507463
AZR M					E	F	G											61.417910
AZS C					E	F	G											60.522388
POR C						F	G	H										55.298507
POL D							G	H	I									53.805970
AZR E							G	H	I	J								49.701493
POS C								H	I	J	K							43.731343
AZR I									I	J	K	L						40.746269
AZR D									I	J	K	L	M					40.447761
AZL I										J	K	L	M	N				36.865672
POR D										J	K	L	M	N				36.641791
POL I										J	K	L	M	N	O			35.746269
POS D											K	L	M	N	O	P		35.000000
AZL C											K	L	M	N	O	P	Q	30.522388
AZS I												L	M	N	O	P	Q	27.014925
AZS H													M	N	O	P	Q	26.716418
POS I														N	O	P	Q	25.820896
POR H														N	O	P	Q	25.298507
POS H														N	O	P	Q	23.880597
POR I														N	O	P	Q	23.208955
POL H															O	P	Q	22.462687
AZR H															O	P	Q	22.388060
AZS D																P	Q	21.194030
AZL H																	Q	18.805970
AZL D																	Q	18.432836

Levels not connected by same letter are significantly different.

Appendix F-ABTS Trolox equivalent

Oneway Analysis of Concentration by Treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZL M	2	492.875	47.729708	33.75	53.04059	910.70941
AZL C	2	563.125	12.374369	8.75	451.94571	674.30429
AZL D	2	401.25	41.542523	29.375	28.005236	774.49476
AZL E	2	538.125	5.3033009	3.75	490.47673	585.77327
AZL H	2	85.25	15.026019	10.625	-38.75343	231.25343
AZL I	2	138.75	41.542523	29.375	-234.4948	511.99476
AZR C	2	186.25	18.561553	13.125	19.481063	353.01894
AZR D	2	245.625	3.5355339	2.5	213.85949	277.39051
AZR E	2	296.25	94.575532	66.875	-553.4774	1145.9774
AZR H	2	175	23.864854	16.875	-39.4172	389.4172
AZR I	2	111.875	14.142136	10	-15.18705	238.93705
AZR M	2	468.125	21.213203	15	277.53193	658.71807
AZS C	2	563.125	12.374369	8.75	451.94571	674.30429
AZS D	2	401.25	41.542523	29.375	28.005236	774.49476
AZS E	2	538.125	5.3033009	3.75	490.47673	585.77327
AZS H	2	96.25	15.127519	10.625	-38.75343	231.25343
AZS I	2	138.75	41.542523	29.375	-234.4948	511.99476
AZS M	2	481.875	48.789709	33.75	53.04059	910.70941
POL C	2	630.625	17.67767	12.5	471.79744	789.45256
POL D	2	195.625	21.213203	15	5.031929	386.21807
POL E	2	594.375	19.445436	13.75	419.66468	769.08532
POL H	2	97.5	4.4194174	3.125	57.79311	137.20689
POL I	2	104.375	8.8388348	6.25	24.96122	183.78878
POL M	2	651.25	45.078057	31.875	246.23972	1056.2603

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POR C	2	598.75	2.6516504	1.875	574.92587	622.57413
POR D	2	237.5	6.1871843	4.375	181.91035	293.08965
POR E	2	580.625	17.67767	12.5	421.79744	739.45256
POR H	2	57.5	9.7227182	6.875	-29.85516	144.85516
POR I	2	181.875	8.8388348	6.25	102.46122	261.28878
POR M	2	706.25	13.258252	9.375	587.12933	825.37067
POS C	2	687.5	0.8838835	0.625	679.55862	695.44138
POS D	2	145.625	8.8388348	6.25	66.21122	225.03878
POS E	2	590.625	15.909903	11.25	447.6802	733.5698
POS H	2	100.625	5.3033009	3.75	52.976732	148.27327
POS I	2	76.25	2.6516504	1.875	52.425866	100.07413
POS M	2	705	4.4194174	3.125	665.29311	744.70689

