Quality of medicinal plants traditionally used in Sudan as affected by ionizing radiation treatments

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DEDICATION

To my mother,,,,

Father,,,,

Brothers and sisters,,,,

Close relatives,,,

With love and respect

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ABSTRACT

This investigation was conducted to study the effect of gamma-radiation doses of 5, 10 and 15 KGy on the microbial and chemical quality as well as antioxidant activity of nine medical plants from 8 plant species grown in Sudan. The plant materials were collected from the country-side of Khartoum State as well as from local markets. Plant parts were selected according to their traditional uses as medicinal plants. Irradiation treatment was carried out for dried ground samples using doses of 5, 10, 15 KGy from experimental cobalt-60 Gamma source. Plants extracts were prepared using 80% methanol.

The control and irradiated samples were analyzed for total bacterial count (cfu/g), secondary compounds, tannin content, total phenol, and antioxidant activity.

Tannins, flavonoids, glycosides, anthraqinones, saponin and phenols were evaluated through major compounds in extracts.

The total bacterial count indicated that the non-irradiated samples of *Trigonella foenum-graecum* L., *Cassia senna* (pods), *Cassia senna* (leaves), *Acacia nilotica L., Brassica nigra* L. Koch, *Lepidium sativum* L., *Cymbopogon citratus and Hibiscus sabdariffa* L. were highly contaminated with bacteria. The sample of *Cymbopogon schoenanthus* L. showed a lower

count of bacteria (9×10^3 CFU/g), which did not exceed the acceptable level. The samples irradiated with 5, 10 and 15 KGy of gamma radiation dose had significantly lower bacterial counts than the non-irradiated control. The highest sensitivity to gamma rays at 5 KGy dose was observed in *Trigonella foenum-graecum* L. and *Acacia nilotica L.* while the lowest sensitivity was in *Cymbopogon schoenanthus* L. At 15 KGy dose Hibiscus *sabdariffa* L. and *Cymbopogon citratus* showed complete absence of microorganisms.

The highest reduction in tannin content (mg/L catechin) due to irradiation with 15 KGy dose was observed in *Cymbopogon citratus*, followed by *Cymbopogon schoenanthus* L., *Cassia senna* L. leaves, *Acacia nilotica* L. and *Hibiscus sabdariffa* L.. Irradiation with 15 KGy dose increased the tannin content in *Brassica nigra* L. Koch, *Trigonella foenum-graecum* L., *Lepidium sativum* L. and *Cassia senna* L. (pods).

Irradiation with 15 KGy dose caused slight increase in phenol content in *Brassica nigra* L. Koch followed by *Cassia senna* L. (pods) with highest increase observed in *Cassia senna* L. (leaves) followed by *Lepidium sativum* L. and *Cymbopogon schoenanthus* L. Irradiation with 15 KGy dose reduced the phenol content of *Trigonella foenum-graecum* L., *Hibiscus sabdariffa* L., *Acacia nilotica* and *Cymbopogon citratus*.

Irradiation with 15 KGy dose resulted in an insignificant increase in the DPPH radical-scavenging ability of the extracts of *Lepidium sativum*, *Cymbopogon schoenanthus* L. and *Trigonella foenum-graecum* L., compared to the non-irradiated samples. *Cassia senna* L. pods, *Cassia senna* leaves, *Brassica nigra*, *Hibiscus sabdariffa* and *Cymbopogon citratus*, showed insignificant decrease in the radical-scavenging ability, and also there was no effect on the antioxidant potential of *Acacia nilotica* L.

It appeared that the high dose 15 KGy of gamma irradiation was the most suitable dose for microbial decontamination of the tested plants. Only *Cymbopogon citratus and Hibiscus sabdariffa* L. achieved commercial sterility (i.e. a total aerobic plate counts of <10 cfu/g). However, gamma radiation at a dose greater than 15 KGy may be required to achieve commercial sterility.

تأثير المعالجة بالأشعة المؤينة علي جودة النباتات الطبية المستخدمة تقليدياً في السودان

هاله أحمد عبدالله موسي

المستخلص:

أجري هذا البحث لدراسة تأثير جرعات أشعة جاما لمستويات 5، 10، 15 كيلو غراي علي الجودة الميكروبية والكيميائية وكذلك النشاط المضاد للأكسدة لتسعة من النباتات الطبية الخام تنتمي لثمانية أنواع نباتية تحت ظروف السودان. تم تجميع المادة النباتية من ريف ولاية الخرطوم ومن الأسواق المحلية. أختيرت الأجزاء النباتية بناءاً علي استخداماتها التقليدية كنباتات طبية. تم تعريض العينات المجففة المطحونة الي الجرع 5، 10، 15 كيلو غراي من أشعة جاما الصادرة من النظير المشع التجريبي كوبالت-60. حضرت المستخلصات النباتية باستخدام 80% ميثانول. تم تحليل العينات المعالجة وغير المعالجة بالأشعة لمعرفة الحمل البكتيري والمركبات الثانوية، محتوي التانينات (ملجم ليتر معادل الكاتيشين) ،الفينولات الكلية (جم ليتر معادل حامض الجاليك) والنشاط المضاد للأكسدة (DPPH).تم تحليل التانينات، الفلافونويدات، الجلوكوسيدات، الانثروكوينونات، السابونينات والفينولات لمعرفة المركبات الرئيسية في المستخلصات. أشارت نتائج الحمل البكتيري أن عينات *Trigonella foenum-graecum* L., *Cassia senna* (pods), *Cassia senna* (leaves), *Acacia nilotica L., Brassica nigra* L. Koch, ملوثة *Lepidium sativum* L., *Cymbopogon citratus, Hibiscus sabdariffa* L. لدرجة عالية بالبكتيريا وأظهرت عينة *Cymbopogon schoenanthus* L. محتوي منخفض من البكتريا لم يتجاوز القيمة المقبولة. وجد أن العينات المعالجة بالأشعة عند الجرع 5، 10، 15 كيلو غراي ذات حمل بكتيري أقل معنوياً من تلك غير المعالجة. لوحظت أعلي حساسية لأشعة جاما عند 5 كيلو غراي في *Trigonella foenum-graecum* L. *Acacia nilotica L.* بينما كانت أدني حساسية في *Cymbopogon schoenanthus* L. *Hibiscus sabdariffa*, *Cymbopogon citratus* أظهرت الإنعدام الكامل للبكتريا عند الجرعة 15 كيلو غراي.

لوحظ أقصي انخفاض في محتوي التانينات نتيجة للتشعيع بالجرعة 15 كيلو غراي في *Cymbopogon citratus* تليها *Cymbopogon schoenanthus* L.، أوراق *Cassia senna* L.، *Acacia nilotica* L. ، *Hibiscus sabdariffa* L. من ناحية أخري أدت المعالجة بالإشعاع عند الجرعة 15 كيلو غراي الي زيادة محتوي التانينات في *Brassica nigra* L. Koch، *Trigonella foenum-graecum* L. ، *Lepidium sativum* L.، قرون *Cassia senna* L. أدت المعالجة بالإشعاع عند الجرعة 15 كيلو غراي الي زيادة طفيفة في محتوي الفينولات في *Brassica nigra* L. Koch تليها قرون *Cassia senna* L. غير أنه قد لوحظت أقصي زيادة في أوراق *Cassia senna* L. تليها *Lepidium sativum* L.، *Cymbopogon schoenanthus* L. من ناحية أخري أدت المعالجة بالإشعاع عند الجرعة 15 كيلو غراي الي انخفاض محتوي الفينولات في *Trigonella foenum-graecum* L.، *Hibiscus sabdariffa* L.، *Acacia nilotica* ، *Cymbopogon citratus* .

أدت المعالجة بالإشعاع عند الجرعة 15 كيلو غراي الي زيادة طفيفة في النشاط المضاد للأكسدة لمستخلصات *Cymbopogon schoenanthus* L.، L*epidium sativum* L.، *Trigonella foenum-graecum* L. ولم تكن الزيادة معنوية عند مقارنتها بالعينات غير المعالجة بالإشعاع. أظهرت قرون وأوراق *Cassia senna* L.، *Brassica nigra*، *Hibiscus sabdariffa* ، *Cymbopogon citratus* انخفاض غير معنوي. لم يكن للمعالجة بالإشعاع أي أثر علي مقدرة *Acacia nilotica* L. المضادة للأكسدة.

أتضح أن الجرعة المرتفعة 15 كيلو غراي من أشعة جاما هي الأنسب لتطهير النباتات من حمولتها الميكروبية. فقط *Hibiscus sabdariffa*, *Cymbopogon citratus* أظهرت الإنعدام الكامل للبكتريا عند الجرعة 15 كيلو غراي ووصلت الي مستوي التعقيم التجاري (أقل من 10 ميكروب/جرام). تحتاج معظم النباتات الي جرعات أشعة أعلي من 15 كيلو غراي للوصول الي مستوي التعقيم التجاري.

CHAPTER ONE

CHAPTER ONE

INTRODUCTION

Despite tremendous advances in modern medicine, plants continue to make important contributions to health care as witnessed by the increasing interest in alternative therapies (Rates, 2001).

In the Sudan, as in many developing countries, medicinal plants have played an important role in the treatment of diseases especially in rural areas. The trade in medicinal and aromatic plants products that caters for the local market is carried by informal trading sector. Collectors of wild medicinal plants may be either those who collect many assorted items in small quantities for the local market or those who collect certain species in large quantities for export purpose.

Medicinal plants in dried form have been exported to different African, Asian, European, North and South American countries since 1952. Plants most commonly collected for export are *Acacia senegal, Cassia acutifolia, Hibiscus sabdariffa, Lawsonia inermis, Boswellia papyrifera, Lupinus termis and Tamarindus indica*. Variation in marketed species and quantity of plant material exported is subject to international demand. It is recently observed that the demand for *Hibiscus sabdariffa*, *Cassia acutifolia* and *Boswellia papyrifera* has increased (personal communication).

Plant materials are highly susceptible to microbial contamination (Kneifel *et al.*, 2002) due to the medium (water and soil) in which they grow. The current practices of harvesting, handling, storage and processing may cause additional contamination and microbial growth. The microbial quality is extremely

important to be achieved according to international requirements to make plant materials suitable for human use and commercialization (WHO, 1994). A good quality of raw material according to the pharmaceutical requirements may be achieved by different methods of decontamination.

The conventional methods of decontamination were fumigation with gaseous ethylene oxide or methyl bromide, which are now prohibited or being increasingly restricted in most advanced countries, e.g. in the European Union for health, environmental or occupational safety reasons (IAEA, 1992). Each decontamination treatment should be safe and fast; be effective against microorganisms; be able to penetrate the product; be adaptable to large quantities with high efficiency and not reduce the sensory and technological qualities of the treated commodities.

Nowadays the use of ionizing radiation is one of the most effective means for microbial decontamination of dried herbs and phytopreparations (Migdal *et al.*, 1998). Irradiation of dried food ingredients, particularly herbs and spices, has a great application potential, and has already been implemented in many countries to replace previous decontamination processes such as fumigation with ethylene oxide and provide an effective residue-free alternative (Farkas, 1998). It is technically feasible, friendly enough to environment and very effective to resolve technical problems in trade and commercialization. It can also control a variety of microorganisms and thus improve the quality of plant materials.

There is an extensive body of research available on the irradiation of spices, herbs and vegetable seasonings. Most products have been studied with at least preliminary if not extensive research, and the effects of irradiation on microbial contamination and sensory properties have been quantified. Since

irradiation is a clearly preferable sanitation method, its use has been allowed by CODEX Alimentarius Commission and by most countries worldwide (WHO, 1994).

Research on the microbiological decontamination of herbs by irradiation, showed that the use of ionizing radiation (a dose of 10 KGy) can ensure satisfactory results of decontamination (Migdal *et al.*, 1998).

Although there is abundant information on the effect of irradiation on the microbial decontamination of medicinal herbs, no research has been carried out in Sudan. There is a lack of information on the effect of gamma rays on both chemical and microbial quality of the plants under study. Therefore, this study was initiated with the following goals:

1- To reduce the microbial load of some Sudanese medicinal plants (Prickly Acacia, mustard, senna, lemon grass, camel's hay, Roselle, pepper cress, fenugreek) available in the local markets.

2- To study the effect of gamma radiation doses of 5, 10 and 15 KGy on the microbiological and chemical quality of the plants.

3- To study the effect of irradiation using a high dose of 15 KGy on the chemical constituents particularly total phenols and tannins.

4- To study the antioxidant activities of the plants prior and after irradiation using a high dose of 15 KGy.

CHAPTER TWO

CHAPTER TWO
LITERATURE REVIEW

2.1 Traditional Herbal Medicine

Herbs are used in many domains, including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances and cosmetics (Djeridane *et al.*, 2006).

Traditional herbal medicine as a major African socio-cultural heritage, obviously in existence for several hundreds of years, was once believed to be primitive and wrongly challenged with animosity, especially by foreign religions. However, today traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also to various extents in all countries of the world (Elujoba *et al.*, 2005).

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world (Kamboj, 2000).

The world Health Organization (WHO) has for several decades, supported, promoted and assisted the development of traditional medicine in the bid to move the African health agenda forward, particularly for the less-developed countries (Elujoba *et al.*, 2005). The WHO has recently defined traditional medicine including herbal drugs as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (Kamboj, 2000).

Plants have a long history of use in the African continent for the treatment of different diseases and complaints. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines (Hostettmann *et al.*, 2000).

Despite the great advances observed in modern synthesis-based pharmacy, medicinal plants still make an important contribution to health care. There has been a growing interest in the alternative therapies in recent years, especially those from plants. The WHO estimates that about 65-80% of the world's population living in developing countries depends essentially on medicinal plants (herbs) for primary health care (Chmielewski and Migdal, 2005).

Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, anti-mutagenic effects and anti-carcinogenic potential (Cai *et al.*, 2004).

2.1.1 Traditional medicine in Sudan

The Sudan has been home to indigenous civilization, such as Meroe, and road for others, namely pharaonic, Christian and Islamic civilizations. The country has been heavily influenced by fusion of different cultures. The immigrant Arab culture and the neighboring cultures (mainly Egyptian and West African cultures) have strongly influenced Sudanese culture. However, there is a wide range of practices, which fall under the umbrella of traditional medicine (Elkhalifa, 2003).

Medicinal plants represent an important component of traditional medicine in Sudan and the flora of Sudan is relatively rich in medicinal plants corresponding to the wide range of ecological habitats and vegetation zones. These are coupled with ample inherited information in

the field of medicinal plants and herbal traditional users which originally were unique blends of indigenous cultures of various nations (Khalid *et al.*, 1986).

Similar to other developing countries, traditional medical practices play an important role in Sudan. Herbal drugs are of major importance in Sudanese folk medicine. This was documented during comprehensive ethno-botanical investigations of El Kamali and Khalid (1996), El Ghazali *et al.* (1994, 1997) and El Kamali and El Khalifa (1999). These authors listed the most common herbal remedies of Sudan, based mainly on interviews with traditional healers.

