

# Introduction

The plant kingdom represents an enormous reservoir of chemical compounds. Malaysia possesses an extremely rich biodiversity and these provide numerous plants with medicinal value. In developing countries like Malaysia, medicinal plants continue to be the main source of medication. Current developments in phytotechnology, phytochemistry and biotechnology have facilitated rapid progress in natural product research. The biological activities of the secondary metabolites can be tested using different kinds of bioassays. Bioassay is a biological testing procedure for estimating the concentration of active substance in the extract. A large number of plant secondary metabolites have been shown to possess antioxidative and antimicrobial properties. There are many bioassay systems to evaluate the plant chemicals such as *in vitro* antimicrobial, antiproliferative, antioxidant and radical scavenging test (Rahman and Choudhary, 2001).

## 1.1 Morphology of *Asparagus officinalis*

Asparagus is a large genus with over 150 different species of herbaceous perennials crop of high economic value with a chromosome number of  $2n=20$  that belongs to the Liliaceae family (Prohens *et al.*, 2008) (table 1.1). The most economically important Asparagus species is garden asparagus (*Asparagus officinalis* L.), which is a highly prized vegetable (Stajner *et al.*, 2002).

**Table 1.1:** Taxonomic classification of *Asparagus officinalis*

Taxonomic classification of <i>Asparagus officinalis</i>	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Liliidae
Order	Liliales
Family	Liliaceae
Genus	<i>Asparagus</i> L.
Species	<i>Asparagus officinalis</i> L.
Common Name	Garden Asparagus

*Asparagus officinalis* L. is a *herbaceous perennial monocot*, and a cool season plant for which 24°-29°C day and 13°-19°C night temperatures are optimal for productivity and longevity. Young *Asparagus* crowns establish a rhizome structure with a large root system and stems. Each rhizome has a few lateral buds that develop into succulent fleshy shoots (spears). The spears are triangular and comprise short internodes and lateral buds. Expansion of individual spear occurs foremost at the base internodes and then in successive internodes, resulting in dynamic growth of the spear into a 4 to 6 feet tall fern.

*Asparagus officinalis* L. is a dioecious species with male and female plants (sex ratio 1:1). The male flowers have perfect anthers and an aborted ovary. The female flowers are smaller than those of male plants and have nonfunctional anthers, a perfect ovary and style, and three stigmas. Female plants produce seed and larger-diameter spears, but have lower yields. Male plants have higher yields, longer productive life, and produce spears earlier. Male plant, however, do not produce seeds. The *Asparagus* fruit is a red berry at maturity and contains up to six small globular seeds. The seed is mostly endosperm tissue surrounding a small embryo.



**Figure 1.1:** *Asparagus officinalis* in the field



**Figure 1.2:** *Asparagus officinalis* plant



**Figure 1.3:** *Asparagus officinalis* leaves

### 1.1.1 History of Asparagus

Asparagus, member of Liliaceae family, is an ancient vegetable and has been cultivated for over 2000 years as a medicinal herb. It is an economically important vegetable crop that is native to the Far East and the Mediterranean regions. The English word “asparagus” is derived from the classical Medieval Latin “sparagus” but the term itself derives from the Greek “aspharagos” which originates from the Persian “asparag” meaning sprout, stalk or shoot.

Ancient Greeks and Romans prized Asparagus for its unique flavor, texture, and medicinal qualities. It was eaten fresh when in season and dried for winter use.

In the sixteenth century, Asparagus gained popularity in France and England. From there, the early colonists brought it to America. It was first planted in California during the 1850s in the San Joaquin Delta (Baayen *et al.*, 2000).

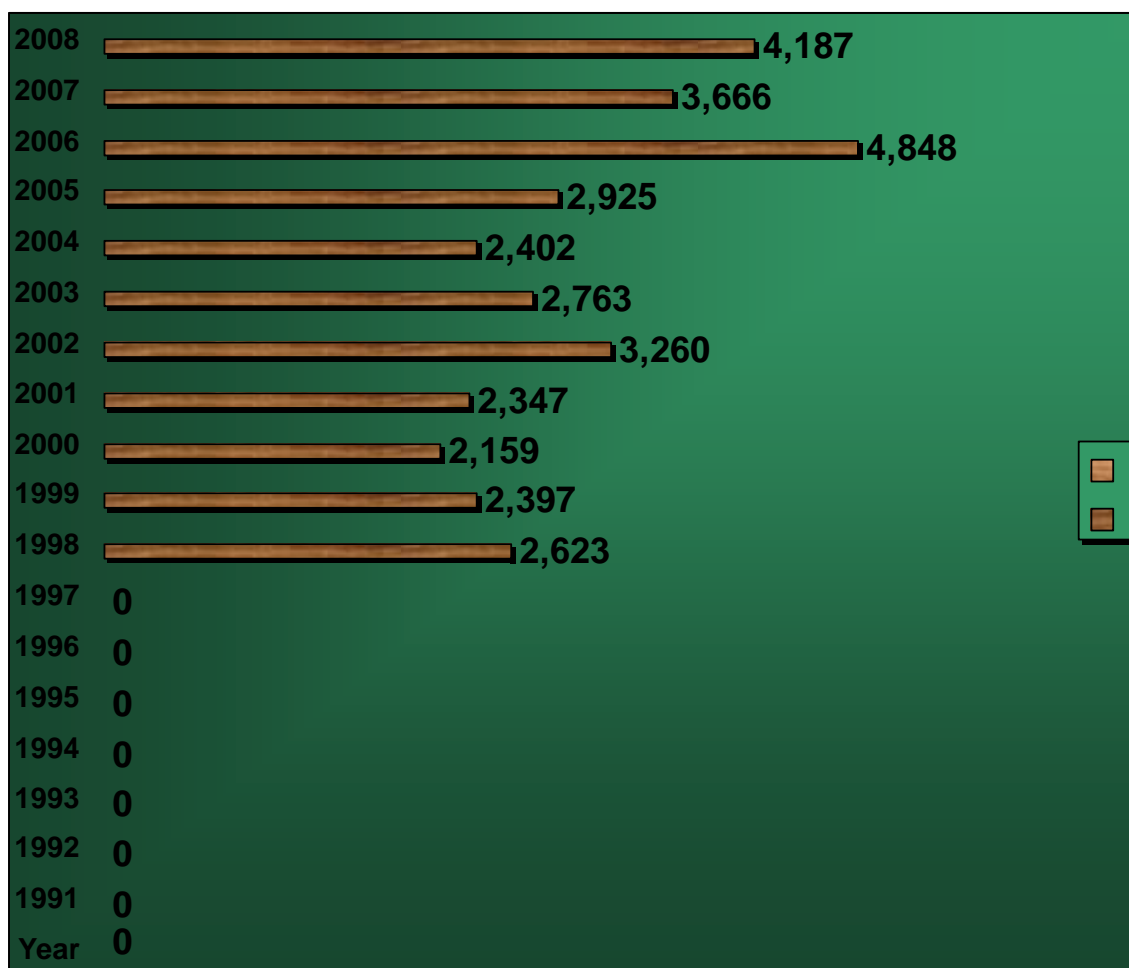
*Asparagus officinalis* production areas in 65 countries are continually changing and are difficult to monitor since Asparagus is considered a minor crop in most countries and the production data is usually reported under the broad term of “vegetable production”. The production areas in the Asparagus industries throughout the world are the result of the worldwide increased consumer demand for this product and the changes in the economic conditions in the producing and consuming nations. International movements of fresh, frozen and canned Asparagus are inter-related and dependent upon the consumption and production periods of the various countries. The largest increases in Asparagus production in the last ten years has occurred in countries in the Southern Hemisphere and China and in countries with low labor rates where they can produce spears relatively cheaply and/or market their production during a higher priced market window in another country or hemisphere.