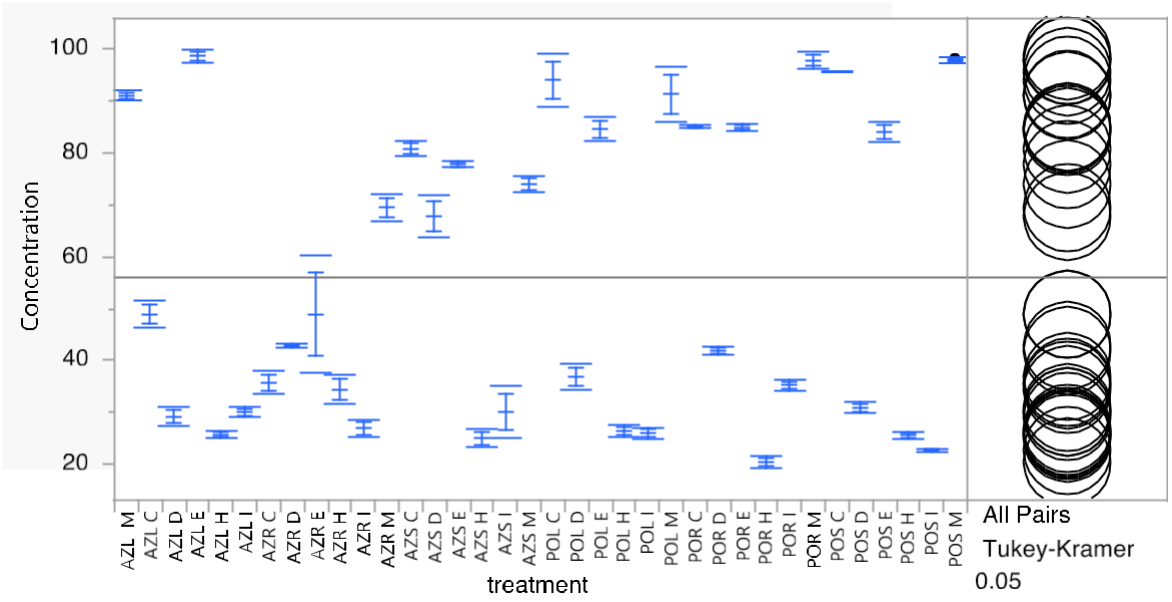
Connecting Letters Report

Level													Mean
POR M	A												706.25000
POS M	A	B											705.00000
POS C	A	B	C										687.50000
POL M	A	B	C	D									651.25000
POL C	A	B	C	D									630.62500
POR C	A	B	C	D									598.75000
POL E	A	B	C	D	E								594.37500
POS E		B	C	D	E								590.62500
POR E			C	D	E	F							580.62500
AZL C				D	E	F							563.12500
AZS C				D	E	F							563.12500
AZL E				D	E	F							538.12500
AZS E				D	E	F							538.12500
AZS M					E	F	G						481.87500
AZL M					E	F	G						481.87500
AZR M						F	G						468.12500
AZL D							G	H					401.25000
AZS D							G	H					401.25000
AZR E								H	I				296.25000
AZR D									I	J			245.62500
POR D									I	J			237.50000
POL D									I	J	K		195.62500
AZR C									I	J	K	L	186.25000
POR I									I	J	K	L	181.87500
AZR H										J	K	L	175.00000
POS D										J	K	L	M 145.62500
AZL I										J	K	L	M 138.75000
AZS I										J	K	L	M 138.75000
AZR I											K	L	M 111.87500
POL I											K	L	M 104.37500
POS H											K	L	M 100.62500
POL H											K	L	M 97.50000
AZL H											K	L	M 96.25000
AZS H											K	L	M 96.25000
POS I												L	M 76.25000
POR H													M 57.50000

Levels not connected by same letter are significantly different.

Appendix G-ABTS radical scavenging activity

Oneway Analysis of Concentration By treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZL M	2	90.956652	0.9512647	0.6726457	82.409877	99.503426
AZL C	2	48.804185	2.642402	1.8684604	25.063145	72.545226
AZL D	2	29.073244	1.7968334	1.2705531	12.929336	45.217151
AZL E	2	98.654709	1.268353	0.896861	87.259009	110.05041
AZL H	2	25.560538	0.6341765	0.4484305	19.862688	31.258388
AZL I	2	29.970105	0.9512647	0.6726457	21.42333	38.516879
AZR C	2	35.650224	2.2196177	1.5695067	15.70775	55.592698
AZR D	2	42.750374	0.4227843	0.2989537	38.951807	46.54894
AZR E	2	48.804185	11.309481	7.9970105	-52.80747	150.41584
AZR H	2	34.304933	2.8537942	2.0179372	8.6646093	59.945256
AZR I	2	26.756353	1.6911373	1.1958146	11.562087	41.950618
AZR M	2	69.35725	2.5367059	1.793722	46.565851	92.148648
AZS C	2	80.717489	1.4797451	1.0463378	67.422506	94.012471
AZS D	2	67.713004	4.0164511	2.8400598	31.626623	103.79939
AZS E	2	77.727952	0.6341765	0.4484305	72.030102	83.425802
AZS H	2	24.887892	1.7968334	1.2705531	8.743985	41.0318
AZS I	2	29.970105	4.9677158	3.5127055	-14.66305	74.60326
AZS M	2	73.916293	1.5854412	1.1210762	59.671669	88.160917
POL C	2	93.946188	5.179108	3.6621824	47.413749	140.47863
POL D	2	36.7713	2.5367059	1.793722	13.979902	59.562699
POL E	2	84.45441	2.3253138	1.6442451	63.562294	105.34653
POL H	2	26.233184	1.1626569	0.8221226	15.787126	36.679242
POL I	2	25.859492	1.0569608	0.7473842	16.363076	35.355908
POL M	2	91.255605	5.3905001	3.8116592	42.823883	139.68733
POR C	2	84.977578	0.3170882	0.2242152	82.128654	87.826503