Several broad-based screenings of many Sudanese medicinal plants were conducted for their antibacterial, antifungal, antiviral, anti-malarial and anthelmintic properties (Khalid *et al.*, 1986; El Tahir *et al.*, 1999a, b; Hussein *et al.*, 1999, 2000; Koko *et al.*, 2000, 2005; Elegami *et al.*, 2001; Ali *et al.*, 2002).

2.1.2 Status of medicinal plants in Sudan

2.1.2.1 Medicinal plants distribution

The Sudan has a very unique geographical position. This is reflected in its diverse climate and its varied natural resources. The climate ranges from completely arid to tropical zones with a wide range of bioclimatic regions, from the almost barren deserts in the north to the tropical rain forests in the extreme south of the country (Eltohami, 1997). Thus the flora of the Sudan consists of 3137 species of flowering plants belonging to 170 family and 1280 genera. Of these, 278 species, 210 genera and 72 families have already been identified as medicinal, culinary and aromatic (MCA) plants (El-Amin, 1990; El Ghazali *et al.*, 1994, 1997).

2.1.2.2 Socio-economic aspects of medicinal plants

In the past people depended exclusively on traditional medicine. They also used some wild plants for cosmetics and perfume by extracting the oils with primitive methods. In recent years, however, medicinal plants have represented a primary health source for the pharmaceutical industry. Large quantities are used for the preparation of infusions and decoctions both in the countries where traditional medicine is still of great therapeutic, social and economic importance, and in the production of important pharmaceutical products.

The government is now giving greater attention to the cultivation of crops in demand on the world market. On the other hand, the government has imported some improved hybrids and varieties which have adapted to the Sudan environment eg. *Datura inermis* and *Hyocyamus niger*. If these plants are cultivated on a large scale, they could represent an important source of hard currency, besides satisfying the market needs (Eltohami, 1997)**.**

2.2 Microbial contamination of medicinal herbs

a. Bacterial contamination

Herbs and spices are exposed to a wide range of microbial contamination during their cultivation, harvest, processing, storage, distribution and sale. Sources of microbial contamination include the macro environment (i.e., soil or plant in which the products are grown), dust, insects, faecal material and contaminated water (Chan, 2003). In addition, microbial contamination can arise from poor handling and poor hygiene practices by handlers (Mckee, 1995; Banerjee and Sarkar, 2004).

Most herbs and spices are known to be contaminated at the point of origin by varying degrees with aerobic and anaerobic bacteria. (Sharma *et al.*, 1984; Mckee, 1995 and Martins *et al.*, 2001). The microbial content of herbs and spices has been thoroughly investigated since 1930's through to present day. Members of anaerobic spore forming species are found but usually in low numbers. Thermophillic organisms are also indigenous to spices but their presence is generally at low levels. (Banerjee, and Sarkar, 2004).

Many of researchers investigated different commodities and levels of contamination by different microorganisms. They found that the contamination levels range from plate counts of zero to 10^8 microorganisms CFU/g (Colony Forming unit/g) for standard plate count, 0 to 10^6 for coliforms and 0 to 10^7 for yeasts and moulds, depending on the product (Chan, 2003). Some products are more susceptible to mould contamination, including pepper (white and black), chilies and chili powder, coriander, caraway and other herbs.

Martins *et al.*, (2001) evaluated the nature and content of microbiological contamination of 62 samples of seven medicinal plants (Chamomile, leaves of orange tree, flowers of linden, corn silk, marine alga, pennyroyal mint and garden sage) using conventional microbiological methods. Practically all samples (96.8%) were contaminated with *Bacillus cereus*; 19.2% of them with levels higher than 10^3 CFU/g. The highest levels were found in corn silk samples (>10^7 CFU/g).

The increasing consumption of natural drugs has made their use a public health issue, due to the possibility of accessing products without quality. The concerns about quality are mainly due to the potential microbiological contamination of the product, by their natural origin. Ninety-one samples of sixty-five herbal species were evaluated in relation

to microbial contamination. Results indicated that 92.3% of the herbal drugs failed to comply with the pharmacopeia parameters of acceptance and therefore, it suggested that regulatory and educational measures are needed in order to guarantee the quality of these products (Bugno *et al.*, 2005).

b. Fungal contamination

Most of herbs and spices are known to be contaminated to varying degrees with fungi species of *Aspergillus*, *Fusarium* and *Alternaria* which were found to be the most common types of fungi in air, soil and many agricultural commodities studied (Aziz *et al.*, 1997).

Martins *et al.* (2001) evaluated the fungal contamination of seven medicinal plants and reported that the mean level of fungal population was 10^5 CFU/g.

Bungo *et al.* (2006) investigated ninety-one samples of medicinal plants for fungal contamination. They found that predominant mycoflora was distributed in 10 genera. From these, 89.9% of the isolates correspond to genera *Aspergillus* and *Penicillium*, which are extremely important from the mycotoxicological standpoint.

2.3 Sterilization methods used for microbial decontamination

A number of conventional methods have, therefore been applied for microbial decontamination and insect disinfestations of herbs and spices.

Both chemical and physical methods have been used with varying degrees of success. Fumigation with ethylene oxide (ETO), methyl bromide (MB) or propylene oxide (PO) is the main chemical method used

commercially in several countries. Physical methods include steam, heat sterilization and irradiation (Lee *et al.*, 2004).

a. Fumigation

Chemical treatment is widely used for reducing bacterial count or for the complete sterilization (Farkas, 1998). Ethylene oxide is commonly used to decontaminate herbs and spices, with varying degree of success. Use of ethylene oxide is, however, prohibited in many countries (Japan, some countries of EEC, the United Kingdom), because it reacts with organic components to leave the harmful residues ethylene chlorohydrins and ethylene bromohydrin on herbs. Ethylene oxide (ETO) is considered by the International Agency for Research on Cancer (IARC) to be a human carcinogen (Fowles *et al.*, 2001). As a fumigant, ETO gas is currently used as a way to disinfect spices potentially contaminated with pathogenic bacteria, such as *Salmonella*, in New Zealand, the United States and Canada. However, in Europe, ETO is banned due to concerns of the potential toxicological risks to workers and consumers. Many countries permit irradiation as an alternative treatment to ETO fumigation (Satomi *et al.*, 2005).

Fumigation may result in reduction of volatile oil content (Satomi *et al.*, 2005) and can dull the colour (Farkas, 1998).

b. Heat treatment

The decontamination of dried powders is difficult, and the difficulty correlates with the presence of a specific micro flora adapted to low water content. Microbial heat resistance depends on the water activity (aw) of the medium. The thermal resistance of spores is more important in dry media than in liquid media, for microorganisms such as

C*lostridium botulinum* type E and *Bacillus megaterium*; there was a substantial decrease in decimal reduction time at 110 c^{o} as the (aw) value approached 1.00 (Leuschner and Lillford, 2002).

Processes used for the decontamination of dried products, such as high pressure steam, often include organoleptic degradation or achieve only a low destruction rate. Indeed, most decontamination processes involving heating induce severe damage to the products.

c. Ultra violet (U.V.), Infrared and Microwave

The U.V. irradiation as a method of sterilization is not effective because of its low penetrating power (Sharma and Demirci, 2003). Infra-red and microwave irradiation have proved to be of limited value because these methods are basically forms of heating and consequently have the same disadvantages of the use of heat.

d. Ionizing radiation

The use of ionizing radiation as a physical method of microbiological decontamination of food, including spices and herbs, was approved by the Codex Alimentarius Commission in 1983. The experts agreed that decontamination by ionizing radiation is a safe, efficient, environmental clean and energy efficient process. Irradiation is used to inactivate food-borne microorganisms, to reduce quality losses during storage and to guarantee the hygienic quality of several foodstuffs such as poultry, meat, spices and herbs (Moy and Wong, 1996).

Herbs and spices, often produced in third world countries, are highly valued for their beneficial properties. They can be exposed to high levels of bacteria, moulds, yeasts and pests which, if left untreated, would result in spoilage. Controlled and low levels of irradiation effectively kill

microorganisms. It also helps retain the colour and flavour, while ensuring the functional properties of the herbs (Sharma *et al.*, 1984). Research clearly indicates that gamma irradiation maintains the sensory properties of herbs, spices and vegetable seasonings better than ETO treatment (Marcotte, 2001).

Gamma radiation has been extensively studied as a mean of reducing the microbial contamination of herbs and spices. Experiments indicate that herbs with water contents of 5-12% are very resistant to physical or chemical change when irradiated. Sensory and food applications analysis indicate no significant difference between irradiated samples and controls for all material tested (Kneifel and Berger, 1994).

Many countries have approved irradiation of herbs and spices for microbial decontamination and insect's disinfestations. Among these countries Argentina, Brazil, France, India, South Africa, U.S.A, etc., Gamma irradiation results in a much lower level of microbial contamination and is often the only treatment effective to meet standards set by processors operating under Hazard Analysis, Critical Control Points (HACCP) or International Organization for Standardization (ISO) (WHO, 1994).

2.4 Types of radiation

Expert committee of WHO/IAEA/FAO approved three types of ionizing radiation to be used in food irradiation and other medicinal material (WHO, 1998). They are:

(1) Gamma rays from radioisotopes cobalt-60 or cesium-137 are most often used in food irradiation application because of their high penetrating power and low cost.

(2) Electron beam generated from machine sources (accelerators) operated at or below an energy level of 10 MeV (million electron-volts).

(3) X-ray generating from machine sources operated at or below an energy level of 5 MeV.

2.5 Radiation Units

When an ionizing radiation emitted from a radioactive source penetrated into a medium (e.g. food) all or part of the radiation energy is absorbed by that medium. The quantity of energy absorbed by the medium is called the absorbed dose which is measured in "rad". It was defined as a unit equivalent to the absorption of 100 ergs/g of matter. The new unit used now according to the international system is the gray "Gy". It is equal to the absorption of 1 J/Kg.

1 Gy = 100 rad

1 KGy = 100 Krad

10 KGy = 10^6 rad = 1 Mrad

The amount of ionizing radiation energy absorbed in a unit of time is called the "dose rate". Radiation exposure was measured in Röentgen (R) and now in Coulomb (WHO, 1998).

2.6 Influence of irradiation

2.6.1. Effect of gamma radiation on the microbial load of herbs and spices

a. Effect on bacteria

Ionizing radiation has the ability to ionize compounds, thereby creating highly reactive free radicals. The killing effect of radiation can

be attributed to breaking of chemical bonds of essential macromolecules such as DNA or to the ionization of water which results in forming highly reactive radicals such as H, OH, etc. These free radicals split carbon bonds of macromolecules in living organisms, thereby killing the organisms. Since no heat is generated in this form of destruction of microorganisms, radiation sterilization of food is commonly known as "cold sterilization" (Abdel-Khalek, 2008). Herbs and spices are suitable for treating with ionizing radiation, as the process does not affect the chemical/ physical properties of the material, yet at a dose of 10 KGy the microbial population is reduced by at least 10^5 CFU/g (Singh *et al.*, 1988).

The effect of different radiation doses on the microbial content of spices has been studied in detail and it has been confirmed that doses between 10 and 20 KGy lead to complete sterilization of spices where the original level of contamination was of the order 10^7 CFU/g (IAEA, 1992).

In general, it was found that moulds, fungi and coli forms are eliminated by doses lower than those required for bacteria. Studies indicated that minimum doses as low as 4-5 KGy will destroy these organisms while some bacteria and yeasts require minimum doses of 10 KGy to reach non-detectable levels (Farkas, 1998; Owczarczyk *et al.*, 2000).

Fang and Wu (1998) showed that the sterilization doses recommended for most herbs was 10 KGy for dry herbs, 7 KGy for herbal medicines and 5 KGy for more sensitive herbal medicines.

Farkas (1998) reported that radiation doses of 3 to 10 KGy proved to be sufficient to reduce the viable cell counts to a satisfactory level in dry ingredients and herbs. In this respect, Kim *et al.* (2000) found that

gamma irradiation at 5–10 KGy inactivated contaminating microorganisms in twenty-one kinds of Korean medicinal herb.

Abdel-Karem *et al.* (2002) studied the effect of gamma irradiation at doses 1, 3, 5 and 7 KGy on microbial load of karkade, tamarind and licorice. They found that radiation at dose levels of 1, 3 and 5 KGy reduced the total bacterial counts by 83, 92 and 97.5%, respectively, while complete elimination was achieved at dose level 7 KGy. Swailam and Abdullah (2002) found that irradiation at doses 5, 10 and 15 KGy greatly reduced the total viable bacterial load in qarad, anise and caraway. Irradiation dose of 5 and 10 KGy reduced the total bacterial counts of garad (the highly contaminated products among material studied) by about 49.9 and 80.9%, respectively.

From a study of the inactivation of microorganisms in 17 kinds of spices in Japan, doses of 5 to 15 KGy of gamma irradiation were required to reduce the total aerobic bacteria below the detectable level, while 4 to 10 KGy doses were required to decrease spore- forming bacteria below the detectable level. Coliforms in various spices were eliminated at 4 to 10 KGy (Ito *et al.*, 1985).

Al-Bachir (2007) reported that gamma radiation at 5, 10, 15 and 20 KGy eliminated the aerobic plate counts of anise seed. Soriani *et al.*, (2005) noticed that after irradiation of two medicinal herbs (ginkgo and guarana) all samples showed reduction of total aerobic counts to a level of $\leq$ 10 CFU/g when subjected to an average dose of 11.4 KGy.

Toofanian and Stegeman (1988) found that doses of 6 KGy for cinnamon and ginger, 6-10 KGy for fennel and 6-8 KGy for fenugreek gave microbial effects similar to those achieved with commercial Ethylene Oxide (ETO) fumigation methods.

Seventeen species of herbs established in Thailand traditional remedies were microbially-decontaminated by gamma-radiation doses of

7.7 and 8.8 KGy. The applied dose was within the regulatory limits in Thailand (< 10 KGy) and the main export country USA (< 30 KGy).

Gamma radiation was reported as an effective treatment for microbial decontamination of Thai export herbs (Phianphak *et al.*, 2007).

b. Pathogenic bacteria

Pathogenic bacteria that may be present in herbs, spices or other vegetable seasonings can be inactivated by a relatively low absorbed dose. Microorganisms belonging to the Enterobacteriaceae family are generally susceptible and can be killed by 4-6 KGy. The number of mesophillic aerobic microbes usually decreases by 2-3 orders of magnitude after being treated with 5 KGy. Of the spore forming bacteria, the most frequent genus in spices is *Bacillius*. The number of anaerobic bacteria spores is normally low and an absorbed dose of 5 KGy kills them (Greez *et al.*, 1986 and Marcotte, 2001).

Kneifel and Berger (1994) reported that a dose of 2 to 3 KGy are sufficient to control or reduce many food borne pathogens, such as *Salmonella, Eschericha Coli* and *Vibrio*, which may be found on herbs and spices.