### 1.1.2 Asparagus in Malaysia

When Asparagus was introduced into Malaysia in the early eighties, it was deemed to be a very profitable crop. However, its initial glamour and appeal have waned, simply because of the limited local market and lower than expected yields. There are many varieties of Asparagus species. Among the most common in Malaysia are the *Asparagus officinalis* and the *Asparagus plumosus*. In Malaysia, locally-grown Asparagus is available almost all year round, coming mostly from the Cameron Highlands and Mount Kinabalu in Sabah. Other states such as Perak and Malacca are also starting to grow the vegetable. The local variety is green and skinny.

The following graph (Figure 1.4) shows the producer price of Asparagus in Malaysia during 1991 to 2008. The data comes from the FAOSTAT database produced by the Food and Agriculture Organization of the United Nation (FAO). The data is displayed with the express written permission of UN/FAO and downloaded from FAOSTAT.





**Figure 1.4:** Producer Price (USD/tonne) of Asparagus in Malaysia 1991-2008

### 1.1.3 Importance of Asparagus

In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. Asparagus has been identified as having marketable value as a medicinal plant with residential and commercial applications. It is a popular vegetable consumed in most parts of the world. Asparagus shoots which are frequently used in salads, vegetable dishes and soups are the edible part of the plant. In Chinese traditional medicine, this plant has been used as a tonic, antifebrile, antitussive, hair growth stimulator and diuretic agent. In recent years the Asparagus extracts have been

demonstrated to possess certain biological activities including antifungal, antimutagenic diuretic, cytotoxic, antiviral and molluscicide properties.

Moreover, *Asparagus* (*Asparagus officinalis* L.) is a kind of health-caring vegetable contributing both to nutrition and fitness and contains manifold functional elements ( Shao *et al.*, 1996).

The *Asparagus* spears (figure 1.5), which are crisp and tender with a distinctive taste, is an abundant source of various nutrients such as vitamins, amino acids and trace elements. Furthermore, it can be used to prepare health foods highly welcomed by consumers worldwide. On the other hand, the edible parts of fresh *Asparagus* are derived from the tender stalks containing top splitting cells and a lot of water, which can cause intense metabolism and respiration (Li and Zhang, 2006).



**Figure 1.5:** Spears of green *Asparagus* in the market

Green *Asparagus* (*Asparagus officinalis* L.) also contains antioxidants, such as rutin, ascorbic acid, tocopherol, ferulic acid and glutathione (Lia *et al.*, 2006).

#### 1.1.4 Properties of Asparagus

The composition of white Asparagus, per 100g of raw edible portion (75% of harvested product) is water 97.4g, energy 103 kJ (25 kcal), protein 2.9g, fat 0.6g, carbohydrate 2.0g, fiber 1.7g, Ca 27mg, Mg 13mg, P 72mg, Fe 0.7mg, Zn 0.7mg, carotene 315µg, thiamin 0.06mg, riboflavin 0.06mg, niacin 1.0mg, folate 175µg, ascorbic acid 12mg (Holland *et al.*, 1991). Green Asparagus scores higher in micro-nutrient (Fe 1.5mg, ascorbic acid 48mg) than white Asparagus. The characteristic flavor of Asparagus is due to sugars and bitter components.

#### 1.2 Plant biotechnology

Plant biotechnology is the application of scientific knowledge to the improvement of plant traits. Improvements are in quantity or quality. It is a specific process in which scientific techniques are used to develop molecular- and cellular-based technologies to improve plant productivity, quality and health; to improve the quality of plant products; or to prevent, reduce or eliminate constraints to plant productivity caused by diseases, pest organisms and environmental stresses. Nearly all commercial improvements have been in productivity. Increased productivity conserves land and water, lowers the cost of production, and can increase the available supply of food, feed, fiber, or medicine.

The field of plant biotechnology is concerned with developing ways to improve the production of plants in order to supply the world's needs for food, fiber and fuel. In addition, plants provide us with many pharmaceuticals and industrial compounds. As our population grows, our needs also grow. To increase the quantity of crop production as well as to produce specific characteristics in plants, biotechnologists are using selective gene techniques. The two major methods of propagation are:

- Plant tissue culture
- Genetic engineering

In addition to food for consumption, food products are also being produced for

- Fuel
- Fiber
- Pharmaceuticals

### 1.2.1 The future of plant biotechnology

Research in the area of plant biotechnology will continue to focus on solving major agricultural problems. Scientists will continue to work toward improving biotechnology tools for even safer, more effective use of biotechnology by all researchers. For example, better models are being developed to evaluate genetically engineered plants and to reduce allergens in foods. Researchers will continue to monitor for potential environmental problems, such as insect pests becoming resistant to biotechnology.

### 1.3 Importance of bioactivities discovery from plants

Study on natural products is constantly an interesting objective for scientists over decades, particularly on plants. Historically, plants (fruits, vegetables, medicinal herbs, etc.) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities, e.g., antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic, antibacterial, or antiviral activities. In many oriental countries (China, Japan, etc), the traditional herbal medicines have been widely used for thousands of years. Herbal plants have become the main object of chemists, biochemist,

and pharmaceuticals. Their research plays an important role for discovering and developing new drugs, which are having hopefully more effectiveness and no side actions like most modern drugs.

Medicinal plants are natural resources which are often used in the treatment of various ailments. From ancient time, plants are rich source of effective and safe medicines. In recent years there has been focus on plants with antimicrobial and antioxidant activities.