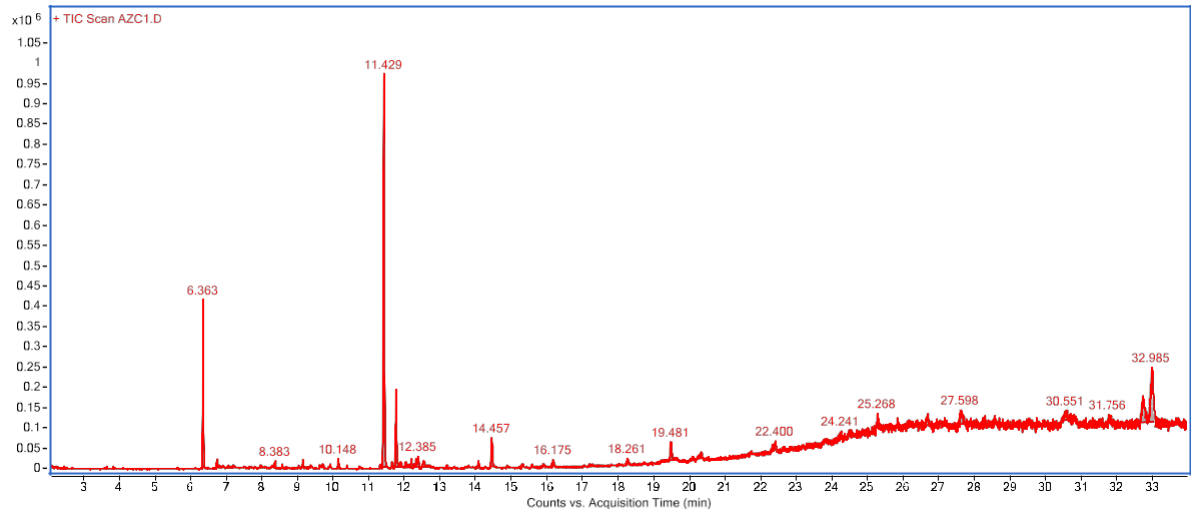
Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POR D	2	41.778774	0.7398726	0.5231689	35.131283	48.426266
POR E	2	84.828102	0.7398726	0.5231689	78.18061	91.475593
POR H	2	20.254111	1.1626569	0.8221226	9.8080529	30.700168
POR I	2	35.127055	1.0569608	0.7473842	25.630639	44.623471
POR M	2	97.832586	1.5854412	1.1210762	83.587962	112.07721
POS C	2	95.590433	0.1056961	0.0747384	94.640792	96.540075
POS D	2	30.792227	1.0569608	0.7473842	21.295811	40.288643
POS E	2	84.005979	1.9025295	1.3452915	66.91243	101.09953
POS H	2	25.411061	0.6341765	0.4484305	19.713212	31.108911
POS I	2	22.496263	0.3170882	0.2242152	19.647338	25.345188
POS M	2	97.683109	0.5284804	0.3736921	92.934901	102.43132

Connecting Letters Report

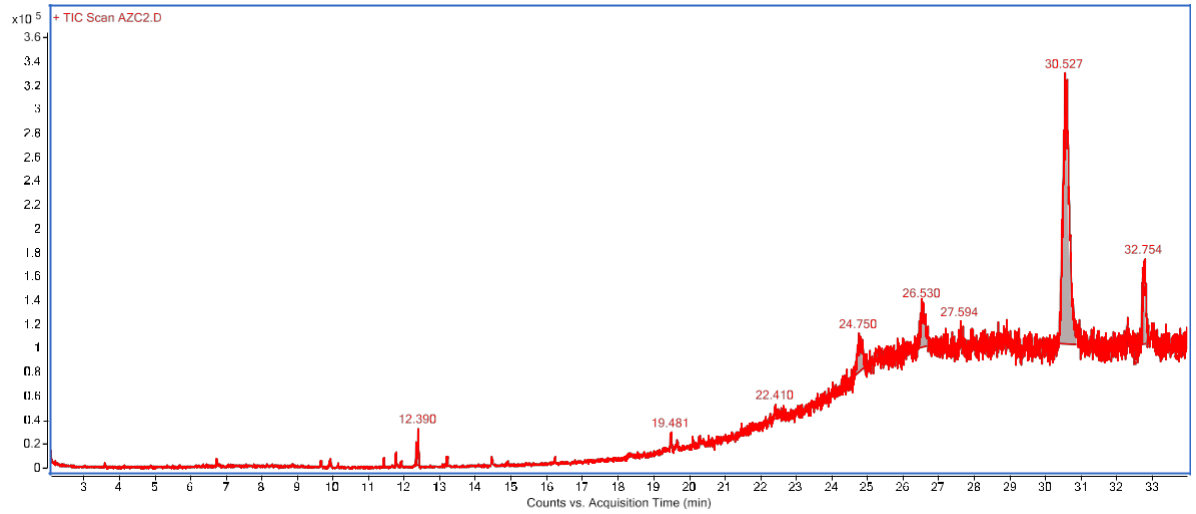
Level											Mean
AZL E	A										98.654709
POR M	A										97.832586
POS M	A										97.683109
POS C	A	B									95.590433
POL C	A	B									93.946188
POL M	A	B	C								91.255605
AZLMABC											90.956652
POR C		B	C	D							84.977578
POR E		B	C	D							84.828102
POL E		B	C	D							84.454410
POS E		B	C	D							84.005979
AZS C			C	D	E						80.717489
AZS E				D	E	F					77.727952
AZS M				D	E	F					73.916293
AZR M					E	F					69.357250
AZS D						F					67.713004
AZL C							G				48.804185
AZR E							G				48.804185
AZR D							G	H			42.750374
POR D							G	H	I		41.778774
POL D								H	I	J	36.771300
AZR C								H	I	J	35.650224
POR I								H	I	J	35.127055
AZR H								H	I	J	K	34.304933
POS D									I	J	K	L	30.792227
AZL I									I	J	K	L	29.970105
AZS I									I	J	K	L	29.970105
AZL D										J	K	L	29.073244
AZR I										J	K	L	26.756353
POL H										J	K	L	26.233184
POL I										J	K	L	25.859492
AZL H										J	K	L	25.560538
POS H										J	K	L	25.411061
AZS H										J	K	L	24.887892
POS I											K	L	22.496263
POR H												L	20.254111