Ramamurthy *et al.* (2004) reported that a dose of 2 KGy was sufficient to eliminate Coliforms, *Listeria* and *Yersinia* in Capsicum. Trampuz *et al.*, (2006) found that doses of 2.8 and 3.6 KGy for *Staphylococcus epidermidis* and *Escherichia coli*, respectively were enough to eliminate them.

c. Fungi

The sensitivity of fungi to ionizing radiation has been established by different investigators. Aziz (1982) noticed that irradiation at 5 KGy

was lethal dose for all fungi and yeast contaminating the dried foods. Aziz *et al.* (1997) evaluated the effect of gamma radiation on the viability of fungi-contaminated medicinal plants. They found that the viable counts of these florae decrease approximately with the level of radiation dose, the effective dose for the elimination of these microorganisms being about 5 KGy, for all the medicinal plants studied e.g. fennel, ginger, black cumin, saffron, cinnamon and chamomile. Swailam and Abdulla (2002) found that irradiation doses of 5 and 10 KGy reduced total fungal counts by about 57.4 and 97.5% respectively in Caraway, anise and qarad (medicinal plants).

El-Bazza *et al.* (1990) studied the effect of gamma radiation on the viability and resistance of the aflatoxigenic *Aspergillus flavus*, and noticed that the fungal counts decreased with increasing the radiation dose. A straight- line curve with a logarithmic decrease was obtained and inhibition of spores was observed at 3 KGy.

2.6.2 Effect of gamma radiation on chemical constituents

Several workers investigated the effect of gamma irradiation on the main components of herbs and spices as well as the minor constituents.

Sharbash (1979) found that the moisture content appeared to be of great importance when seeds were irradiated. The moisture content of seed grains decreased as a result of irradiation with 3.2 KGy.

Ragab (1994) studied the effect of gamma radiation (1, 2 and 10 KGy) on fatty acids and peroxide value of anise seed. He found that gamma irradiation induced remarkable change in fatty acids and the palmitic acid was more susceptible and less stable than others. The peroxide value increased by increasing dosage of gamma rays.

Fats are ranked among the less stable components and hence very sensitive to ionizing radiation (Hammer and Wills, 1979) that may induce many auto-oxidizing and hydrolytic reactions.

Abdel-Khalek (2008) reported that no distinct changes in lipids fraction composition were noticed in the samples of caraway, coriander, black pepper and mustard exposed to gamma irradiation up to 20 KGy.

Gupta *et al.* (1995) studied the effect of gamma irradiation (7.5-10 KGy) on major fatty acids (Oleic, linoleic, linlenic, palmitic, stearic), Saponins including Ginsenosides Rb1, Rc, Rg1, Rb2, Re (major effective components) in ginseng-red powder. No effect of irradiation on major fatty acid composition and no significant changes in saponins concentration. Swailam and Abdullah (2002) reported that gamma irradiation doses (5, 10 and 15 KGy) used resulted in fluctuation changes in the relative percentage of the major fatty acids identified in irradiated samples.

Extensive research has shown that proteins, essential amino acids, minerals, trace elements and most vitamins do not represent significant losses during irradiation even at doses over 10 KGy. Pradeep *et al.*, (1993) reported that the amino acids lysine and histidine were resistant to gamma irradiation (5 KGy) in Ginseng-Panax leaf tea (herb). Al-Jassir (1992) found that the contents of arginine, methionine, lysine, phenylalanine and norleucine of garlic- bulbs were slightly increased. However, reduction in other amino acids in irradiated samples was also showed especially at higher doses.

Sharabash *et al.* (1999) found no significant effects regarding the contents of protein of coriander seeds in response to irradiation with gamma rays (10 KGy).

2.6.3 Effect of gamma radiation on bioactive agents

The content of essential biologically active substances such as essential oils flavonoids, glycosides, anthocyanins, anthra-compounds polyphenoloacids, triterpene saponins, oleanosides and plants mucus did not change significantly after irradiation. Pharmacological activity of medicinal herbs has been found satisfactory after microbiological decontamination by irradiation (Owczarczyk *et al.*, 2000). Koseiki *et al.*, (2002) studied the effect of radiation doses (0, 10, 20 and 30 KGy) on the flavonoids, essential oils and phenolic compounds of Brazil medicinal herbs. From the described pharmacological tests of Brazil medicinal herbs carried out by this study, it is concluded that phytotherapy showed identical therapeutically action as non-irradiated preparations after exposure to a dose of 10, 20 and 30 KGy of ionizing radiation.

The irradiation of traditional medicines and herbal products does not result in any negative chemical changes or important losses of active components. After irradiation up to 17.8 KGy, the content of the main biologically active substances of two medicinal herbs (ginko and quarana) was not modified (Soriani *et al.*, 2005).

Lai *et al.* (1994) noticed that the total volatile compounds were decreased by more than 50% in irradiated (5 and 10 KGy) dry shiitake (*Lentinus edodes* Sing). Irradiation increased the concentration of some minor volatile compounds, such as 3-methyl- 2- butanol and 1- hexanol. However, the major flavour compounds including eight-carbon and sulphur-containing compounds were significantly reduced. The ratio of the eight-carbon compounds, such as 3-acetone, 3-octanol and 1-octen-3-o1, to total volatiles decreased from 72% in the control to 21% in the 10 KGy irradiated samples.

Concerning the effect of gamma radiation on contents and components of volatile oils, different results were reported. The differences were mainly due to the species of the plant and the dose applied. Uchman *et al.* (1983) reported that the treatment of herbs with higher doses (> 6 KGy) had been suggested to have a dose-dependent reduction effect on the volatile oil content of black pepper.

Mishra *et al.* (2004) reported that the dose of 5 KGy led to a decrease in 6-gingerol, the compound responsible for the pungency of ginger.

Lee *et al.* (2005) showed that the pungency and red colour caused by capsanoids and capsanthin, were not altered by irradiated (3, 7 and 10 KGy) red pepper powder.

2.7 Antioxidant activity of medicinal plants and spices

Antioxidants are regarded as compounds that are able to delay, retard or prevent oxidation processes. They can interfere with oxidation by reacting with free radicals, chelating metals and also by acting as oxygen scavengers, triplet as well as singlet form and transferring hydrogen atoms to the free radical structure (Kitazuru *et al.*, 2004).

The synthetic antioxidants such as butylated hydroxyl toulene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate (PG) are widely used in many foods in order to retard development of lipid rancidity and extend the shelf-life (Agbor *et al.*, 2005).

Today much attention has been focused on using natural antioxidants. Consumers prefer natural antioxidant because they are safer and harmless natural products.

Herbs and spices have been used as food and traditional medicines for centuries. Recent research interest has focused on their antioxidant

activities that are associated with reducing the risk of some chronic diseases, such as cardiovascular disease and cancer (Kempaiah, 2004). The herbs and spices posses antioxidant activity because they contain chemicals including the flavonoids, terpenoids lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins and phthalids (Kaur and Kapoor, 2001). They are proper materials to search for safe antioxidants in such a time of worldwide tendency for consumers to prefer natural additives (Dorman *et al.*, 2000). Naturally occurring compounds in rosemary extracts have been reported to exhibit antioxidant properties greater than BHA and equal to BHT (Kim *et al.*, 1994).

Korean medicinal herbs are known to include high amounts of antioxidants such as tocopherols, ascorbic acid and carotenoids (Chmielewski and Migdal, 2005).

Khadri *et al.* (2008) reported the antioxidant activity and acetylcholinesterase inhibition properties of essential oil of *Cymbopogon schoenanthus* obtained from fresh and dried plant material.

Yu *et al.* (2002) evaluated the water soluble rosemary extracts for their inhibitory effects on lipid oxidation and colour change in cooked turkey products during storage. The rosemary extracts showed significant protection of lipid oxidation and colour change.

Tsai *et al.* (2002) reported that roselle petals are a good source of antioxidants and this activity is relatively stable over a storage period, although provided by different compounds. The nutritional benefit of roselle as an antioxidant will ultimately depend on the bioavailability of anthocyanins.

The polyphenolic compound kaempferol which has been reported for Acacia nilotica, showed a significant radical scavenging activity in

different *in vitro* assays which provide scientific validations of this plant to be used in Ayurvedic preparations (Singh *et al.*, 2008).

Surveswaran *et al.* (2007) revealed that a wide range of total antioxidant capacities and phenolic contents exist among the 133 Indian medicinal plants assayed.

Tsao and Deng (2004) reported that phytochemicals in spices and traditional herbal medicinal plants have been found to play protective roles against many human chronic diseases including cancer and cardiovascular diseases (CVD). These diseases are associated with oxidative stresses caused by excess free radicals and other reactive oxygen species. Antioxidant phytochemicals exert their effect by neutralizing these highly reactive radicals. Among the tens of thousands of phytochemicals found in our diets or traditional medicines, polyphenols and carotenoids stand out as the two most important groups of natural antioxidants.

2.7.1 Effect of gamma radiation on the antioxidant activity

Not many contributions were concerned with the study of influence of irradiation procedure on antioxidant activity of herbs and spices.

Byun *et al.* (1999) reported that the gamma irradiation for hygienic quality of Korean medicinal herbs at 10 KGy had no effect on antioxidant capacity.

Effect of gamma-irradiation at 10 KGy on the free radical and antioxidant contents in nine aromatic herbs and spices (basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary and sage) were studied by Calucci *et al.* (2003). Irradiation resulted in a general increase of quinone radical content in all of the investigated samples, as

revealed by EPR spectroscopy, and in a significant decrease of total ascorbate and carotenoids content of some herbs.

Polovka *et al.* (2006) reported that irradiation (5 to 30 KGy) of ground black pepper shows some significant influences on the antioxidant activities with respect to the non-irradiated samples. The most significant changes of antioxidant activity were observed in creation of thiobarbituric acid reactive substances.

2.8 Plants under study

2.8.1 Prickly Acacia (Sunut, Garad)

Acacia nilotica (L.) willd. Ex Del., subsp. nilotica, belongs to the family mimosaceae. The plant is widely spread in north tropical Africa. It spreads from Egypt south to Mozambique and Natal. In West Africa it is found in Senegal, North Nigeria, Angola and Botswana. It is also found in Arabia and India. In Sudan it forms forests starting from Khartoum down the shore of the Blue Nile to Damazin (Eltayeb, 2005).

The tree ranges from 2.5 to 14 meters in height. Bark is of twigs grey to brown in colour. Branches are spreading, their bark is thin, rough and deep red-brown (Eltayeb, 2005). Leaves bi-pinnate up to 5 cm long, pinnate 4-5 paired, sessile, linear, 3-5 ×1 mm, apex rounded-obtuse, base un-equally rounded, margin entire. Inflorescences auxiliary heads, up to 1.5 cm across, peduncles 0.7-1.5 cm, brown 2-7 seeded (El Ghazali *et al.*, 1994).

It is rich in phenolics consisting of condensed tannin and phlobatannin, gallic acid, protocatechuic acid pyrocatechol, catechin, epigallocatechin-5, 7-digallate (Singh *et. al.*, 2008; Raghavendra *et al.*, 2006). This plant offers variety of bioactive components such as ellagic

acid, isoquercitin, leucocyanadin and kaempferol-7-diglucoside (Malan, 1991; Singh *et al.*, 2009).

It is a multipurpose tree that has been used extensively for the treatment of various diseases eg. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma (Kaur *et al.*, 2005; Singh *et al.*, 2008). It is widely used for tanning.

2.8.2 Black Mustard (Khardal Aswad)

Brassica nigra Koch. Belongs to the family Brassicaceae. It is a native of Eurasia and long cultivated in Europe (Purseglove, 1974). In Sudan it is cultivated in Northern state and Kordofan (El Ghazali *et al.*, 2004).

It is abranched herb up to 1 m high; leaves all stalked up to 16 cm long; flowers bright yellow, about 8 mm long; fruits about 2 mm long are held erect and apprised to rachis; seeds 1 mm or more in diameter, dark brown, self-sterile (Purseglove, 1974).

Mustard contains large quantities of prop-2-enylglucosinolate (sinigrin) and upon hydrolysis this give rise to allyl isothiocynate (Bellostas *et. al.*, 2007), in addition to flavonols, kaempferol and quercetin (Lako *et al.*, 2007; Surveswaran *et al.*, 2007). The oil of the seeds is used as a counter-irritant, stimulant; it is useful for chronic rheumatism (Purseglove, 1974).

2.8.3 Senna (Sanamaka)

Cassia senna L. belongs to the family Leaguminosae (Ceasalpiniaceae). Wild and cultivated. C. *senna* is a native shrub of Egypt, the Sudan and the Sahara region (Purseglove, 1974). Sudan senna is grown in the African Sahel zone (Müller and Basedow, 2007).

Senna is a bushy herbaceous plant, leaves, paripinnate with 3-9 pairs of lanceolate leaflets, 1.5-3.5 x 0.5-1.2 cm; flowers yellow in erect racemes, pods flattened, 4-6 x 1.8 – 2.5 cm (Purseglove, 1974). Rich in anthraquinones, anthrones, flavonoids and triterpenoids (T.Sob *et al.*, 2008). Dried leaves and pods of C. senna contain sennoside A & B which hydrolyse to give many other components of senna eg. sennoside C & D (Elamin, 1999; Morinago *et al.*, 2009; Müller and Basedow, 2007). Seeds are used for their mild purgative properties. The leaves have purgative, abortifacient properties, treat parasitic and fungal skin diseases (Nazif *et al.*, 2000). A number of diseases have been mentioned to be treated with senna e.g. piles, scapis, acre, wounds, Gout, Sciatica and Arthritis convulsions (Elamin, 1999).

2.8.4 Lemon Grass (Hashishat El-lemon)

Cymbopogon citratus L. belongs to the family gramineae (poaceae). It is a widely cultivated tropical perennial shrub whose origin could be traced to Malaysia, Indochina and SiriLanka (Puresglove, 1974). It is now widely distributed throughout the tropics and is grown for oil extraction in the West India, Brazil, The Congo and Tanzania. In Sudan it is cultivated in many parts of the country (El Ghazali *et al.*, 2004).

Erect perennial herbs, culms clutered, up to1m. high (El Ghazali *et al.*, 2004). The plant tillers strongly, making large tussocks. Leaf blade narrow, linear, glaucous, dropping, 50-100 x 0.5-1.5 cm, with scabrid margins; ligule truncate, 0.2 - 0.8 cm long. Inflorescences rarely produced, a large loose panicle; spathe bracts long and narrow; sessile spikelets awnless, linear- lanceolate, concave on lower part of the back (Purseglove, 1974).

The major component of the volatile oil of lemon grass is the citral (Rauber *et al.*, 2005). Phytochemical studies of plant leaf aqueous extract showed that the extract contain alkaloids, saponins, tannins, simple sugars (Adeneye and Agbaje, 2007), phenolic acids (caffeic and p-coumaric acid derivatives) and flavone glycosides (apigenin & luteolin derivatives). Thirteen compounds of flavonoids (O-and C-glycosyl-flavones) were identified by Figueirinha *et al.* (2008), nine of which were identified for the first time in this plant, all of them being C-glycosylflavones (mono-C-di-C- and O,C-diglycosylflavones).