Several studies have shown that aromatic and medicinal plants are sources of various nutrient and non nutrient molecules, many of which show antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Mothana and Lindequist, 2005, Bajpai et al., 2005; Wojdylo et al., 2007).

Therefore, studies on herbal plant profoundly not only to discover active compounds but also to find the effective mechanism of them to develop into drugs for treatment diseases. Furthermore, the studies also supplied general constituents and effects that can encourage the use of herbal plants as “food” for intensifying health and prevent diseases.

There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, and as a result, plants are still recognized as the foundation for modern medicine to treat infectious diseases. Antimicrobial and antioxidant properties of medicinal plants are being increasingly reported from different parts of the world.

## 1.4 Problem statements

Asparagus has a low multiplication rate using conventional methods. It has a short postharvest life and is highly perishable because of its high rate of respiration (Saito *et al.*, 2003). Garden Asparagus (*Asparagus officinalis* L.) is a vegetable of high economic value. The main cause of Asparagus crop decline in the world is the disease known as ‘crown and root rot’, caused by *Fusarium spp.* (Farr *et al.*, 1989). Recovery of resistant Asparagus cultivars through conventional breeding is hindered because *Fusarium* is ubiquitous in the soil, Asparagus is perennial and dioecious, and disease resistance is subject to polygenic control. The application of *in vitro* selection techniques has facilitated the generation of disease resistant plants in other pathosystems (Pontaroli and Camadro 2005).

## 1.5 The objectives of study

This study consists of two major parts; 1.To study the micropropagation in tissue culture system and 2. To investigate the antioxidant components and antibacterial activities. In the first part of the project, a tissue culture system (micropropagation) was performed in order to mass propagate *Asparagus officinalis* and following that, in the second part, a comparison of antioxidant and antibacterial activities of *Asparagus officinalis* L. extracts which was obtained from *in vitro* and *in vivo* plants was made to determine whether there is any significant difference between the *in vitro* and the *in vivo* systems.

*CHAPTER*  
*II*  
*Literature review*

## 2.1 Plant biotechnology and tissue culture

Often, the problem with commercially production of unique or uncommon plants is in availability or insufficient of plant materials required for traditional vegetative propagation and also to obtain sufficient quantities of uniform compounds. It is, however, possible to use a biotechnological approach in order to improve horticultural crop production (Chebet *et al.*, 2003). Biotechnology can also be defined as the branch of molecular biology that examines the use of microorganisms to carry out specific industrial processes. Plant tissue culture is a form of biotechnology that is a powerful tool for plant breeders (Stewart, 2007). Propagation *in vitro* is a promising method of rapidly producing numerous, uniform plants that are free of microbial contamination. It is important to develop a micropropagation method or protocol in the case of plant production via micropropagation (Razdan *et al.*, 2008). The development of an *in vitro* protocol is advantageous not only for maintainable utilization of a species, but also for germplasm conservation and genetic improvement (Chen *et al.*, 2006). It is possible to store germplasm collections in cold storage *in vitro*, which keeps plants available for future study or distribution (Kovalchuk, 2009).

Tissue culture protocol development is challenging due to interaction of biotic and abiotic factors. Example of biotic factors warranting critical consideration includes the variability that exists in culture response with regards to the source of plant tissue or explant material (Skof *et al.*, 2007). A second concern is fungal, bacterial and viral contamination that can occur *in vitro*, either from the explant materials or environmentally. Sources of microbial contamination in plant tissue culture are extremely difficult to determine (Kyte and Kleyn, 2001). Failure to control contamination will ultimately result in severe losses of *in vitro* grown plant cultures (Anjali *et al.*, 2007).



Tissue culture involves the use of small pieces of plant tissue (explants) which are cultured in a nutrient medium under sterile condition. Using the appropriate growing conditions for each explant type, plant can be induced to rapidly produce new shoots, and, with the addition of suitable plant growth regulators (PGRs) new roots. The most important and complex biotic factor affecting tissue culture of plant is biochemical activity, specifically plant responses that result from the interaction of plant growth regulators. Plant responses elicited from phytohormones are dependent on type, mode of application, concentration, tissue sensitivity and species genotype (Goncalves *et al.*, 2008). Research has shown that every species explicitly requires a particular concentration and combination of phytohormones in order to achieve optimal development (Ramirez-Malagon, 2008).

### 2.1.1 Benefits of tissue culture

*In vitro* culture of cells and tissues has a great value in providing powerful ways to study developmental processes in plants. Merits of tissue culture include: precise control over growth conditions, uniformity, increased levels of control and the ability to create recombinant proteins using acceptable manufacturing practices (Hellwig *et al.*, 2004). The production of plant recombinant proteins, also known as molecular farming, was first reported in 1989 by Hiatt *et al.* Recombinant proteins are encoded by recombinant Deoxyribonucleic acid (DNA) or generated from a recombinant gene. Products targeted for bioengineered plants include those for food, feed additives, human and animal health, industrial enzymes and technical reagents (Abranches *et al.*, 2005). Furthermore, tissue culture techniques can be used to commercially produce and can save rare plants of medicinal value. Such propagation techniques could be necessary as a result of species physiological factors such as low productivity due to low seed germination rates (Chen *et*

*al.*, 2006). Similarly, seedless plants and varieties where seedlessness is commercially important can benefit from tissue culture techniques (Zhang *et al.*, 2007).

Seed culture *in vitro* is a viable method for improving germination rates and assisting in breaking seed dormancy (Chen *et al.*, 2006; Nikolic, 2006). Further, micropropagation methods can prove beneficial in rescuing species at risk of becoming extinct (Stephenson and Fahey, 2004). *In vitro* propagation is also beneficial for plants with seeds that are slow rooting or those with reduced multiplication rates (Chen *et al.*, 2006).

In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions. *In vitro* propagation also called micropropagation is infact the miniature version of conventional propagation, which is carried out under aseptic conditions. The advent of *in vitro* tissue culture technique has offered a new approach to the morphogenetic investigations. It allows a living system to be studied under controlled environmental conditions. This enables a study of the complex biological phenomenon in parts. Moreover these partial processes are amenable to controlled investigations.

Plants raised through micropropagation are supposed to have the following characteristics:

- i. Uniform quality
- ii. Pathogen free
- iii. Can be produced much more rapidly as new cultivars could become commercially available within 2 to 3 years from development rather than 5 to 10 years needed using conventional propagation.
- iv. Produce uniformly superior seeds
- v. Show improved vigor and quality
- vi. year round production

- vii. Clean of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture

## 2.2 Explant materials

Selection of explant material is a crucial aspect of micropropagation that requires three important considerations: 1) genetic and epigenetic characteristics of the source plant 2) pathogen control 3) physiological conditions of the plant prior to explant excision in order to optimize its ability to establish in a culture (Hartmann *et al.*, 1990). Studies have revealed that somatic embryogenesis culture could be improved by choosing the most favourable female or male parent in specific cases (Park *et al.*, 2006). The source of explant material can be from seed, leaf, stem, flower or root tissue.