Levels not connected by same letter are significantly different.

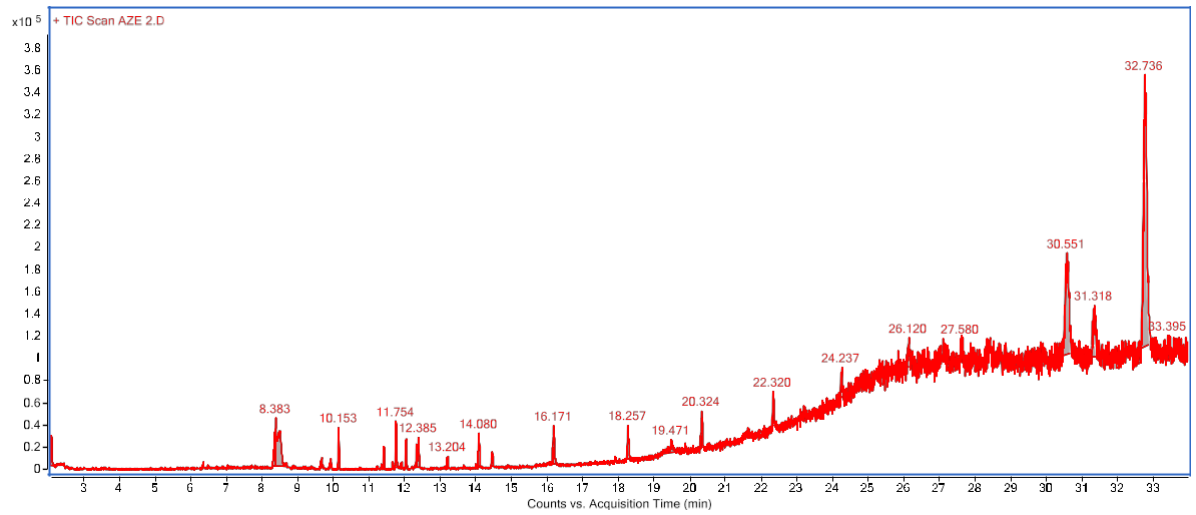
Appendix H-GC MS Chromatograms



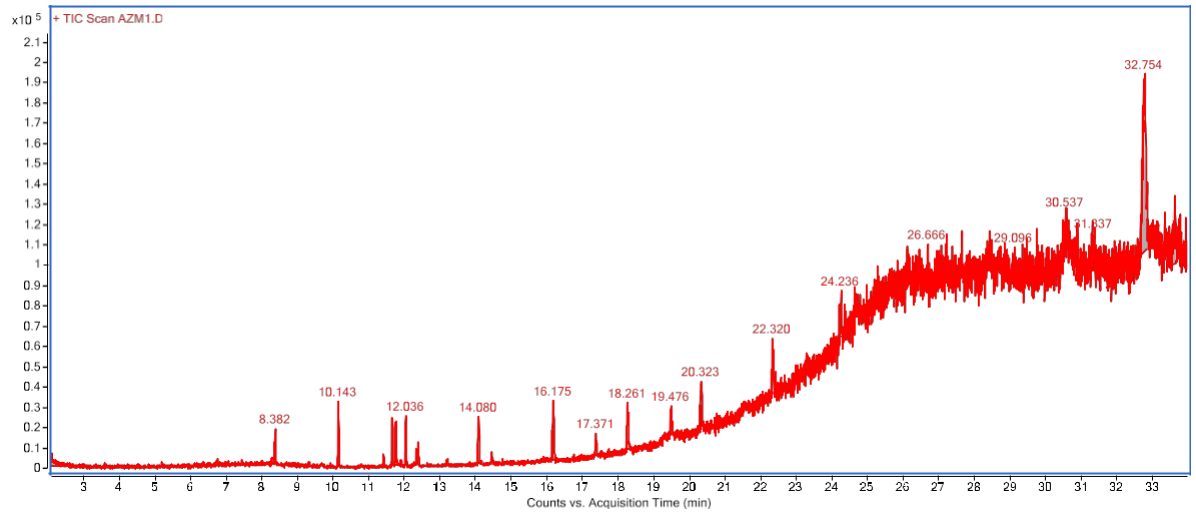
Appendix H1- figure showing GC MS chromatogram of *P. oleracea* leaf chloroform extract