Infusions or decoctions of dry leaves have been used as stomachic, antispasmodic, carminative and antihypertensive agents (Borrelli and Izzo, 2000). In many countries it is used to treat feverish conditions and as a relaxant and sleeping aid. Studies on extracts from C. *citratus* leaves have demonstrated anti-inflammatory, hypotensive, vasorelaxating, diuretic activities, efficiency against oxidative damage and cancer chemo preventive properties (Runnie *et al.*, 2004). Also it has been found effective in malaria and *Diabetes mellitus* treatment (Tchoumbougnang *et al.*, 2005; Adeneye and Agbaje, 2007). The volatile oil obtained from fresh leaves of this plant is widely used by the perfume and cosmetics industries (Rauber *et al.*, 2005).

2.8.5 Camel's Hay (Al-Maharaib, Hamareb)

Cymbopogon schoenanthus L. Subsp. proximus belongs to the family poaceae (gramineae). It is widely spread throughout northern and central Sudan (El Ghazali *et al.*, 1997). It grows as wild plant in dry areas and valleys.

Glabrous compactly tufted much-branched perennial herbs up to 60 cm high, with slender, erect, 3-4 nodded culms. Leaves alternate; laminas

linear, 10-30 x 3 cm, tapering to a long setaceous point; ligule membraneous, ciliolate, truncate; sheath 5-8 cm long, firm. Inflorescences spatheate panicle, 6-20 x 3-5 cm; spathes lanceolate, apex acuminate, up to 2.5 cm long (El Ghazali *et al.*, 1997).

The essential oil extracted from *C. schoenanathus* leaves contains piperitone (69%), 2-carene (17%) and elemol (5.8%) as major components. The oil is characterized by a high percentage of oxygenated monoterpenes (Ketoh *et al.*, 2005; Ketoh *et al.*, 2006). The plant contains the sesquiterpenes cryptomeridiol (proximodiol), elemol, β-eudesmol in addition to p-sistosterol (Elgamal and Wolf, 1987). It is also proved to contain alkaloids (El Ghazali *et al.*, 1997).

Besides its use in culinary *C. schoenanthus* is also used in folk medicine for the treatment of rheumatism and fever. The infusions are taken as a diuretic, it cures intestinal troubles and, in the form of decoction, it acts against food poisoning and helps also in digestion (Khadri *et al.*, 2008).

2.8.6 Roselle (Karkadeh)

Hibiscus sabdariffa L. belongs to the family malvaceae. It is a native of West Africa and is now widely cultivated throughout the tropics. In Sudan it is grown as a cash crop in rainfed areas. The principal production area is in eastern Kordofan, El-Rahad and Umruaba. It is grown on a small scale around El-Obeid, in western Kordofan, near El-Fashir, Nyala and in the southern of the country (Al-Shoosh, 1997).

Roselle is an annual plant up to 100 cm high with terete reddish brownish glabrous stems, radical leaves ovate not parted. Upper leaves are 3-parted into lanceolate oblong, crenate lobes. Flowers are solitary

sessile, yellow in colour. Calyx & bracts are red; Fruit capsule more or less deliscent (El Ghazali *et al.*, 1998).

The chemical components contained in the flowers of *H. sabdariffa* include anthocyanins, flavonoids and polyphenols (Tzu-Li Lin *et al.*, 2007). The petals are potentially a good source of antioxidant agents as anthocyanins and ascorbic acid (Prenesti *et al.*, 2007).

It is used effectively in folk medicines for treatment of hypertension, inflammatory diseases and cancer (Haji Faraji and Haji Tarkhani, 1999; Chewonarin *et al.*, 1999; Tzu-Li Lin *et al.*, 2007). Many studies demonstrated cardioprotective effect and decrease of the serum cholesterol level. The calyces are used to decrease blood viscosity and reduce hypertension (Christian *et al.*, 2006). Hibiscus pigments reduce the incidence of liver lesions including inflammation, leucocyte infiltration and necrosis (Kong *et al.*, 2003).

2.8.7 Pepper Cress (Hab El Rashad)

Lepidium sativum L. belongs to the family brassicaceae (cruciferae). It has been cultivated since very early times in Europe and is now cultivated as a salad and spice all over the world (Maier *et. al.*, 1998). It is distributed in central Sudan (El Ghazali *et al.*, 2004).

Pepper Cress is an erect glabrous annual herb, 15-30 cm tall; basal leaves long-stalked, lyrate, with toothed obovate lobes; stem leaves once or twice pinnate; uppermost leaves sessile, linear; entire; flowers minute, white, protogynous. Fruit a silicula, 5-6 x 3-4 mm (Purseglove, 1974).

The major secondary compounds of this plant are glucosinolates and phenylpropanoid derivatives (Brock *et al.*, 2006 and Mohn *et al.*, 2007). The seeds afforded five dimeric imidazole alkaloids lepidine B, C, D, E and F in addition to monomeric imidazole alkaloids

semilepidinoside A and B (Maier *et al.*, 1998). Phenylacetonitrile, benzyl- sulphur oil, sesquiterpene lactones and stigmast 5-en-3, β 27-doil 27-benzoate (steryl ester) were isolated from the plant (El Ghazali *et al.*, 2004). They are used for wound treatment and gastrointestinal disorders (El Ghazali, 1998).

2.8.8 Fenugreek (Al-Hilba)

Trigonella foenum- graceum L. belongs to the family fabaceae (papilionaceae). It is an annual herbaceous aromatic leguminous, widely cultivated in Mediterranean countries, Asia and North African regions (Eidi *et al.*, 2007).

Fenugreek is an annual plant 15-140 cm. Stems erect, stipules triangular- lanceolate acuminate, entrire- leaves obovate to oblong dentate (El Ghazali *et al.*, 1998). The seeds are rich in proteins and with a pleasing appetizing aroma. Besides, the seeds contain sapogenins (particularly diosgenin), steroidal saponins as well as the free amino acid 4-hydroxyisoleucine (near 80% of free amino acids present in fenugreek seeds) (Mebazaa *et al.*, 2009), phenolic acids mainly neochlorogenic acid, p-coumaric acid and flavonoids, quercetin, luteolin and apigenin (Wojdylo *et al.*, 2007). Two alkaloids trigonelline and choline were detected on the seeds (El Ghazali *et al.*, 1998).

The seeds of fenugreek are commonly used in India and in oriental countries as a spice in food preparations due to their strong flavour and aroma. The seeds are reported to have restorative and nutritive properties and to stimulate digestive processes (Kaviarasan *et al.*, 2007). Fenugreek seeds are used as a traditional remedy for the treatment of diabetes (Miraldi *et al*., 2001 and Vats *et al*., 2004). Amin *et al.* (2005) established that the *T. foenum graecum* has appreciable anti-cancer activity.

CHAPTER THREE

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

The plant material was collected from the country-side of Khartoum state as well as the local markets, where herbs and plants collected from Northern and Western parts of the country are available. Plant parts were selected according to their traditional uses as medicinal herbs. Only the parts of the plants which were indicated in ethno medicine were sampled e.g. pods of prickly Acacia, leaves and pods of senna, leaves of lemon grass and camel's hay, seeds of mustard, pepper cress, fenugreek and calyces of roselle. (Table 3.1).

Table (3.1): Most common traditionally used Sudanese medicinal plants used in this study

Latin Name	Family	Part used	English name	Vernacular name (s)
Acacia nilotica L.	Mimosaceae	Pods	Prickly Acacia	Sunut, Garad
Brassica nigra L. Koch	Brassicaceae	Seeds	Black Mustard	Khardal aswad
Cassia senna L.	Ceasalpiniaceae	Leaves & pods	Senna	Sanamaka
Cymbopogon citratus	Poaceae	Leaves	Lemon Grass	Hashishat el-lemon
Cymbopogon schoenanthus L.	Poaceae	Leaves	Camel's hay	Mahareb
Hibiscus sabdariffa L.	Malvaceae	Calyces	Roselle	Karkadeh
Lepidium sativum L.	Brassicaceae	Seeds	Pepper Cress	Hab El-rashad
Trigonella foenum-graecum L.	Fabaceae	Seeds	Fenugreek	El-Hilba

3.2 Sample preparation

The selected parts of the plants were dried at room temperature. Some of the dried samples were ground by mortar and pestle and those with hard seeds were ground by an electric blender into a coarse powder. Each of the dried, coarsely ground samples was sub-divided into 4 portions (150g each), packed in plastic and paper packages and stored in a dry, well ventilated room.

3.3 Irradiation treatment

Irradiation treatment was carried out for the samples at different doses 0, 5, 10, 15 KGy using an experimental cobalt-60 Gamma source (Nordion gamma cell 220-Excell) at the Sudan Atomic Energy Commission, Khartoum. The activity of the source was 6.345Kci and the energy 1.25 MeV. The irradiation time varied from 1 to 3 hours depending on the dose applied.

3.4 Preparation of plant material for phytochemical screening

3.4.1 Preparation of plants extracts

A 50 g of each plant sample was macerated using 500 ml of 80% methanol and was allowed to shake for 4 hours, then left for 72 hours. The extracts were filtered using whatman no. 1 filter papers. The solvent was evaporated under reduced pressure using a rotary evaporator (Buchi Laboratourisms-Technik AG). The extracts were placed in Petri dishes and then air-dried until the solvent was completely evaporated. The different dried extracts were stored at 4°C.

3.4.2 Preparation of samples for analysis

The dried extracts were further processed by addition of 1 ml of 80% methanol to 1 g of the crude extract, and then 4 ml of methanol were added to obtain a suitable dilution (1:5) for the samples. From these samples 1:10 dilution were prepared using 80% methanol. The dilutions were stored at -20°C.

3.5 Preparation of Test Reagents for phytochemical screening

3.5.1 Tannin test reagents

The reagents for the tannin assay were prepared according to Hagerman and Butler (1978), as follows:

(i) Buffer A (Washing buffer)

1.2 g (200 mM) acetic acid was added to 0.993g (170 mM) NaCl, the PH adjusted to 4.9 with NaOH.

(ii) Buffer B

5g/L potassium sodium-tartrate tetrahydrate $C_4H_4KNao_6.H_2o$ (0.5 g/100 ml), the pH adjusted to 3.3 with HCl.

(iii) Buffer C (Re-suspension buffer)

5 ml triethanolamine (5%) and 5 g (5%) Sodium Dodecyl Sulphate (SDS), were dissolved in 100 ml of H_2o, the pH adjusted to 9.4 with HCl.

(iv) Protein Solution

1 mg/ml bovine serum albumin was dissolved into buffer A.

(v) Ferric Chloride Reagent

0.2703 g $FeCl_3$ (10 mM) was dissolved in 1 ml HCl (0.01 N) and 99

ml H_2O.

(vi) Catechin Standard

1.4 mg catechin was dissolved in 1260 µL H_2O and 140 µL ethanol (1 mg/ml catechin solution in a 10% ethanol).

3.5.2 Total phenols test reagents

The reagents for the total phenols assay were prepared according to Slinkard and Singleton (1977) as followed:

(i) Gallic acid stock solution

In a 100-ml volumetric flask, 0.5 g of dry gallic acid was dissolved in 10 ml of ethanol and diluted to volume with water (500mg/100ml), then stored in a refrigerator.

(ii) Sodium carbonate solution

100 g of anhydrous sodium carbonate were dissolved in 400 ml of water and brought to a boil. After cooling, few crystals of sodium carbonate were added, and after 24 hours filtered and the volume completed to 500 ml with distilled water.

(iii) Folin Ciocalteu reagent (2N)

3.6 Thin Layer Chromatography (TLC)

The TLC analysis of the 1:10 dilution was carried out according to Nitzsche *et al.* (2004) using silica gel plates 20×20 cm (Silica gel F254, Merck, Darmstadt, Germany). From each extract 10µl were spotted on the plate.

3.6.1 Tannins

The TLC solvent system was 98% acetic acid/ether/ethyl acetate (20:20:40; v/v/v). After evaporation of the solvent for 10-15 min. at room temperature, the plate was sprayed with Fast Blue salt B solution (50 mg dissolved in 10 ml of H_2O). The tannins showed a red-brown colour.

3.6.2 Flavonoids

The TLC solvent system was formic acid/H_2O/ethyl acetate (6:9:90; v/v/v). The plate was dried for 10 min. at 100-105º C and then sprayed with diphenylboryloxy-ethylamine (0.25 g DPBA in 25 ml methanol). After 30 min. the yellow-brown and yellow-orange spots were visualized under UV light at 366 nm.

3.6.3 Phenols

The TLC solvent system was formic acid/H_2O/ethyl acetate (6:6:88; v/v/v). Formic acid was evaporated by heating to 105-110º C and then the plate was sprayed with $FeCl_3$ solution [2 g $FeCl_3$ in 98 ml of 96% ethanol (2% w/v)]. Phenols showed a light blue to brownish-blue colour.

3.6.4 Anthraquinones

The TLC solvent system was H_2O/methanol/ethyl acetate (13:17:100; v/v/v). After evaporation of the solvent for a maximum of 5 min., the plate was sprayed with KOH reagent (5g KOH/100 ml methanol) and then heated to 100-105ºC for 15 min. The brownish-yellow spots were visualized under UV light at 366 nm.

3.6.5 Saponins

Saponins were detected with anisaldehyde reagent (10 ml of anisaldehyde reagent was mixed with 90 ml of ethanol and, after the addition of 10 ml of H_2SO_4 mixed again). The TLC solvent system was ethyl acetate/H_2O/butanol (25:50:100; v/v/v). After evaporation of the solvent, the plate was sprayed with 10 ml of anisaldehyde reagent and then heated to 105-110ºC for 5-10 min. Saponins showed brown to grey-violet spots.

3.6.6 Glycosides

The TLC solvent system was butanol/acetic acid/H_2O (4:1:1; v/v/v). The plate was dried for 10 min. at 100-105º C and then sprayed with diphenylboryloxy-ethylamine (0.25 g DPBA in 25 ml methanol). After 30 min. yellow-brown spots were visualized under UV light at 366 nm (Slimestad *et al.*, 1994).

3.7 HPLC Separation of metabolites

From the extracts, which were diluted 1:10 dilution in methanol, 100 µl of each sample (0 and 15 KGy treatments) were analyzed by HPLC (Jasco PU 2080 plus pumps and Jasco DE 2080-53 degasser; coupled to a Jasco AS-1550 auto sampler) on a 4 mm × 250 mm Reverse phase column Hyper clone ODS C_{18} 5µm (Phenomenex, Aschaffenburg, Germany), and a Multi-wavelength Diode Array Detector (Jasco MC-919). The HPLC equipment was linked to a computer (IBM ThinkPad) using the Jasco LC-Net II/ADC. As software the Jasco ChromPass Chromatography Data System software was used (Jasco, Gross-Umstadt, Germany).