Generally, adventitious shoot meristems are genotypically independent and are preferred for *in vitro* examination due to their ease of handling compared to other sources of explants. Axillary shoot tip meristems are easier to grow than true apical meristems in culture (Sai *et al.*, 2006).

## 2.3 Growth regulators

Hormones also known as plant growth regulators are chemicals used to alter the growth of a plant or plant parts. They are natural chemicals produced within plant tissues, which control normal plant functions such as root growth, fruit set and drop, growth and other development processes. Good development of tissues and organs are influenced by the present of hormones in medium though a few monocotyledonous species find it optional (Mukhambetzhayev, 1997). In addition, if the explants already have existing endogenous hormones, therefore there is no need to supplement the medium with exogenous hormones (Hoe, 1992).

The use of plant growth regulators in agricultural production grown exponentially since they were first discovered and has been a major component of some agricultural commodity production (Frederick, 2006). It is acknowledged that without these regulators, *in vitro* culture is often impossible.

Some of the natural growth substances are prepared synthetically or through the fermentation processes and can be purchased from chemical suppliers. There are several recognized classes of plant growth substances:

- Auxins
- Cytokinins
- Gibberellins
- Ethylene
- Absciscic acid

Auxins and Cytokinins are the most important for regulating growth and morphogenesis in plant tissue and organ cultures; in these classes, synthetic regulators have been discovered with a biological activity which equals or exceeds that of the equivalent growth substances (George, 1993).

Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) from Auxins and

6-Benzylaminopurine (BAP) and Kinetin (Kn) from Cytokinins were used for this study.

IBA with the molecular formula  $C_{12}H_{13}NO_2$  and NAA with the molecular formula  $C_{12}H_{10}O_2$  are among the auxins and BAP with the formula  $C_{12}H_{11}N_5$  and Kinetin with the formula  $C_{10}H_9N_5O$  from cytokinins are the most favored and common hormones employed in various experiments for tissue culture and micropropagation (Keng and Stanley, 2007; Loc *et al.*, 2005; Miachir *et al.*, 2004; Georg, 1993).

Auxins can promote the growth of plant tissues. Binding of auxin leads to lipid breakdown and acidification of the wall, increasing its extensibility and increasing the water potential of the cell; so water enters and cell expands.

Auxins also affects the mRNA transcription and they cause the cell to produce biologically active cell oligosaccharides. Auxins are able to remove genetically the original physiology of whole plant tissues which had previously determined their differentiated state; therefore auxin causes the cell to divide (George, 1993).

Auxins are generally used to stimulate callus production and cell growth in a culture medium or to initiate shoots, particularly roots, and to induce somatic embryogenesis and stimulate growth from shoot apices and shoot tip cultures (George, 1993).

The second group, cytokinins, can stimulate protein synthesis. The cytokinins are used to stimulate cell division in the culture medium and induce shoot formation or auxiliary shoot proliferation (George, 1993).

## 2.4 Culture Media

Plant tissues and organs for an *in vitro* micropropagation grow on artificial media which is supplied with nutrients necessary for growth. The success level of a plant tissue culture work is strongly influenced by the environment and formation of the culture medium. For healthy and vigorous growth, intact plants need large amounts of some inorganic elements (the so-called major plant nutrients and macronutrients) salts of nitrogen (N), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg) and sulphur (S); and small quantities of other elements (minor plant nutrients or trace elements) like iron (Fe), Sodium (Na). Chlorine (Cl), Manganese (Mn), Zinc (Zn), boron (B), Copper (Cu), molybdenum (Mo) and (possibly) nickel (Ni) (George, 1993).

Plant tissue culture media is therefore made up from some or all of the following components:

- Macronutrients (always used)
- Micronutrients (most of time is used; sometimes just iron, has been used)
- Vitamins (can be included/not included when purchase a ready media from suppliers)
- Amino acids and other nitrogen supplements
- Sugars
- A solidifying agent ( Agar is the most common agent )

Ready commercial MS media (Murashige and Skoog, 1962) with vitamins was used in this study due to the lack of some ingredients for making a manual media and also the cost of making the media in the lab.

## 2.5 *In vitro* propagation of medicinal plants

Currently, biotechnology is recognized for its importance with respect to plant production, protection and improvement. One of the main biotechnological applications in plant biotechnology is tissue culture. Micropropagation by means of tissue culturing is commonly referred to as cell, tissue or organ culture *in vitro* (in glass) (Debergh and Read, 1991 in Rout *et al.*, 2006). This technology utilizes plant cells, tissues or organs in a culture medium that contains plant growth regulators to produce plants in a controlled environment. *In vitro* culture is one of the main implements of plant biotechnology responsible for exploiting the totipotency character of plant cells (Rout *et al.*, 2006). Additionally, *in vitro* propagation can be utilized to rapidly multiply cultivars with

desirable traits and create healthy, disease-free plants without seasonal constraints (Pati *et al.*, 2006).

Tissue culture systems offer better environmental containment than whole plants grown in the field so that regulatory requirements can be more easily met; production times are also shorter and, for proteins that are secreted from the cells, downstream processing and product purification are simpler and cheaper (Doran, 2006).

In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. On the order of 40% or more of the pharmaceuticals currently used in Western countries are already derived or at least partially derived from natural sources. The forest harbour a large number of plant species, but deforestation has been responsible for the rapid loss of medicinal plant wealth, such that many valuable medicinal plants are under the threat of extinction. Pharmaceutical companies depend largely upon materials procured from naturally occurring stands that are being rapidly depleted. Plant tissue culture is an alternative method of commercial propagation (George and Sherrington, 1984) and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants. Application of traditional medicinal plants for human use has also been reported (Shimomura *et al.*, 1997).

Experimental approaches used for propagation of medicinal plants through tissue culture can be divided into three broad categories. The most common approach is to isolate organized meristems like shoot tips or axillary buds and induce them to grow into complete plants. This system of propagation is commonly referred to as micropropagation. In the second approach, adventitious shoots are initiated on leaf, root and stem segments or on callus derived from those organs. The third system of propagation involves induction of somatic embryogenesis in cell and callus cultures. This system is theoretically most efficient as large numbers of somatic embryos can be obtained once the whole process is

standardized. Biotechnology involving modern tissue culture, cell biology and molecular biology offers the opportunity to develop new germplasms that are well adapted to changing demands.