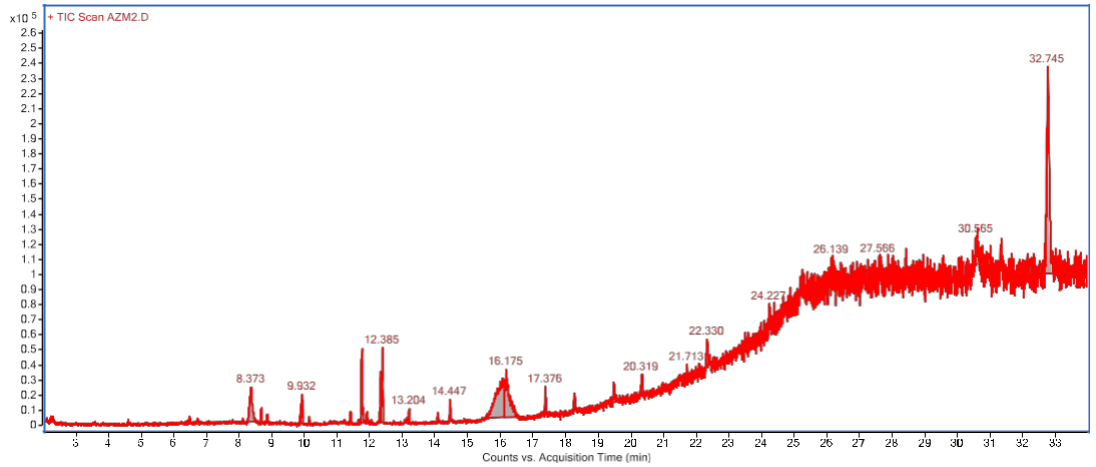
Appendix H2-figure showing GC-MS chromatogram of *P. oleracea* stem chloroform extract.



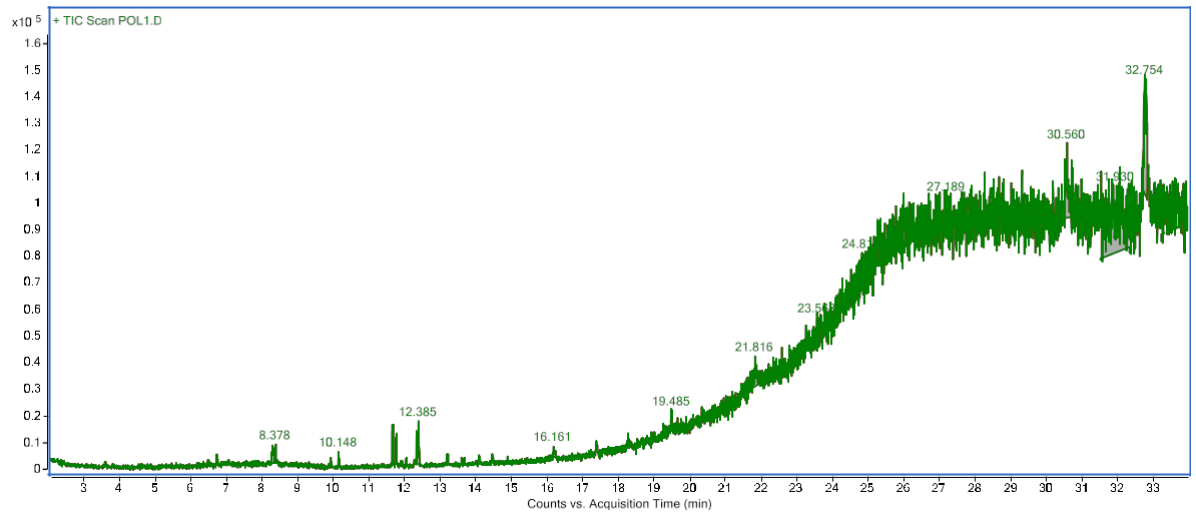
Appendix H3-figure showing GC-MS chromatogram of *P. oleracea* root methanol extract.