A linear gradient of aqueous 1% acetic acid and 100% methanol was used. The gradient was started with 20% methanol/80% aqueous acetic acid and run within 70 min. to 100% methanol. Then the initial conditions were re-established over a period of 10 min. samples were analyzed at different wavelengths. The wavelength of the highest absorption was used for the chromatograms.

3.8 Phytochemical Screening

3.8.1 Test for Tannins

According to protein precipitation method as described by Hagerman and Butler (1978), 50 to 300 µl of standard catechin solution were taken for standard curve preparation, and the volume adjusted to 875 µl with buffer C (e.g. 100 µl catechin solutions plus 775 µl of buffer C). 125 µl of the ferric chloride reagent was added and mixed. A zero tannin sample was made with 875 µl buffer C and 125 µl of ferric chloride reagent. The absorbance value was recorded at 510 nm using a spectrophotometer (Unicom UV/Vis spectrometer). The standard samples and the zero tannin were incubated at room temperature for 10 minutes then the absorbance was read.

The samples were diluted with buffer B 1:1 (750 µl sample: 750 µl buffer B). For each sample, 1 ml of the protein solution was pipette into a microfuge tube with 500 µl of the diluted sample, and then incubated for 15 minutes with slow agitation (300 rpm) in thermo mixer compact (Eppendorf, Hamburg, Germany).

The samples were centrifuged for 5 minutes in micro-centrifuge, 14.000 RPM (Eppendorf, Hamburg, Germany). The supernatant were carefully poured off, retaining the pellet in the microfuge tube. 250 µl of

buffer A was slowly added to the pelleted samples, and then centrifuged for 1 minute (14.000 RPM). The last 3 steps were repeated to wash the pellet a second time. The supernatants were poured off and then 875 µl of buffer C added, and then the tubes incubated for 10 min. in thermomixer (950-1000 rpm) for complete mixing. 10 min. after dissolving the pellet, the absorbance was read at 510 nm and the value recorded. Then 125 µl of ferric chloride reagent was added, the sample mixed, incubated for 10 minutes and the absorbance was re-read at 510 nm.

The concentration of tannin (mg/L CE) was calculated using the standard curve (fig 3.1) and following the general equation:

$$2\times \frac{(\text{Absorbance} - \text{Intercept})}{\text{Slope}} \text{ (Dilution)}$$

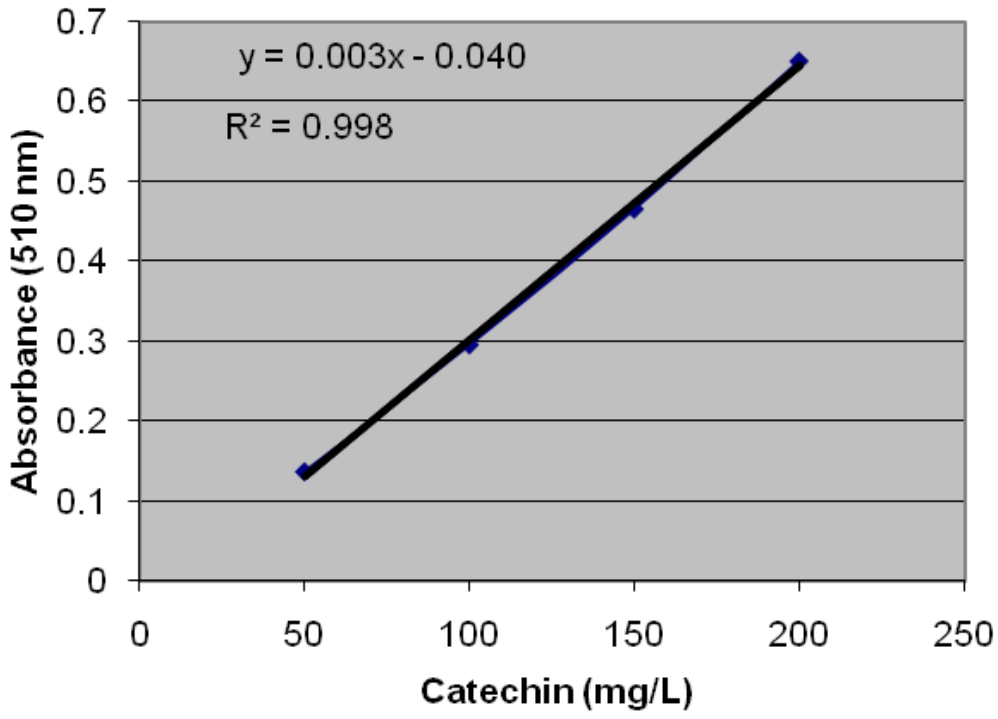


Fig 3.1 Standard curve for determination of Catechin equivalents in tannin assay

3.8.2 Test for Total Phenols

To prepare the calibration curve, 0, 1, 2, 3, 5 and 10 ml of the gallic acid stock solution (500mg/100ml) were added into 100 ml volumetric flasks, and then diluted to volume with water following folin-ciocalteau micro method described by Slinkard and Singleton (1977). These solutions had phenol concentrations of 0, 50, 100, 150, 250 and 500 mg/L gallic acid.

From each calibration solution, 20 µL were pipette into separate cuvettes, and to each 1.58 ml water, 100 µL of folin-ciocalteau reagent were added, and then 300 µL of sodium carbonate solution after 8 minutes.

The cuvettes were shaken until mixing, and then left at 20 ºC for 2 hours. The absorbance for each solution was determined at 765 nm against the blank (the "0 ml" solution) using a spectrophotometer (UNICAM UV/Vis spectrometer), then plotted against concentration. A calibration curve was created with the standards and used to determine the levels in the samples (fig. 3.2).

For the samples, 20 µL was added as for the calibration solutions; with different dilutions (values for dilutions 1:60 and 1:100 were too high, so for all samples dilution 1:30 was used).

Results were reported as gallic acid equivalent. The observed concentrations were multiplied by the dilution factor.

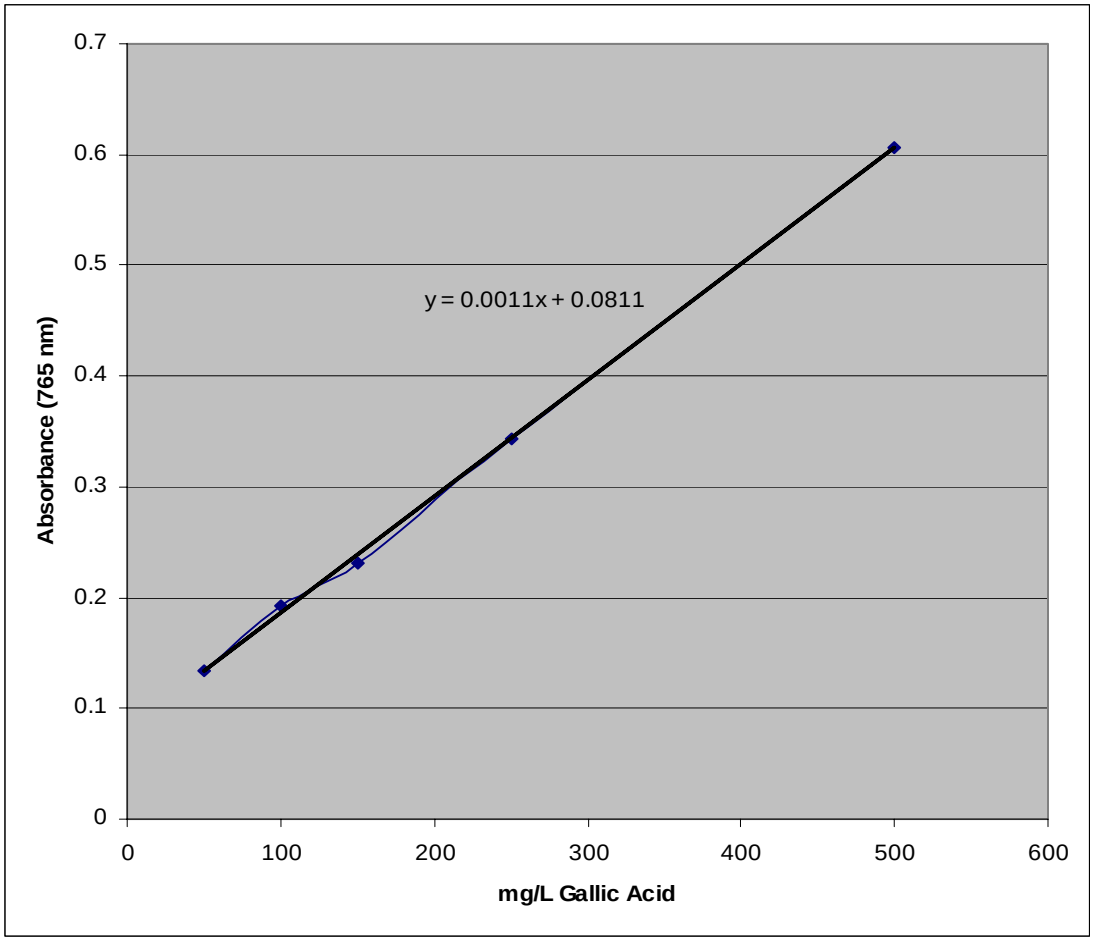


Fig 3.2 Folin Ciocalteau Gallic Acid Standard Curve

3.9 Antioxidant activity

Free radical scavenging activities were determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, Fluka, Germany) as described by Wang *et.al.* 2002. The flavonol quercetin (1 mM) was analyzed in parallel as positive control. The assays were performed from a mixture containing 100 µl of freshly made 0.1 mM DPPH solution in 96% ethanol, 900 µl of samples at different dilutions (0, 1, 2, 5, 10, 20, 40 and 80 µM). The mixture was shaken and left to stand for 30 min. at

room temperature, the absorbance measured at 517 nm using a spectrophotometer (UNICAM UV/Vis spectrometer).

3.10 Microbial Analysis

Test of the total viable count of bacteria on all irradiated plant material in comparison to the control was carried out following the pour plate count method (Harrigan, 1998); a 10 g of the powdered sample was weighed under aseptically conditions and homogenized in 90 ml of sterile diluents (0.1% peptone solution) to give 1:10 dilution (10^{-1}). Five test tubes each containing exactly 9 ml of sterile diluents were prepared. One ml of the dilution 10^{-1} was aseptically removed and transferred to a tube containing 9 ml of the diluents yielding the dilution 10^{-2}. The procedure was repeated to prepare serial dilutions up to 10^{-6}. From each of the six dilution tubes 1 ml was transferred aseptically to each of six sterile empty Petri dishes beginning with the dilution 10^{-6}. To each plate 15 ml of sterile plate count agar were added. The plates were then incubated at 37° for 48 hours. The plates containing between 30-300 colonies were counted using colony counter (Scientific & Cook Electronics L.T.D), then the viable count of bacteria in 1 ml of the original sample was calculated from the colony count and the respective dilution. The results were presented as colony forming units (cfu/g).

3.11 Statistical Analysis

Each sample was analyzed in triplicate following factorial experiment. Data were assessed by the analysis of variance (ANOVA) described by Sendecor and Corchan (1987).

CHAPTER FOUR

CHAPTER FOUR

RESULTS

4.1 Influence of irradiation on the bacterial load

Table (1) shows the effect of different doses of gamma rays 5, 10 and 15 KGy compared to the control (0 KGy) on the bacterial count of nine medicinal plant species. There was a significant reduction on the bacterial load in the irradiated samples compared to the un-irradiated and the species were significantly different (appendix1). The highest mean count of the un-irradiated samples was detected in *Trigonella foenum-graecum* L. followed by *Cassia senna* (pods), *Cassia senna* (leaves) and *Acacia nilotica L.* and the lowest mean counts were detected in *Brassica nigra* L. Koch followed by *Lepidium sativum* L., *Cymbopogon citratus, Hibiscus sabdariffa* L. and *Cymbopogon schoenanthus* L.

The interaction between dose and species was significant (appendix1). The highest sensitivity to gamma rays at 5 KGy was observed in *Trigonella foenum-graecum* L. (up to 99%) followed by *Acacia nilotica L.* (97.4), *Cassia senna* pods (95.7%) and Cymbopogon *citrates* (94.7%). While the lowest was in *Brassica nigra* L. Koch (77.5%) followed by Lepidium *sativum* L. (77.1%), *Cassia senna* (leaves) (70%), *Hibiscus sabdariffa* L. (69.2%) and *Cymbopogon schoenanthus* L (55.6%).

At 15 KGy dose Hibiscus *sabdariffa* L. and *Cymbopogon citratus* showed complete absence of microorganisms.

Regarding the LD_{50}, significant differences were detected among the nine plant species (fig.4.1). *Acacia nilotica, Cymbopogon citratus, Trigonella foenum-graecum* L. and *Cassia senna* L. (pods) had a relative low LD_{50} values which ranged between 3.41 and 3.69 KGy. The plant species *Brassica nigra* L. Koch, *Lepidium sativum* L., *Cassia senna* L. (leaves)

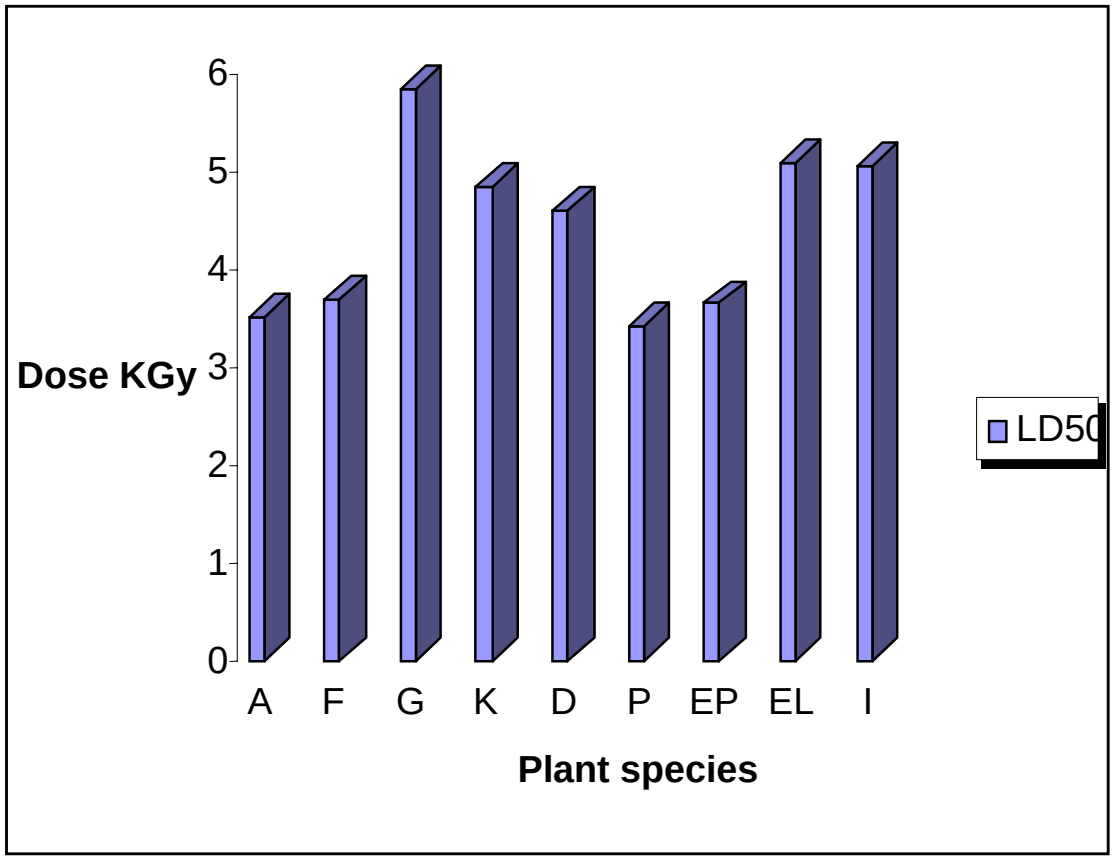
and *Hibiscus sabdariffa* L. required a moderate dose ranging between 4.6 and 5.09 KGy, to achieve 50% reduction in the microbial load. However the highest estimates of LD_{50} (5.84) was recorded for *Cymbopogon schoenanthus* .The same pattern was observed for LD_{75} (fig. 4.2), in which the plant species followed the same arrangement detected in the $LD_{50.}$

Table (1): Bacterial profile (cfu×10^2) of nine medicinal plant species irradiated with gamma ray.