Micropropagation of various plant species, including many medicinal plants, has been described by many authors during the last three decades (Murashige, 1978; Saurabh *et al.*, 2005; Nishritha *et al.*, 2008; Chitra *et al.*, 2009). From a practical and pharmaceutical point of view, propagation from existing meristems is not technologically difficult, and it yields plants that are genetically identical with the donor plants. Micropropagation of medicinal plants has been achieved through rapid proliferation of shoot-tips and axillary buds in culture. Numerous factors are reported to influence the success of *in vitro* propagation of different medicinal plants and, therefore, it is unwise to define any particular reason for the general micropropagation of medicinal plants (Rout *et al.*, 2000). The factors that influence micropropagation of medicinal and aromatic plants have been reviewed by Murashige (1977), Hu and Wang (1983), Bhagyalakshmi and Singh (1988) and Rout (2000). The influence of plant regulators and their interactions on micropropagation of different plant species have been discussed in detail by Mor and Zieslin (1987), Skirvin *et al.* (1990), Rout *et al.* (1989). Shoot multiplication is viewed as the most significant stage of plant micropropagation. Shoot multiplication is achieved by exploiting the use of PGRs including auxins and cytokinins (Rout *et al.*, 2006). Cell elongation, apical dominance and root initiation are among the many responses mediated by auxins (Kyte and Kleyn 2001). Cytokinins are responsible for promoting cell division, organ formation and cell and organ enlargement. PGRs alter various physiological processes that affect morphogenic responses. The concentration of PGRs required for a given response is cultivar and genotypically specific and depends on the unique cytokinin or auxin used (Bhatia *et al.*, 2004). The effect of auxins and cytokinins on shoot multiplication of various medicinal



plants were reported (Murashige and Skoog, 1962; Huang and Murashige, 1977; Inden and Asahira, 1988; Sharma and Singh, 1997; Sahoo *et al.*, 1997; Rout *et al.*, 1999; Handique and Bora, 1999; Sagare, 2000; Singh and Tiwari, 2010). Cytokinins levels were shown to be the most critical for multiplication of medicinal plants. Benjamin *et al.*, (1987) reported that BAP at high concentration (1-5 ppm) stimulated the development of the axillary meristems and shoot tips of *Atropa belladonna*. Lal *et al.* (1988) reported a rapid proliferation rate in *Picrorhiza kurroa* using kinetin at 1.0-5.0 mg/l. Singh and Tiwari (2010) indicated that the production of multiple shoots was higher in *Clitoria ternatea* L. on a medium having 1.5 mg/l BAP. Multiple shoot formation were reported from shoot tips (1-2cm) of field grown plants of *Paederia foetida* and *Centella asiatica* on MS medium supplemented with BAP (1.0 mg/l) within 7 days of culture (Singh *et al.*, 1999). Addition of BAP (0.3 mg/l) and kinetin (0.2 mg/l) has been found to give a good response of shoot proliferation in *Withania somnifera* with a regeneration of 85 % (Kulkarni *et al.*, 2007). In *Crataeva magna*, rapid multiplication was achieved on MS added with BAP (8.8 µm) (Benniaamin *et al.*, 2004). Similarly, it was observed that cytokinin was required in optimal quantity for shoot proliferation in many genotypes but including of low concentration of auxins along with cytokinin triggered the rate of shoot proliferation. Faria and Illg (1995) reported that the addition of 10 µM BAP together with 5 µM IAA or 5 µM NAA induced a high rate of shoot proliferation in *Zingiber spectabile*. The rates of multiplication of *Zingiber officinale* cvs. Suruchi and Suprabha were higher in a medium containing BA (4.0–6.0 mg/L), IAA (1.0–1.5 mg/L) and 100 mg/L adenine sulfate (Palai *et al.*, 1997). MS medium with growth regulators such as BAP (0.5 mg/l) in conjunction with NAA (0.01 mg/l) has been reported to give optimum results in *Utleria salcifolia* (Gangaprasad *et al.*, 2003). In *peltophorum pterocarpum*, highest number of multiple shoots was observed on MS with kinetin (2.0 mg/l) + NAA (0.5 mg/l) (Uddin *et al.*, 2005).

A rapid rate of propagation depends on the subculturing of proliferating shoot cultures. In the case of prolonged cultures, the nutrients in the medium are gradually exhausted, and at the same time, the relative humidity in the vessels decreases leading to the drying of the culture medium. Subculturing also decreases the effects of competition of the developing shoots for nutrients. Upadhyay *et al.*, (1989) reported a propagation profile for *Picrorhiza kurroa* and observed that the shoot multiplication rate gradually improved as the number of subcultures increased. Biswas *et al.*, (2007) demonstrated that the number of shoots per culture was increased with the number of subculturing in *Aristolochia tagala* Champ. Rout *et al.*, (2000) reported that, a rapid rate of propagation depends on the sub-culturing of proliferating shoots.

The induction of callus growth and subsequent differentiation and organogenesis is accomplished by the differential application of growth regulators and the control of conditions in the culture medium. With the stimulus of endogenous growth substances or by addition of exogenous growth regulators to the nutrient medium, cell division, cell growth and tissue differentiation are induced. Several reports are available on the regeneration of various medicinal plants via callus culture. For instance callus cultures initiated from shoot base explants of *Curcuma aromatica* Salisb were maintained on Murashige and Skoog (MS) media supplemented with 2 mg dm<sup>-3</sup> 2,4-dichlorophenoxyacetic acid alone or with 0.5 mg dm<sup>-3</sup> benzyladenine and 0.5 mg dm<sup>-3</sup>  $\alpha$ -naphthalene acetic acid. Approximately 8-10 plantlets were produced after 30-40 days of culture per 50 mg of callus inoculated (Mohanty *et al.*, 2008). karami *et al.*, (2009) reported the regeneration of shoots from callus of *Elaeagnus angustifolia* L. using appropriate concentration of BAP alone or BAP combined with NAA or IAA.

Thomas and Maseena, (2006) described *in vitro* regeneration of *Cardiospermum halicacabum* Linn. via callus culture. Shoot regeneration was maximum on media

supplemented with 8  $\mu$ M kinetin and 0.5  $\mu$ M IAA. Saxena *et al.*, (1997) reported plant regeneration via organogenesis from callus cultures derived from mature leaves, stems, petioles and roots of young seedlings of *Psoralea corylifolia*. The calli differentiated into green nodular structures which developed into dark green shoot buds in the media supplemented with 2.5 mg/L BA and 1.0 mg/L NAA. Successful plant regeneration was reported from stem and leaf-derived callus of *Centella asiatica* on MS medium supplemented with 4.0 mg/L BA, 2.0 mg/L kinetin, 0.25 mg/L NAA, and 20 mg/L adenine sulfate. The regeneration frequency varied from 62.8% to 73.4% (Patra *et al.*, 1998). Bui-Dang *et al.*, (1975) described regeneration of *Asparagus officinalis* from callus cultures derived from protoplasts.