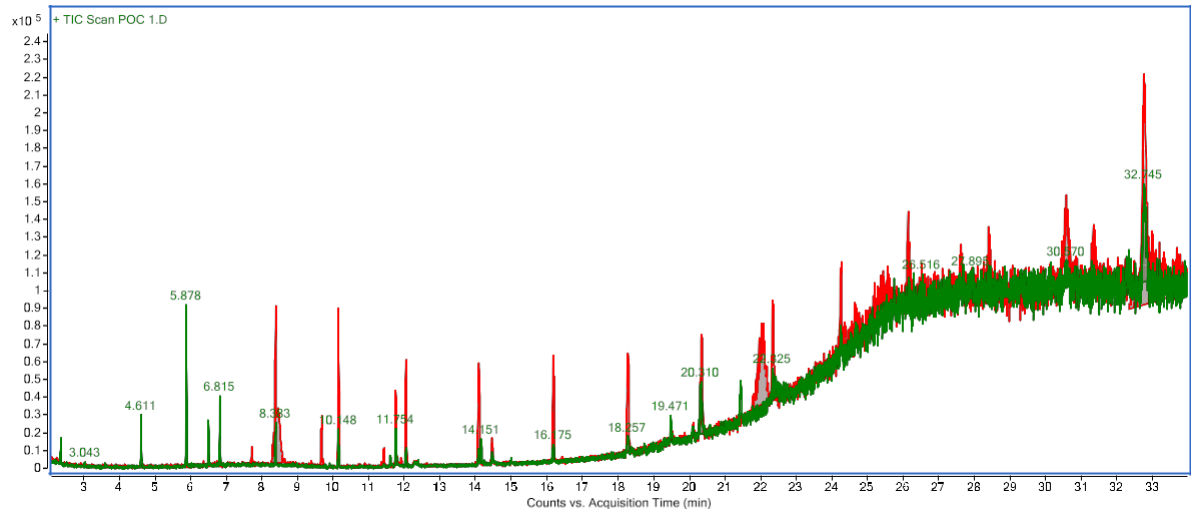
Appendix H4-figure showing GC-MS chromatogram of *P. oleracea* leaf ethanol extract.



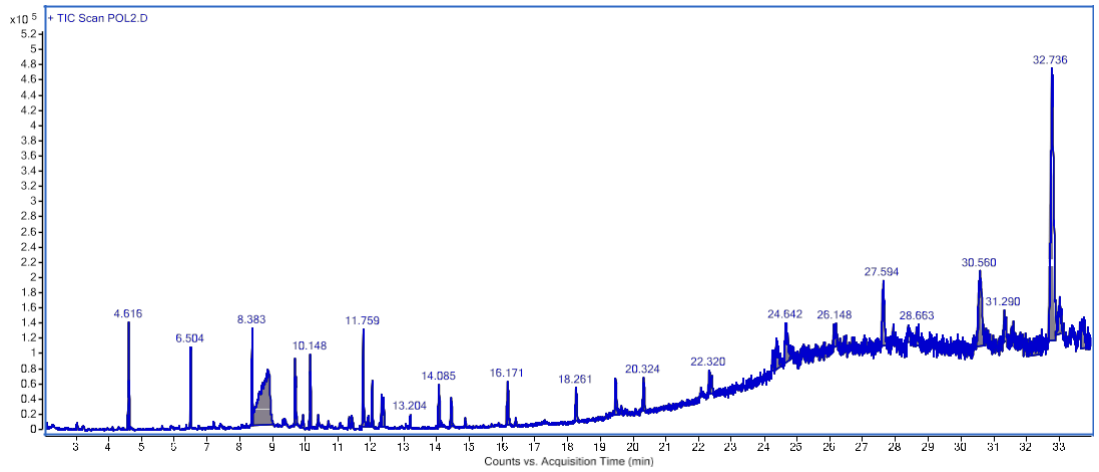
Appendix H5-figure showing GC-MS chromatogram of *A.garckeana* leaf methanol extract.



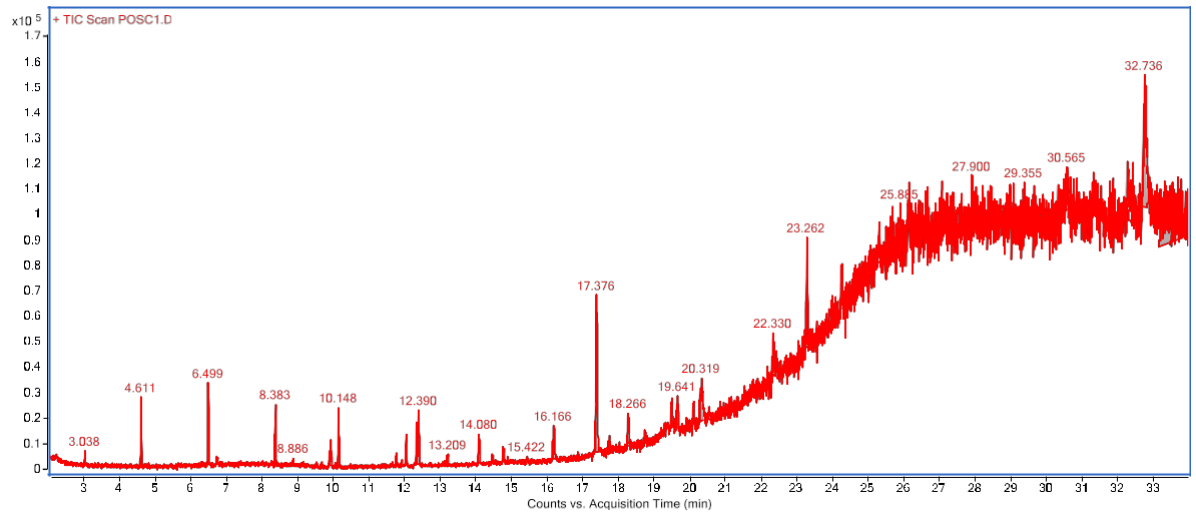
Appendix H6-figure showing GC-MS chromatogram of *P. oleracea* leaf methanol extract.