Species	Dose KGy			
	0	**5**	**10**	**15**
Acacia nilotica L.	700**d**	18**h** (2.6)	8**h** (1.1)	3**h** (0.4)
Cymbopogon citratus	300**efg**	16**h** (5.3)	7**h** (2.3)	0**h** (0)
Cymbopogon schoenanthus L.	90**h**	40**h** (44.4)	8**h** (8.9)	3.4**h** (3.8)
Lepidium sativum L.	350**ef**	80**h** (22.9)	36**h** (10.29)	3.8**h** (1.1)
Brassica nigra L. Koch	400**e**	90**h** (22.5)	12**h** (3)	4**h** (1)
Trigonella foenum-graecum L.	14400**a**	150**fgh** (1)	90**h** (0.6)	5**h** (0.03)
Cassia senna L. (pods)	3000**b**	130**gh** (4.3)	87**h** (2.9)	4**h** (0.1)
Cassia senna L. (leaves)	1200**c**	360**e** (30)	95**h** (7.9)	3**h** (2.5)
Hibiscus sabdariffa L.	130**gh**	40**h** (30.8)	7.6**h** (5.8)	0**h** (0)

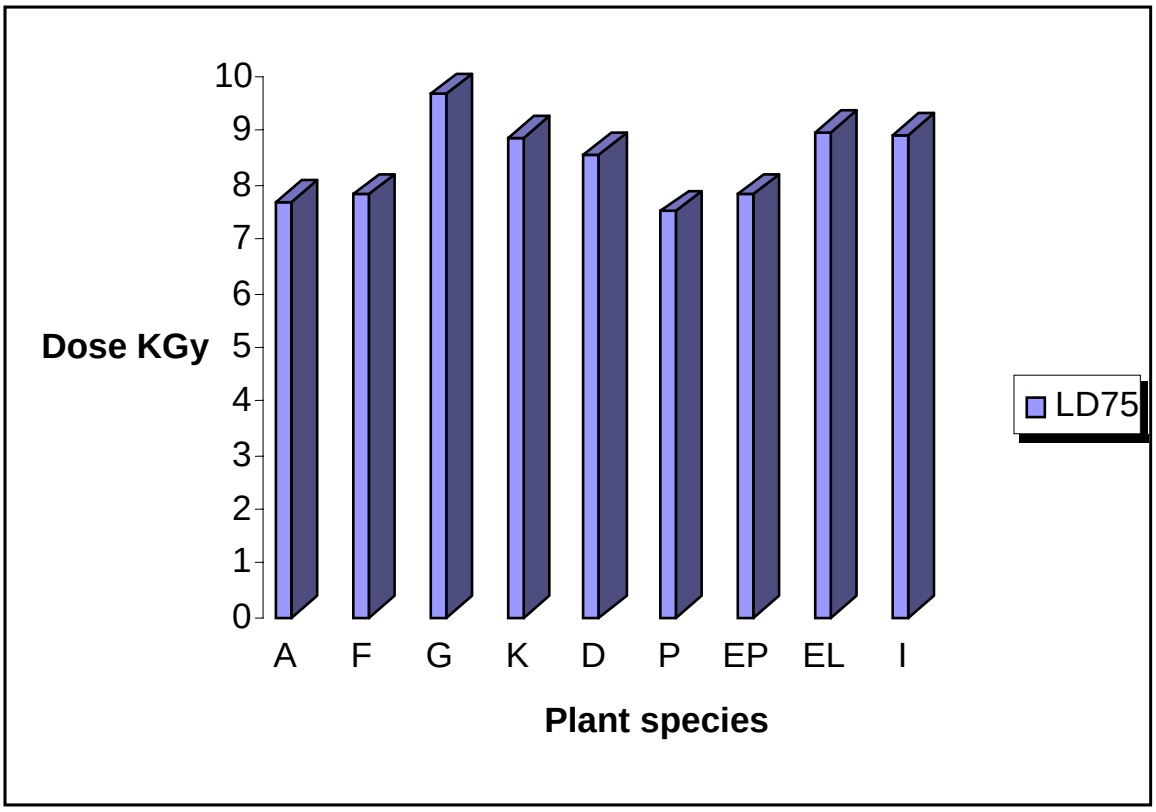
Means with the same following letter in a column or a row are not significantly different, according to Duncan's Multiple Range test.

Figure 4.1 Lethal dose (LD_{50}) of the plant species



[(A) *Acacia nilotica* L., (D) *Brassica nigra* L. Koch, (K) *Lepidium sativum* L**,** (F) *Cymbopogon citratus*, (G) *Cymbopogon schoenanthus* L., (I) *Hibiscus sabdariffa* L., (P) *Trigonella foenum-graecum* L., (EL) *Cassia senna* L.(leaves), (EP) *Cassia senna* L. (pods)]

Figure 4.2 Lethal dose (LD75) of the plant species



[(A) *Acacia nilotica* L., (D) *Brassica nigra* L. Koch, (K) *Lepidium sativum* L**,** (F) *Cymbopogon citratus*, (G) *Cymbopogon schoenanthus* L., (I) *Hibiscus sabdariffa* L., (P) *Trigonella foenum-graecum* L., (EL) *Cassia senna* L.(leaves), (EP) *Cassia senna* L. (pods)]

4.2 Effect of gamma irradiation on the qualitative analysis of herbal raw material

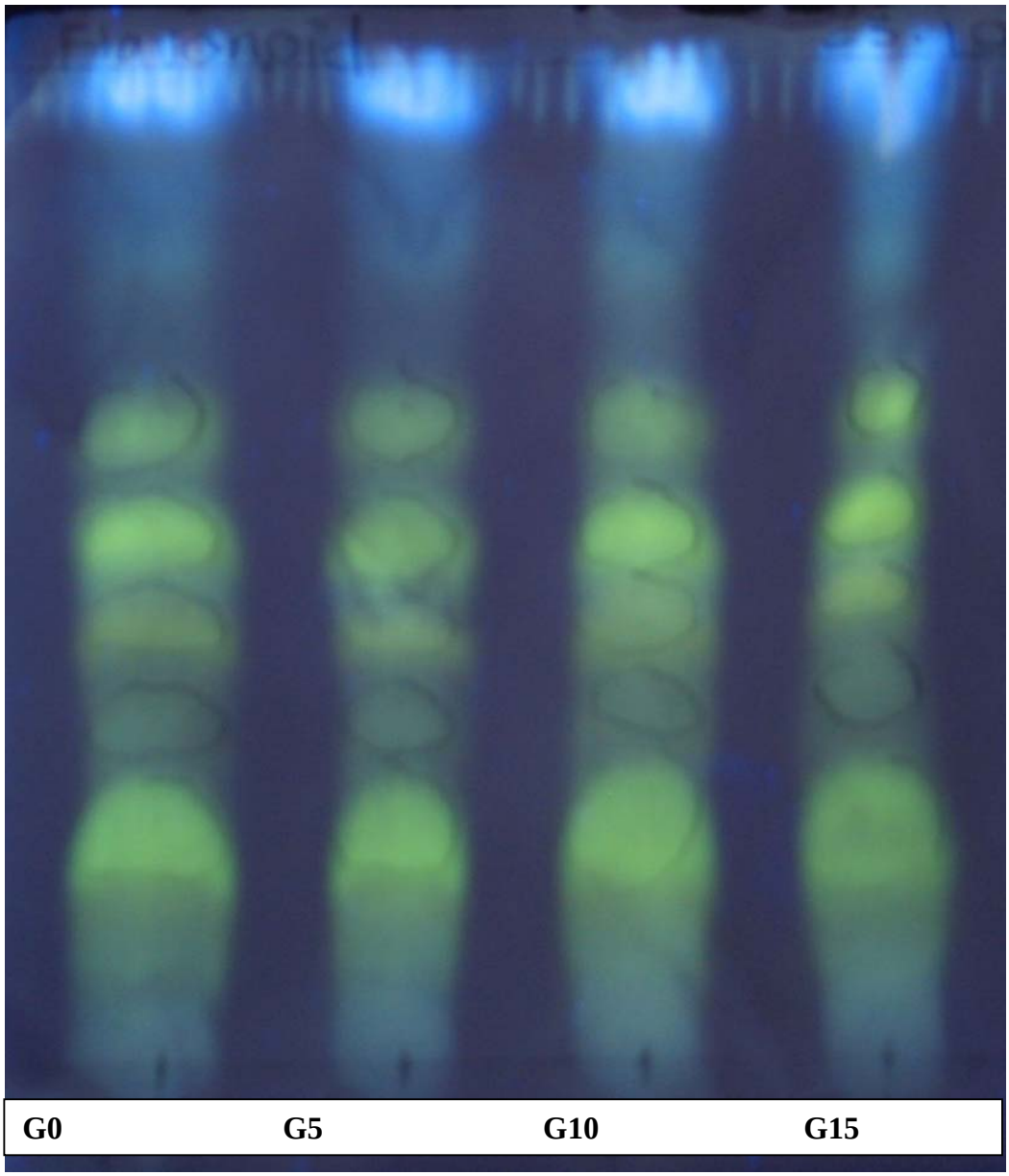
Table (2) represents the chemical finger print as RF values of the major compounds in the extracts of different plant species. No differences in the RF values were observed among the irradiated and un-irradiated samples in the qualitative analysis by TLC, as observed in plates 1 and 2.

The results indicated that application of different doses of gamma radiation caused no effect on the qualitative analysis of herbal material as presented by their major important secondary compounds.

Table (2): Occurrence of different classes of secondary compounds by TLC

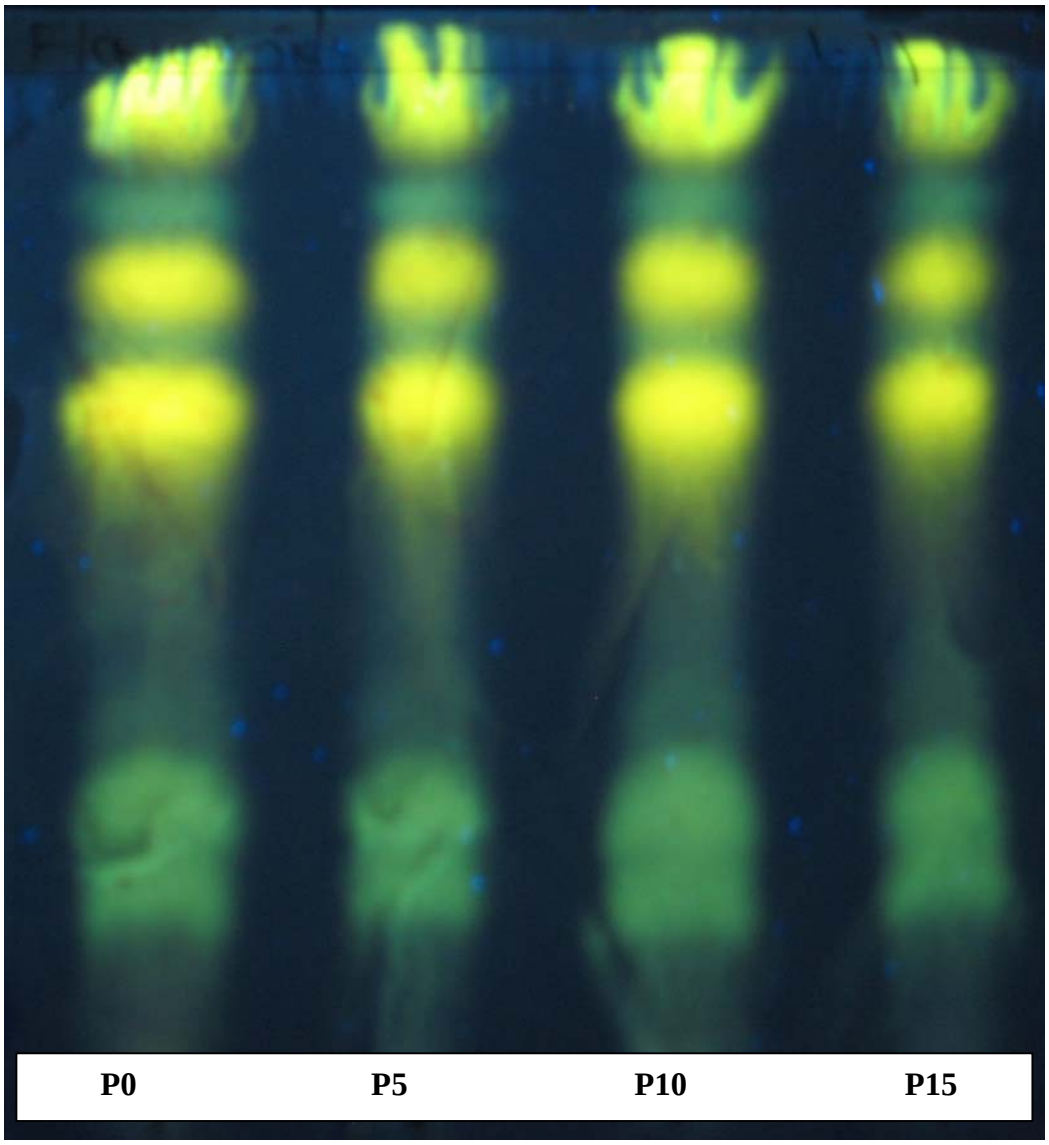
Herbal raw material	Classes of secondary metabolites					
	Anthra-quinones	Phenols	Flavonoids	Tannins	Saponin	Glycosides
	Rf values					
Acacia nilotica L. (pods)	-	0.23 0.46 0.66 0.87	0.24 0.39 0.65 0.76	0.71	0.90	-
Brassica nigra L. Koch (seeds)	-	-	0.14 0.27 0.36 0.52 0.69 0.78	-	-	0.27 0.36 0.52 0.69 0.78
Cassia senna L. (leaves)	0.14 0.39 0.54 0.63 0.71 0.93	-	0.06 0.27 0.39 0.65 0.73	-	-	0.48 0.56 0.64 0.77 0.86

Cassia senna L. (pods)	0.83 0.24 0.40 0.54 0.67	-	0.18 0.24 0.30 0.48 0.59 0.76	-	-	0.54 0.64 0.75
Cymbopogon citratus (leaves)	-	0.25 0.54 0.12	0.17 0.30 0.40 0.50 0.65	-	-	0.53 0.68 0.81
Cymbopogon schoenanthus L. (leaves)	0.21 0.48 0.54 0.75	-	0.21 0.32 0.44 0.53 0.66	-	-	0.64 0.76
Hibiscus sabdariffa L. (calyces)	-	0.72	0.03 0.11 0.14 0.20 0.33 0.66 0.75 0.88	-	-	0.37 0.49
Lepidium sativum L. (seeds)		0.08	0.16			0.44 0.54
Trigonella foenum-graecum L. (seeds)	-	-	0.28 0.66 0.79 0.98	-	-	0.47 0.79 0.89



G0: Control sample
G5, G10, G15: irradiated samples (at 5, 10, 15 KGy)

Plate 1 Flavonoids profile of *Cymbopogon schoenanthus* L. by TLC method



P0: Control sample
P5, P10, P15: irradiated samples (at 5, 10, 15 KGy)

Plate 2 Glycosides profile of *Trigonella foenum-graecum* L. by TLC method

4.3 Effect of gamma irradiation on herbal raw material components

The influences of gamma radiation dose (15 KGY) on the stability of the major compounds in herbal raw materials of nine plant species, as shown by HPLC retention times (min) at different wave lengths are shown in tables 3 to 11.