*In vitro* induction of roots from growing shoots is dependent on the interactions of internal and external factors. Factors include; species/cultivar, age and size of microshoots, media, inorganic salts, carbohydrates, activated charcoal, PGRs, culture vessel, light and temperature (Pati *et al.*, 2006). Commercially, indole-3-acetic-acid (IAA) and Indole-3-butyric acid (IBA) are natural and synthetic auxins respectively that are used extensively to improve adventitious root development. However, IBA is the auxin of choice in micropropagation because IAA tends to break down during the autoclaving process. IBA is used extensively for rooting commercially and in agriculture due to its availability and reasonable cost. Anis *et al.*, (2003) obtained 80% rooting from shoots cultured of mulberry (*Morus alba* L.) on the MS supplemented with NAA (1.0 mg/l). Agrawal *et al.*, (1997) reported induction of rooting in *in vitro* shoots of cotton (*Gossypium hirsutum* L.) on half-strength agar-solidified MS basal medium or with 0.05 or 0.1 mg/l NAA. Rout *et al.*, (1999) reported induction of rooting in microshoots of *Plumbago zeylanica* on half-strength MS medium supplemented with 0.25 mg/L IBA with 2% sucrose.

### 2.5.1 *In vitro* propagation of Asparagus

Asparagus is a large genus with over 150 different species of herbaceous perennials crop of high economic value with a chromosome number of  $2n=20$ . They are grown throughout the world but they originated mainly from Asia, Africa and Europe (Prohens *et al.*, 2008). The most economically important Asparagus species is garden Asparagus (*Asparagus officinalis* L.), which is a highly prized vegetable (Stajner *et al.*, 2002). The edible organs of garden Asparagus are tender and unexpanded shoots that commonly called spears (Rubatzky and Yamaguchi, 1997). Asparagus has a low multiplication rate using conventional methods. The traditional method of propagation of Asparagus adopted by Mauritian growers, involves the division of the parent crown into 2-4 parts, which are then cultivated separately to give new plants. However, this form of propagation is very slow as one plant gives only 2-4 new plants per year under optimum conditions in absence of any pest invasion of injured surface (Ornstrup, 1997). One method that allows cloning of high-yielding varieties at a fast rate is micropropagation. Early reports of tissue culture of *Asparagus officinalis* dated back to 1945 and since then, this sector has experienced a great deal of development. Within the Asparagus genus, micropropagation protocols have been extensively studied in *A. officinalis* (Murashige *et al.*, 1972) and other species used mainly as ornamental or medicinal plants, using media supplemented with MS (Murashige and Skoog, 1962) medium and various concentrations of auxins and cytokinins. Several methods of *in vitro* regeneration of Asparagus have been established namely: direct organogenesis (Murashige *et al.*, 1972), indirect organogenesis (Reuther, 1977b, 1984) and somatic embryogenesis (Reuther, 1977). Among the existing pathways of Asparagus *in vitro* regeneration, none of them are used on a large commercial scale for propagation, as regenerated plants have poor survival rate either at hardening or at field level (Sarabi and

Almasi, 2010). Somatic embryogenesis has been recognized as the most efficient method for clonal micropropagation of plants and thus could be useful for asexual *Asparagus* multiplication. *Asparagus* was one of the first monocot species to be regenerated through somatic embryogenesis which has been obtained from different types of explants such as hypocotyls, terminal buds, stems, crowns of seedlings, cladophylls and mesophyll cell cultures (Limanton and Jullien, 2000).

Benmoussa *et al.*, (1996) reported efficient establishment of callus and shoot culture in *A. densiflorus* cv. Sprengeri on MS medium supplemented with several auxins and cytokinins. Callus formation with subsequent shoot regeneration has also been reported for three other species: *A. plumosus* (Ghosh and Sen, 1994a), *A. verticillatus* (Ghosh *et al.*, 1996) and *A. robustus* (Nayak and Sen, 1998); micropropagation without callus formation of *A. cooperi* was reported by Ghosh and Sen (1994b). Benmoussa *et al.*, (1996) reported the effects of growth regulators on induction and growth of callus of *Asparagus densiflorus* CV. They showed that growing of callus were more rapidly on Murashige and Skoog basal medium supplemented with 5.4  $\mu\text{M}$  p-chlorophenoxyacetic acid (pCPA) and 4.4  $\mu\text{M}$  BAP as compared to the same medium with 11.3  $\mu\text{M}$  2,4-D and 4.6  $\mu\text{M}$  kinetin. They also indicated that BAP was more effective than kinetin in the initiation of *Asparagus densiflorus* shoots. Mamiyaa and Sakamoto, (2000) reported the influence of sugar concentration and strength of basal medium on plants production from somatic embryos in *Asparagus officinalis* L. They noted that there was a significant difference between concentrations of sugar in growth of shoots and roots and also there was a significant difference among strength of basal medium in shoot growth but not in root growth. Saurabh *et al.*, (2005) reported that very high multiple shoots was obtained from nodal explants of *Asparagus adscendens* Roxb. on MS supplemented with 0.27  $\mu\text{M}$  NAA, 0.46  $\mu\text{M}$  Kn and 0.6% agar. They also reported that good rooting response was observed when individual

regenerated shoots were inoculated on to MS with 1.48  $\mu\text{M}$  IBA, 3.90  $\mu\text{M}$  ancymidol and 3% sucrose. Bopana and Saxena (2008) reported *in vitro* shoot proliferation by culturing single node segments in Murashige and Skoog's (MS) medium supplemented with 3.69  $\mu\text{M}$  2-isopentyl adenine and 3% sucrose with a multiplication rate of 3.5. for proper root formation, the *in vitro*-formed shoot clusters were cultured on half strength MS medium with 1.61  $\mu\text{M}$  NAA, 0.46  $\mu\text{M}$  Kn, 98.91  $\mu\text{M}$  adenine sulfate, 500 mg/l malt extract, 198.25  $\mu\text{M}$  phloroglucinol, and 3% sucrose. They showed that in this medium, 85% rooting was observed within 20 days. Shigeta *et al.*, (1996) reported that *in vitro* propagated stem explants of *Asparagus officinalis* L. produced thick storage roots by using high concentration of gellan gum for solidifying Murashige and Skoog (MS) medium containing 0.1 mg/l NAA, 0.1 mg/l kinetin and 30g/l sucrose. They showed that the rooting frequency (40-96%) using 8g/l gellan gum was approximately twice that using 2 g/l (20-45%). They also showed that the addition of 20 g/l glucose effectively increased root formation (70%) for the clone with a relatively low rooting frequency (40%) at a high gellan gum concentration.