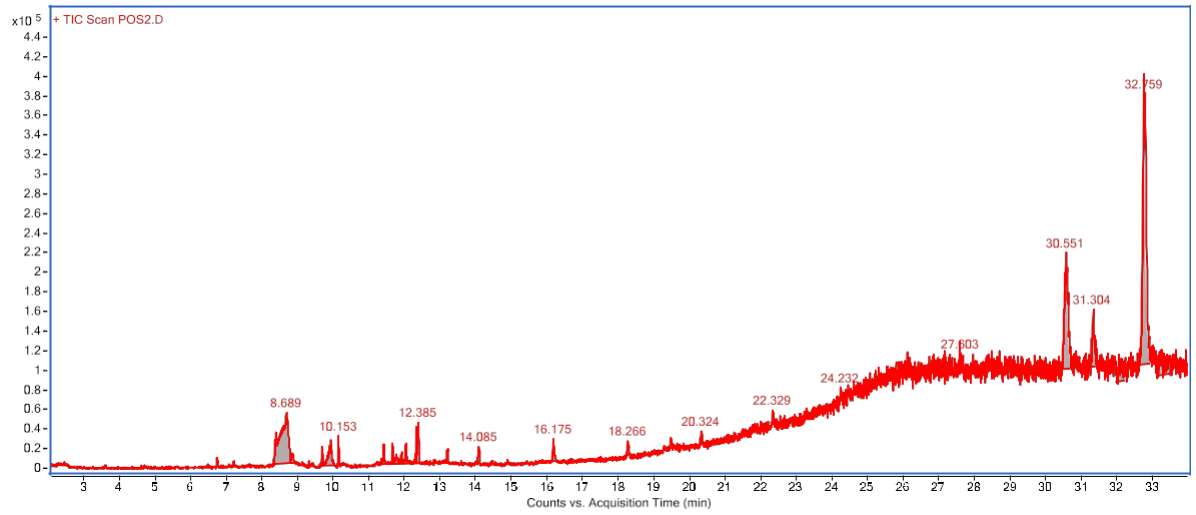
Appendix H7-figure showing GC-MS chromatogram of *P. oleracea* stem methanol extract.



Appendix H8-figure showing GC-MS chromatogram of *P. oleracea* stem ethanol extract.



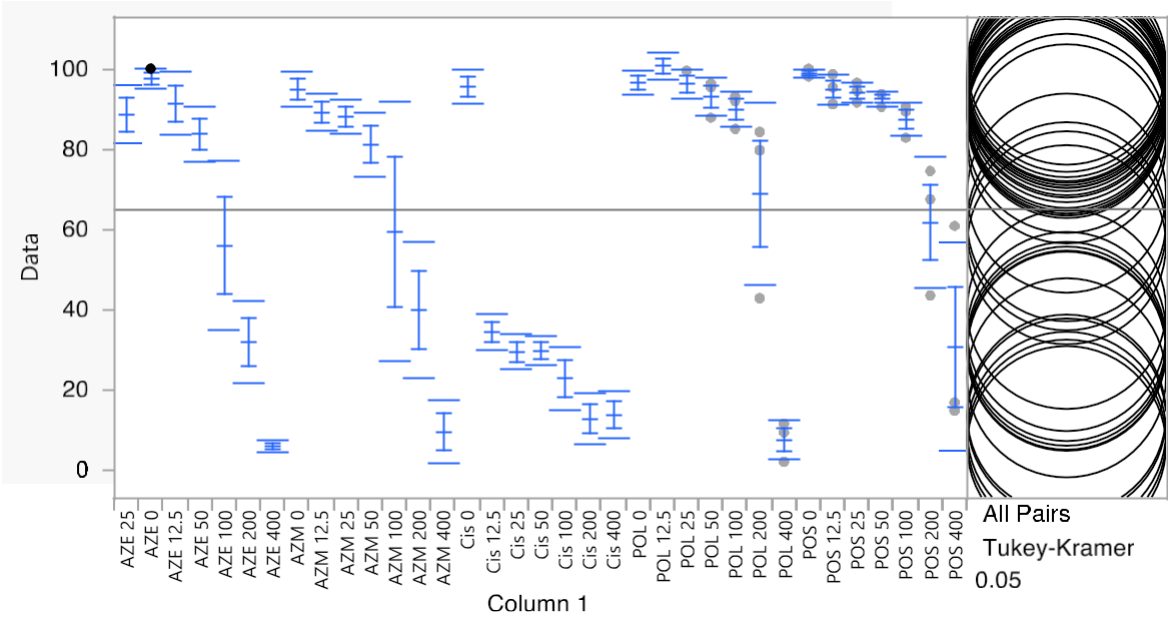
Appendix H9-figure showing GC-MS chromatogram of *A. garckeana* root methanol extract.