As shown in table (3), noticeable changes could be observed in the chemical constituents of *Acacia nilotica* (Pods) as affected by gamma irradiation. The compounds of the retention times 2.06, 7.53, 6.69, 25.60, 2.19 min were decreased from 44.87 to 6.07, 8.91 to 0.62, 8.60 to 2.12, 20.09 to 10.09 and 29.95 to 20.85, respectively. While that of the retention times 2.13 and 6.14 min, were increased from 8.46 to 16.38 and 7.22 to 14.22, respectively. However, the compounds of the retention times 20.66, 11.92, 12.48, 21.95, 31.18 and 10.93 min were not detected. The results also revealed that at all wave lengths 280, 320 and 370 nm, the compounds of retention time 2.07 min of *Brasica nigra* L. (Seeds) were decreased as affected by gamma irradiation. The reduction was from 50.9 to 32.32, 19.37 to 9.13 and 31.21 to 5.52, respectively. Also the compounds of retention times 26.22, 25.43, 23.47, 19.46 min were increased from 8.98 to 12.41, 7.96 to 13.40, 27.12 to 32.38 and 2.76 to 12.37, respectively. Moreover, slight changes were observed in the other chemical constituents. However, for the compounds of retention times 22.81, 3.57, 33.63, 37.52, 37.68 and 24.57 min, complete elimination was detected (table 4).

Table (5) illustrates the results of the effect of gamma irradiation on the chemical constituents of *Cassia senna* L. (Leaves). The compound of retention time 22.76 min was decreased from 20.47

to 4.75, while that of the retention time 12.72 min increased from 2.90 to 9.03. No great changes were observed in the other chemical constituents. However, the compounds of retention times 22.78, 26.04, 23.94, 21.82, 26.06, 29.31, 21.82 and 22.77 min disappeared after irradiation.

The data of the chemical constituents of *Cassia senna* L. (Pods) as affected by gamma irradiation were presented in table (6). No noticeable differences were observed. However, the compounds of the retention times 29.84, 26.27 min were decreased from 19.46 to 11.38 and 14.41 to 5.67, respectively. Complete elimination was detected for the compounds of retention time 24.98 min, at all wave lengths.

Table (7) gives the relative percent distribution of the major constituents of *Cymbopogon citratus* L. (Leaves) as affected by gamma irradiation. Generally the results indicated that no qualitative changes based on the retention time in the major HPLC peaks were noted. Only one compound of the retention time 7.02 min was not detected. The compounds of the retention times 22.50, 15.83, 18.37 min, at different wave lengths, were decreased from 22.22 to 14.10, 13.04 to 7.93 and 17.67 to 12.30, respectively. The compounds of the retention times 8.18, 26.51, 24.46 and 28.35 min showed slight increase from 6.40 to 7.21, 9.63 to 10.67, 7.68 to 8.14 and 1.23 to 3.01, respectively. While that of the retention times 2.07 and 35.47 min showed obvious increase from 4.70 to 16.28 and 13.41 to 23.61, respectively. The effect of gamma irradiation on the chemical constituents of *Cymbopogon schoenanthus* (Leaves) is presented in table (8). The compounds of retention times 8.42, 29.08, 8.38, 16.93, 14.80 and 42.80 min remained approximately unchanged, while the compound of retention times 16.95 min was eliminated. On the other

hand, the compounds of retention times 9.66, 2.03 and 29.35 min were decreased from 16.56 to 11.77, 14.06 to 9.88 and 8.25 to 2.88, respectively. An increase in the chemical constituents, was observed in the compounds of the retention times 14.37 and 35.42 min from 33.76 to 40.11 and 5.64 to 10.05, respectively. Slight changes were observed in the other compounds. Table (9) summarizes the effect of gamma irradiation on the chemical constituents of *Hibiscus sabdariffa* (calyces). Great reduction was noted in the compounds of retention times 2.17, 4.19, 4.23 and 2.19 min from 14.05 to 1.49, 14.46 to 4.47, 38.36 to 33.61 and 16.93 to 2.14, respectively. The compounds of the retention times of 3.35, 7.27 and 2.17 min were increased from 38.76 to 56.67, 28.62 to 39.36 and 18.26 to 24.96, respectively. Slight changes were observed in the other constituents while the compounds of the retention times 2.52, 51.69, 11.45 and 23.03 min totally disappeared. As shown in table (10), no noticeable changes could be observed in the chemical constituents of *Lepidium sativum* L. (seeds) as affected by gamma irradiation. However, the compounds of retention time 14.81 min showed reduction at wave lengths 280, 320 and 370 nm. The reduction was from 19.60 to 13.10, 20.11 to 9.71 and 24.49 to 9.71, respectively. Moreover, the compounds of retention time 15.50 remained substantially unchanged and total elimination was occurred for the compounds of retention times 16.07, 5.56, 35.67, 6.42 and 41.25 min. The results of the chemical constituents of *Trigonella foenum- graceum* L. (Seeds) as affected by gamma irradiation were presented in table (11). The compounds of retention times 2.67, 12.17, 13.36, 17.44, 20.81 and 16.81 min, remained approximately unchanged, while the compound of retention time 16.27 min was not detected. Slight changes could be observed in the other chemical constituents.

Table (3): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Acacia nilotica* (Pods)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	**15**
280	1	2.06	44.87	6.07
	2	15.24	9.27	7.39
	3	7.53	8.91	0.62
	4	6.69	8.60	2.12
	5	20.66	7.05	-
	6	19.69	5.12	7.57
	7	17.05	3.72	5.39
	8	19.59	3.28	7.57
	9	4.53	2.42	0.17
	10	11.92	1.51	-
320	1	25.60	20.09	10.09
	2	16.41	12.18	9.58
	3	11.38	11.52	7.46
	4	2.13	8.46	16.38
	5	18.63	8.14	6.27
	6	21.46	7.85	10.84
	7	6.14	7.22	14.22
	8	15.28	6.69	6.49
	9	19.65	6.49	5.82
	10	12.48	4.74	-
370	1	21.95	46.00	-
	2	2.19	29.95	20.85
	3	23.83	9.15	10.34
	4	23.09	4.96	3.20
	5	28.16	2.60	0.26
	6	1.80	1.86	5.91
	7	31.18	1.51	-
	8	15.24	0.78	2.82
	9	3.04	0.65	1.16
	10	10.93	0.53	-

- : Not Detected

Table (4): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Brasica nigra* L. (Seeds)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	2.07	50.90	32.32
	2	26.22	8.98	12.41
	3	25.47	8.97	9.74
	4	23.74	7.43	7.75
	5	3.12	4.25	0.73
	6	10.10	4.21	8.16
	7	24.87	3.35	1.87
	8	19.43	2.13	2.76
	9	22.81	1.84	-
	10	3.57	1.70	-
320	1	23.74	20.48	20.67
	2	2.07	19.37	9.13
	3	10.09	8.62	6.47
	4	25.43	7.96	13.40
	5	33.63	7.59	-
	6	8.65	7.27	6.99
	7	37.52	7.25	-
	8	19.45	4.70	7.07
	9	34.00	3.47	2.21
	10	15.90	3.23	2.75
370	1	2.08	31.21	5.52
	2	23.74	27.12	32.38
	3	25.48	9.38	11.66
	4	10.10	7.98	11.51
	5	37.68	7.16	-
	6	8.66	3.91	8.52
	7	19.46	2.76	12.37
	8	32.27	2.05	0.26
	9	24.57	1.41	-
	10	29.98	1.10	0.06

- : Not Detected

Table (5): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Cassia senna* L. (Leaves)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	**15**
280	1	22.78	20.32	-
	2	24.46	8.88	4.96
	3	26.04	8.48	-
	4	6.49	8.10	8.86
	5	23.94	6.90	-
	6	12.65	6.49	10.03
	7	2.08	5.90	6.37
	8	29.30	5.26	4.46
	9	21.82	4.16	-
	10	26.91	4.15	8.06
320	1	22.76	20.47	4.75
	2	26.06	10.71	-
	3	12.65	9.73	12.82
	4	24.46	8.70	7.76
	5	23.93	8.29	-
	6	26.91	7.49	11.96
	7	29.31	6.76	-
	8	6.48	5.36	3.09
	9	21.82	3.74	-
	10	2.20	3.27	0.36
370	1	22.77	31.12	-
	2	23.91	13.75	-
	3	26.06	12.87	13.74
	4	24.46	12.77	8.46
	5	26.92	9.24	13.74
	6	29.30	5.82	5.17
	7	12.72	2.90	9.03
	8	19.26	2.03	2.87
	9	21.83	1.80	-
	10	19.69	1.54	-

- : Not Detected

Table (6): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Cassia senna* L. (Pods)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	29.84	16.01	13.05
	2	6.69	14.37	11.97
	3	24.98	14.10	-
	4	5.69	10.42	7.58
	5	23.35	10.03	13.98
	6	2.07	8.58	5.71
	7	26.27	5.80	7.24
	8	13.05	4.32	3.51
	9	22.23	4.22	6.35
	10	4.32	2.56	2.82
320	1	29.84	19.46	11.38
	2	24.99	18.82	-
	3	23.38	9.16	12.96
	4	6.69	8.07	8.93
	5	26.28	7.03	6.00
	6	13.05	6.59	8.01
	7	22.23	6.00	9.39
	8	5.69	5.79	6.16
	9	26.59	5.03	6.00
	10	27.43	4.42	3.47
370	1	24.98	25.29	-
	2	29.84	19.98	16.64
	3	26.27	14.41	5.67
	4	23.37	13.41	17.67
	5	27.44	5.75	6.63
	6	13.05	4.04	3.96
	7	22.23	3.61	4.62
	8	6.69	2.45	2.93
	9	19.79	2.31	3.19
	10	5.69	2.01	2.12

- : Not Detected

Table (7):Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Cymbopogon citratus* L. (Leaves)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	22.50	22.22	14.10
	2	35.45	14.58	14.43
	3	15.83	13.04	7.93
	4	14.38	8.77	6.43
	5	26.50	6.05	4.21
	6	8.25	5.82	4.77
	7	2.07	4.70	16.28
	8	21.73	4.43	2.49
	9	7.01	3.91	2.07
	10	24.47	3.50	2.93
320	1	18.37	17.67	12.30
	2	22.51	14.69	14.19
	3	35.47	13.41	23.61
	4	15.86	9.78	5.5
	5	14.40	8.85	6.43
	6	8.18	6.40	7.21
	7	7.02	5.42	-
	8	26.48	4.44	4.39
	9	21.75	3.87	3.11
	10	24.46	2.93	2.46
370	1	18.37	32.38	27.40
	2	22.51	25.61	23.60
	3	26.51	9.63	10.67
	4	24.46	7.68	8.14
	5	15.86	6.91	5.61
	6	21.77	6.19	6.50
	7	14.24	3.74	3.40
	8	2.09	2.12	0.87
	9	16.85	1.75	2.10
	10	28.35	1.23	3.01

- : Not Detected

Table (8): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Cymbopogon schoenanthus* (Leaves)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	14.37	33.76	40.11
	2	9.66	16.56	11.77
	3	2.03	14.06	9.88
	4	18.33	12.94	11.78
	5	35.41	3.36	4.76
	6	13.26	2.89	3.51
	7	8.42	2.75	2.37
	8	29.10	1.94	2.11
	9	26.48	1.83	1.31
	10	16.95	1.24	-
320	1	14.37	39.43	38.77
	2	9.66	24.93	21.46
	3	18.34	9.52	7.03
	4	35.42	5.64	10.05
	5	29.08	4.24	4.66
	6	8.38	3.78	3.44
	7	26.46	2.25	1.46
	8	13.28	1.58	1.34
	9	42.80	1.36	1.56
	10	2.17	1.10	0.99
370	1	18.34	9.52	7.03
	2	26.50	12.62	7.35
	3	29.35	8.25	2.88
	4	8.40	7.19	11.29
	5	22.46	5.97	4.88
	6	16.93	5.34	5.77
	7	9.64	4.25	7.85
	8	23.33	3.06	1.77
	9	14.80	2.64	2.20
	10	24.46	2.52	1.17

- : Not Detected

Table (9): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Hibiscus sabdariffa* (calyces)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	3.35	38.76	56.67
	2	4.73	14.93	13.44
	3	7.72	14.73	13.71
	4	2.17	14.05	1.49
	5	4.19	14.46	4.47
	6	5.91	4.23	0.45
	7	2.52	3.96	-
	8	23.03	1.16	0.36
	9	51.69	1.47	-
	10	11.45	0.91	-
320	1	4.23	38.36	33.61
	2	7.72	28.62	39.36
	3	2.19	16.93	2.14
	4	5.93	4.15	1.44
	5	23.03	2.18	-
	6	24.67	1.91	2.68
	7	10.72	1.75	1.66
	8	9.74	1.51	0.59
	9	11.42	1.50	1.86
	10	19.77	0.93	2.24
370	1	23.03	26.06	-
	2	2.17	18.26	24.96
	3	19.77	15.12	7.20
	4	4.24	10.60	16.83
	5	7.73	10.49	14.52
	6	16.09	5.52	2.11
	7	18.82	4.01	1.95
	8	16.59	2.50	1.08
	9	26.79	1.78	1.04
	10	29.52	1.69	0.75