In this study a rapid and efficient protocol for the large-scale propagation of *Asparagus officinalis*, through *in vitro* culture of nodal explants obtained from 2 month-old seedlings was described. Nodal explants treated with different hormones, 6 benzylaminopurine (BAP), 1-Naphthalene acetic acid (NAA), kinetin (Kn) and IBA, mixed or separately, evaluated as supplements to Murashige and Skoog (MS) medium, in order to obtain callus, shoot and root formation. In this study, indirect organogenesis was induced at two different conditions (in light and in dark) for the regeneration of *Asparagus officinalis in vitro* system.

## 2.6 Importance of antioxidant

Antioxidants are a group of compounds that are produced by the human body and that occur naturally in many foods. Antioxidants work together in the body to maintain our health and vigor well into the late decades of life. They are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. They do this by protecting us from damage caused by free radicals, which can injure healthy cells and tissues. The need for antioxidants becomes even more critical with increased exposure to free radicals. The body produces free radicals in the normal course of energy production, but there are also substances in our surrounding environment-certain chemicals, smoke, pollutants, solar radiation-that trigger the production of free radicals. Scientists now believe that free radicals are causal factors in nearly every known disease, from heart disease to arthritis to cancer to cataracts. In fact, free radicals are a major culprit in the aging process itself.

By controlling free radicals, antioxidants can make the difference between life and death, as well as influence how fast and how well we age. The more we understand about antioxidants and how they work, the more we will understand and appreciate the profound role they play in keeping us healthy and happy. Their role in the human body is nothing less than miraculous.

There is overwhelming scientific evidence demonstrating that those of us who eat a diet rich in antioxidants and take antioxidant supplements will live longer and healthier lives

### 2.6.1 Antioxidant assay

Broadly defined, an antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate (Halliwell and Gutteridge, 2007). The scavenging of reactive oxygen species (ROS) is one of possible mechanism of action. Others include the prevention of ROS formation by metal binding or enzyme inhibition. Chain breaking antioxidants prevent damage by interfering with the free radical propagation cascades.

The antioxidant compounds can be recycled in the cell or are irreversibly damaged, but their oxidation products are less harmful or can be further converted to harmless substances (Halliwell and Gutteridge, 2007). At the cellular and organism level the antioxidant protection is provided by numerous enzymes and endogenous small molecular weight antioxidants such as ascorbic acid, uric acid glutathione, tocopherols and several others. Many compounds contain antioxidant activity in addition to their specialized physiological function, and their importance as antioxidants *in vivo* is sometimes ambiguous (Azzi *et al.*, 2004). The antioxidant activity of plant secondary metabolites has been widely established in *in vitro* systems and involves several of the above mentioned mechanisms of action.

In a majority of tissue culture research reporting the production of secondary metabolites, their antioxidant activity has not been actually determined. The knowledge of their properties usually comes from studies involving dietary antioxidants and compounds isolated from ethnomedicinal plants with both food conservation and chemoprevention in mind. Thus, in most cases the antioxidant power of many secondary metabolites is so well established, that real time monitoring of activity during the culture seems to be redundant. On the other hand, the enormous variability among antioxidants, and their



complex structure–activity relationships suggest that antioxidant and any other biological activities should be paid more attention during research. There are a number of simple, slightly more complex, and quite sophisticated methods for antioxidant testing. The matter has been reviewed by many authors with respect to different aspects of the problem (Aruoma, 2003; Sanchez-Moreno, 2002).

The antioxidant testing can reveal various mechanisms of action, depending on features of the particular assay. Simple methods include free radical scavenging with use of colored, artificial stable free radicals such as 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS used in the TEAC assay — Trolox equivalent antioxidant capacity) (Re *et al.*, 1999) and DPPH• (1,1-diphenyl-2-picrylhydrazyl free radical) (Molyneux, 2004), as well as transition metal reduction that can be monitored by colorimetry. The metal-based methods include the reduction of ferric ions: FRAP — (ferric reducing ability of plasma) and ferric thiocyanate assays (Aruoma, 2003), or molybdenum ion — phosphomolybdenum (P-Mo) assay (Prieto *et al.*, 1999). These tests are easy and affordable and can be used in high throughput screening. Their main drawback is that their relevance to the real oxidizing life is somewhat limited. The first issue is the chemical context of the assays, which use artificial compounds or are conducted in unrealistic conditions. This problem is eliminated in methods based on naturally occurring reactive oxygen species, but the fate of a free radical is observed either indirectly with chromophore reagents or with more expensive ESR techniques (electron spin resonance). The scavenging of superoxide radical anion, hydroxyl radical, or nitric oxide can be observed (Aruoma, 2003).

## 2.6.2 Antioxidant activity of plants

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants exist within the body which many of them are derived from dietary sources like fruits, vegetables and teas. In recent decades, interest in chemopreventive plant natural products has grown rapidly. The etiology of several degenerative and aging-related diseases has been attributed to oxidative stress, and numerous studies have been undertaken to search for the most effective antioxidants (Aruoma, 2003; Soobrattee *et al.*, 2005).

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Winston, 1999).

Some researchers suggest that two-thirds of the world's plant species have medicinal value; in particular, many medicinal plants have great antioxidant potential. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases (Duduku *et al.*, 2010).

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are currently used as food additives, and many plant species have similar antioxidant potentials as these synthetics. These species include *Diospyros abyssinica*, *Pistacia lentiscus*, *Geranium sanguineum* L., *Sargentodoxa cuneata* Rehd. EtWils, *Polyalthia cerasoides* (Roxb.) Bedd, *Crataeva nurvala* Buch-Ham., *Acacia auriculiformis* A. Cunn, *Teucrium polium* L., *Dracocephalum moldavica* L., *Urtica dioica* L., *Ficus microcarpa* L. fil., *Bidens pilosa* Linn. *Radiata*, *Leea indica*, the *Lamiaceae*

species, *Uncaria tomentosa* (Willd.) DC, *Salvia officinalis* L., *Momordica Charantia* L., *Rheum ribes* L., and *Pelargonium endlicherianum*. The literature reveals that these natural antioxidants represent a potentially side effect-free alternative to synthetic antioxidants in the food processing industry and for use in preventive medicine (Chung, 1997).