Appendix H10-figure showing GC-MS chromatogram of *A. garckeana* stem methanol extract.

Appendix I-MTT Cell viability and IC_{50}

Oneway Analysis of Data by Column 1



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
AZE 25	3	88.608783	7.376384	4.2587573	70.28483	106.93274
AZE 0	3	97.666667	2.5166115	1.4529663	91.415057	103.91828
AZE 12.5	3	91.42828	7.9177623	4.5713222	71.759468	111.09709
AZE 50	3	83.779279	6.8930632	3.9797119	66.655961	100.9026
AZE 100	3	56.005957	21.157006	12.215003	3.4490407	108.56287
AZE 200	3	31.873941	10.315261	5.9555188	6.2494112	57.49847
AZE 400	3	5.880978	1.4955867	0.8634774	2.1657346	9.5962214
AZM 0	3	95	4.3588989	2.5166115	84.171895	105.82811
AZM 12.5	3	89.272129	4.572415	2.639885	77.91362	100.63064
AZM 25	3	88.111191	4.2067999	2.4287971	77.660921	98.561461
AZM 50	3	81.165783	8.01032	4.6247604	61.267045	101.06452
AZM 100	3	59.482792	32.46652	10.744554	-21.16851	140.1341
AZM 200	3	39.880551	17.022401	9.8278881	-2.405438	82.166541
AZM 400	3	9.4638155	7.9030694	4.5628393	-10.1685	29.096128
Cis 0	3	95.666667	4.163332	2.4037009	85.324377	106.00896
Cis 12.5	3	34.359734	4.4168689	2.5500804	23.387624	45.331845
Cis 25	3	29.445718	4.3921658	2.5358181	18.534973	40.356463
Cis 50	3	29.11127	3.6662041	2.1166839	20.603914	38.818625
Cis 100	3	22.810214	7.8727489	4.5453337	3.2532216	42.367206
Cis 200	3	13.775842	6.3253791	3.6519593	-2.937271	28.488955
Cis 400	3	12.751337	5.8221494	3.3614195	-0.711684	28.214358
POL 0	3	96.666667	3.0550505	1.7638342	89.077501	104.25583
POL 12.5	3	96.499938	3.3826217	1.9529575	92.39703	109.20283
POL 25	3	96.274324	3.6950591	2.1333434	87.095289	105.45336
POL 50	3	93.166389	4.6796456	2.7017947	81.541505	104.79127

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
POL 100	3	89.999841	4.4104127	2.546353	79.043768	100.95591
POL 200	3	68.868776	22.757928	10.139296	12.334948	125.4026
POL 400	3	7.4976138	4.9719519	2.8705578	-4.853399	19.848627
POS 0	3	99	1	0.5773503	96.515862	101.48414
POS 12.5	3	94.998864	3.7350834	2.1564514	85.720402	104.27733
POS 25	3	94.179759	2.5187046	1.4541748	87.92295	100.43657
POS 50	3	92.593777	1.8397435	1.0621764	88.0236	97.163953
POS 100	3	87.480711	4.1321721	2.3857107	77.215827	97.745596
POS 200	3	61.734146	16.298914	9.4101823	21.245399	102.22289
POS 400	3	30.705289	13.023312	10.024566	-33.9402	95.350781

Connecting Letters Report

Level									Mean
POL 12.5	A								100.79993
POS 0	A								99.00000
AZE 0	A								97.66667
POL 0	A	B							96.66667
POL 25	A	B							96.27432
Cis 0	A	B							95.66667
AZM 0	A	B							95.00000
POS 12.5	A	B							94.99886
POS 25	A	B	C						94.17976
POL 50	A	B	C						93.16639
POS 50	A	B	C						92.59378
AZE 12.5	A	B	C						91.42828
POL 100	A	B	C	D					89.99984
AZM 12.5	A	B	C	D					89.27213
AZE 25	A	B	C	D					88.60878
AZM 25	A	B	C	D					88.11119
POS 100	A	B	C	D					87.48071
AZE 50	A	B	C	D					83.77928
AZM 50	A	B	C	D					81.16578
POL 200	A	B	C	D	E				68.86878
POS 200		B	C	D	E	F			61.73415
AZM 100			C	D	E	F			59.48279
AZE 100				D	E	F	G		56.00596
AZM 200					E	F	G	H	39.88055
Cis 12.5					E	F	G	H	34.35973
AZE 200						F	G	H	31.87394
POS 400						F	G	H	30.70529
Cis 50						F	G	H	29.71127
Cis 25						F	G	H	29.44572
Cis 100							G	H	22.81021
Cis 400								H	13.75134
Cis 200								H	12.77584
AZM 400								H	9.46382
POL 400								H	7.49761
AZE 400								H	5.88098

Levels not connected by same letter are significantly different.