## 2.7 Importance of antibacterial

There has been a significant increase in the emergence of disease causing microorganisms in the recent past and this has brought about an urgent need for scientists to discover new antimicrobial compounds with variable chemical structures and mechanisms of actions. There is also another important fearful matter which is the development of serious infections caused by bacteria that have become resistant to commonly used antibiotics which has become a major global healthcare problem in the twenty first century so far. Antibiotic resistance is actually nowadays problem of the hospitals and patients and it has now caused severe infections which are difficult and expensive to diagnose and treat. The Bacteria has developed resistance to all different classes of antibiotics discovered to date (Rojas *et al.* 2003); this makes the present work more remarkable.

This must be pointed out that, even with optimal antibiotic use, antibacterial resistances would not decline quickly and existing resistances are unlikely to vanish but we must avoid the emergence of new strains of resistant bacteria and limit the existing antibacterial resistance (Guillemot, 1999).

### 2.7.1 Antibacterial assay

Plants contain a variety of active chemical compounds. For instance, the roots of ginseng plants contain saponins and essential oils, while eucalyptus leaves contain essential oils and tannins (Cowan, 1999). Regardless of the plant part and species, the antimicrobial effect, and the degree of the effect, will vary (Benli *et al.*, 2007). Therefore, numerous studies are conducted worldwide to test the effectiveness of natural products prior to their usage as antimicrobials (Periago *et al.*, 2006).

There are several various biological assay techniques which are used for measuring and monitoring the antimicrobial activity of natural compounds especially within plant extracts (José *et al.*, 2006).

There are two standard microbial techniques for studying the antibacterial activities, which have been used the most among the similar previous works so far; those two methods are considered as “agar-well diffusion method” and “filter paper disc method” and in some cases they both resemble each other. Niamsa & Sittiwet (2009) used the term “agar-well diffusion Method” while Chen *et al.*, (2008) and Habsah *et al.*, (2007) used the term “filter paper disc method”.

While the final results of antimicrobial susceptibility testing are greatly influenced by the method of choice, test strains also influence the outcome. The test strains should represent common pathogenic species of different classes. Various combinations are possible, but should at least include a Gram-positive bacterium and Gram-negative bacterium.

### 2.7.2 Antimicrobial activity of plants

The idea that certain plants have healing potential and they contain what we would currently call antimicrobial compounds was well accepted before humans actually

discovered the existence of microbes (Ríos and Recio, 2005). Since then, mankind have used plants to treat common infectious diseases and some of these traditional medicines are still being performed as antimicrobial treatments; for example, the usage of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) are used to treat urinary tract infections, or lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) has a broad-spectrum of antimicrobial agents (Heinrich and Gibbons, 2004). It has been proven that the main compounds for antibacterial activities generally contain the essential oils of these plants rather than their extracts which bring a great and vast area of usage in the treatment for infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin. For example, in the case of *Melaleuca alternifolia*, the usage of the essential oil (tea tree oil) is a common therapeutic tool to treat acne and other infectious problems of the skin (Ríos and Recio, 2005).

The experimental methods used for studying the activity of both plant extracts and essential oils were published in 1988 proposing the use of diffusion methods for studying polar compounds of small or medium molecular size and determining the antimicrobial spectrum which allowed researchers to test different compounds against one microorganism and it has been widely accepted by many research groups.

Kalemba and Kunicka (2003) reviewed the classical methods commonly used for the evaluation of the antibacterial and antifungal activities of essential oils including the agar diffusion method (paper disc and well) which was used in this work field.

Microorganism growth was reported in the presence of tested essential oils and the *in vitro* antimicrobial activity of essential oils and their mechanisms of action were well monitored and concluded (Ríos and Recio, 2005).

Mahesh and Satish, (2008) reported that the methanol leaf, root/bark extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* showed the activity against *B. subtilis*, *E. coli*, *P. fluorescens*, *S. aureus*, *X. axonopodis* pv. *Malvacearum*, *A. flavus*, *D. turcica* and *F. verticillioides* and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The antimicrobial activity from *Mikania triangularis*, known as “thin leaf guaco”, was tested against five genera of bacteria and three genera of yeast, and showed it had activity against *Bacillus cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* (Cruz *et al.*, 1996).

## 2.8 Antioxidant and antibacterial activity of *Asparagus officinalis*

Production of secondary metabolites using *in vitro* propagation techniques has been studied since the 1970s. For example, Al-Abta *et al.*, (1979) showed that production of phthalides, the main flavour compounds of celery, can be detected in differentiated calli of celery plant but was undetected in the undifferentiated callus tissues. Based on several studies, a compound produced in an *in vivo* plant could be produced at the same or different levels or not produced at all (Verpoorte *et al.*, 2002).

The variety of compounds produced in *in vivo* and *in vitro* plants can show different bioactivity potentials. A study by Grzegorzczuk *et al.*, (2007) showed that the concentration of carnosic acid in acetone extract of *in vitro* grown *Salvia officinalis* shoots was higher than acetone extract of the *in vivo* plant, while amount of carnosol reduced from *in vivo* to *in vitro* plant shoot extracts. They have then reported a higher antioxidant potential for acetone extract of *in vitro* *S. officinalis* shoots compared to *in vivo* shoot extracts using linoleic acid oxidation prevention assay.

*Asparagus officinalis* or vegetable Asparagus has been traditionally consumed as a medicinal plant for many years (Flory, 1931). The medicinal and nutritional values of Asparagus were also confirmed in different bioactivity studies as investigations showed it to be a rich source of proteins with gullible activities. For example, antifungal activity of Asparagus was reported due to the presence of a novel deoxyribonuclease in its seeds (Wang and Ng, 2001). The anthocyanins isolated from spears of *A. officinalis* cv. Purple Passion showed a high antioxidant potential (Sakaguchi *et al.*, 2008). Rodriguez *et al.*, (2005) evaluated the antioxidant activity of eight different Asparagus cultivars and byproducts, using three different methods (antiradical activity, inhibition of primary oxidation, and ferric reducing power). They also studied the correlation between antioxidant activity and total phenol content. Six standards were also tested to validate the modified methods for antioxidant activity determination. Results obtained for antiradical capacity and reducing power were very similar, and a high correlation with phenols was found. They also reported that Asparagus inhibits lipid primary oxidation, but no correlation between the inhibition percentage and phenols was observed. Asparagus origin was the only factor that led to significant differences. The results obtained suggested that byproducts could be considered as an excellent source of natural antioxidants.

The present study aimed to investigate if plant tissue culture can be a reliable tool for the mass production of pharmaceutical compounds produced in a medicinal plant. Hence, the antioxidant and antibacterial activities of ethanolic extracts of *in vivo* grown *Asparagus officinalis* cv. Mary Washington were investigated using superoxide dismutase, erythrocyte haemolysis, and 2,2-diphenyl-1-picrylhydrazil free radical scavenging methods.