- : Not Detected

Table (10): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Lepidium sativum* L. (seeds)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	14.81	19.60	13.10
	2	16.07	14.51	-
	3	5.56	10.93	-
	4	15.50	9.56	9.56
	5	13.48	8.95	7.19
	6	35.67	7.95	-
	7	2.07	6.01	8.74
	8	6.42	5.41	-
	9	28.27	4.08	3.60
	10	27.36	3.95	3.19
320	1	16.09	32.16	33.76
	2	14.81	20.11	9.71
	3	15.55	8.12	8.96
	4	35.67	7.29	-
	5	20.05	5.69	3.20
	6	27.36	5.37	6.50
	7	26.35	5.11	5.66
	8	28.26	4.37	5.62
	9	41.25	3.29	-
	10	2.27	2.05	7.39
370	1	14.81	24.49	9.71
	2	16.07	19.10	-
	3	13.48	18.67	20.91
	4	15.49	11.00	11.59
	5	2.25	4.49	3.29
	6	20.05	3.54	2.93
	7	19.26	3.10	2.92
	8	27.38	2.92	2.84
	9	28.27	2.22	2.33
	10	1.99	2.02	0.35

- : Not Detected

Table (11): Quantities of major compounds recorded at different wave lengths (nm) as percentage areas of treated and un-treated methanolic extracts of *Trigonella foenum- graceum* L. (Seeds)

Wave length (nm)	Major compounds (Unknown)	RT (min.)	Quantity (% Area) Radiation dose (KGy)	
			0	15
280	1	17.45	16.76	17.86
	2	15.13	15.61	18.89
	3	2.67	12.38	12.10
	4	20.81	12.27	9.73
	5	12.17	7.29	8.04
	6	16.78	6.75	8.27
	7	13.34	6.31	7.33
	8	27.30	4.67	3.98
	9	16.27	3.53	2.42
	10	29.30	2.60	3.19
320	1	17.45	17.77	19.46
	2	15.12	16.93	19.55
	3	20.81	14.37	13.52
	4	12.17	8.86	8.78
	5	13.36	6.90	6.67
	6	16.78	6.81	8.19
	7	27.23	6.32	4.92
	8	29.30	5.66	4.62
	9	16.27	4.42	-
	10	28.15	3.66	3.01
370	1	17.44	25.98	25.46
	2	15.13	16.59	16.37
	3	20.81	13.44	13.87
	4	12.17	8.50	8.92
	5	16.81	7.72	7.50
	6	16.29	7.17	-
	7	13.34	7.07	6.01
	8	27.23	4.09	3.95
	9	28.15	2.63	2.34
	10	2.24	2.19	1.58

- : Not Detected

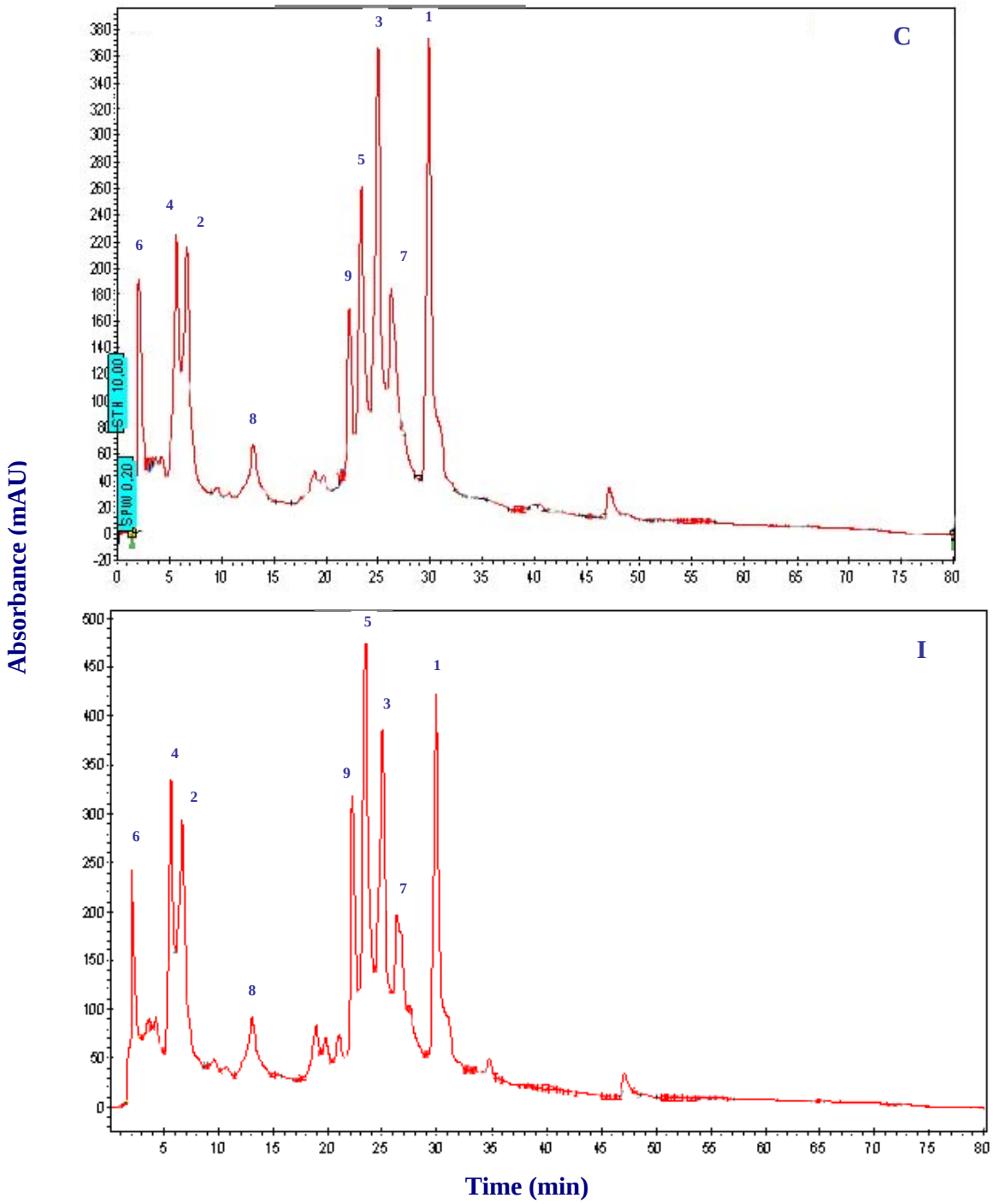


Figure (4.3) Chromatograms of methanolic extract of un-irradiated (C) and 15 KGy- irradiated (I) *Cassia senna* pods at wave length 280 nm.

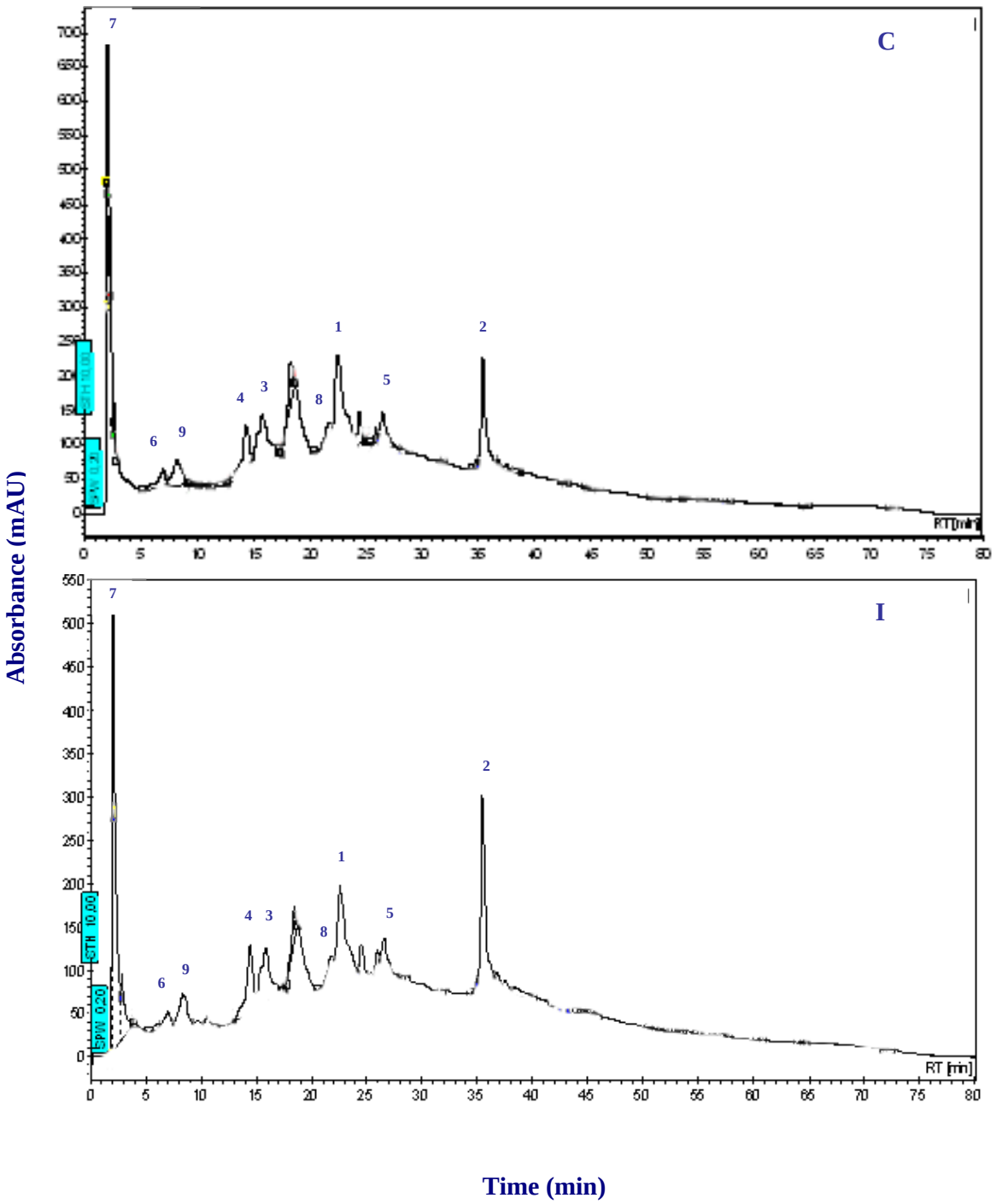


Figure (4.4) Chromatograms of methanolic extract of un-irradiated (C) and 15 KGy-irradiated (I) *Cymbopogon citratus* leaves at wave length 280 nm.

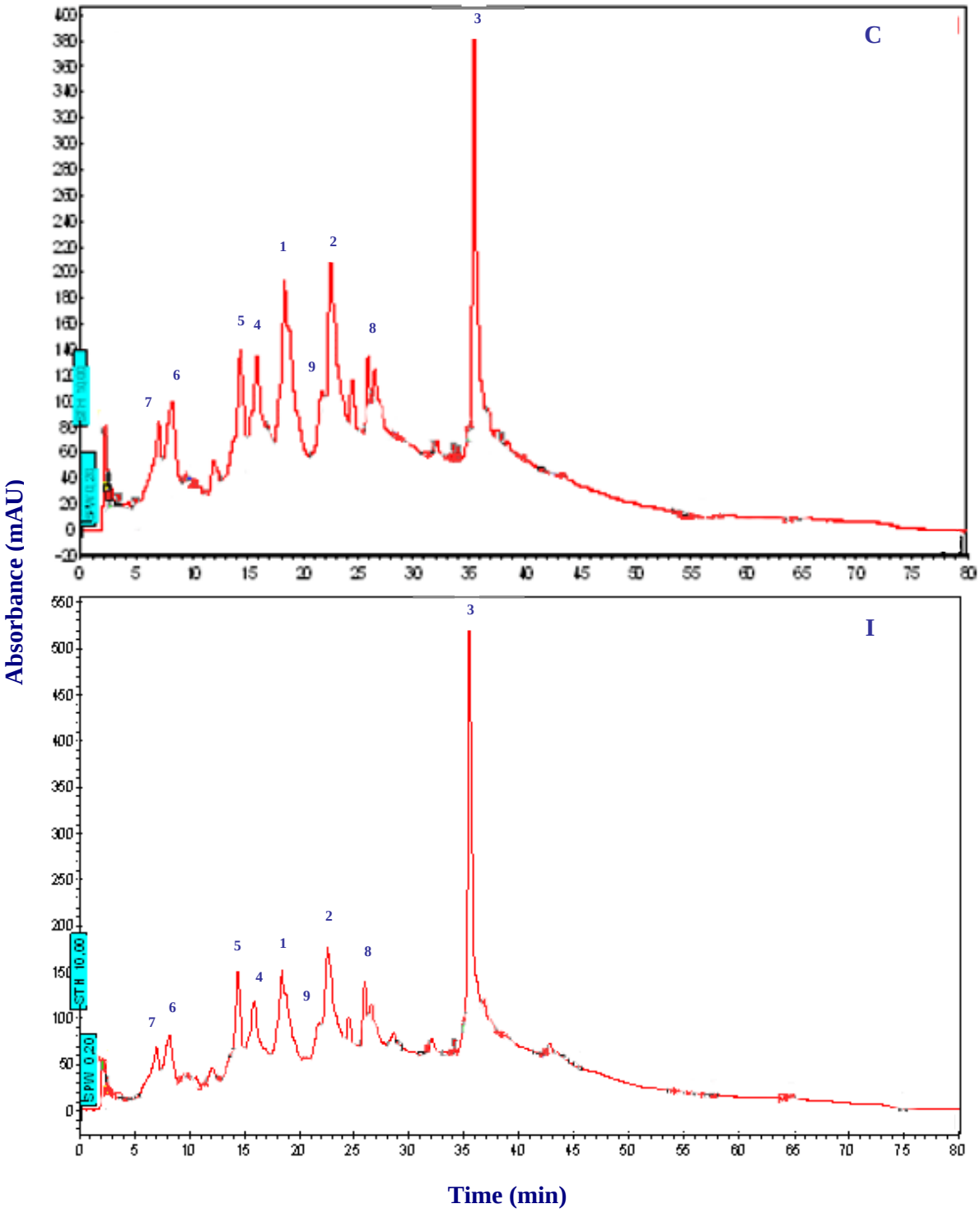


Figure (4.5) Chromatograms of methanolic extract of un-irradiated (C) and 15 KGy-irradiated (I) *Cymbopogon citratus* leaves at wave length 320 nm.

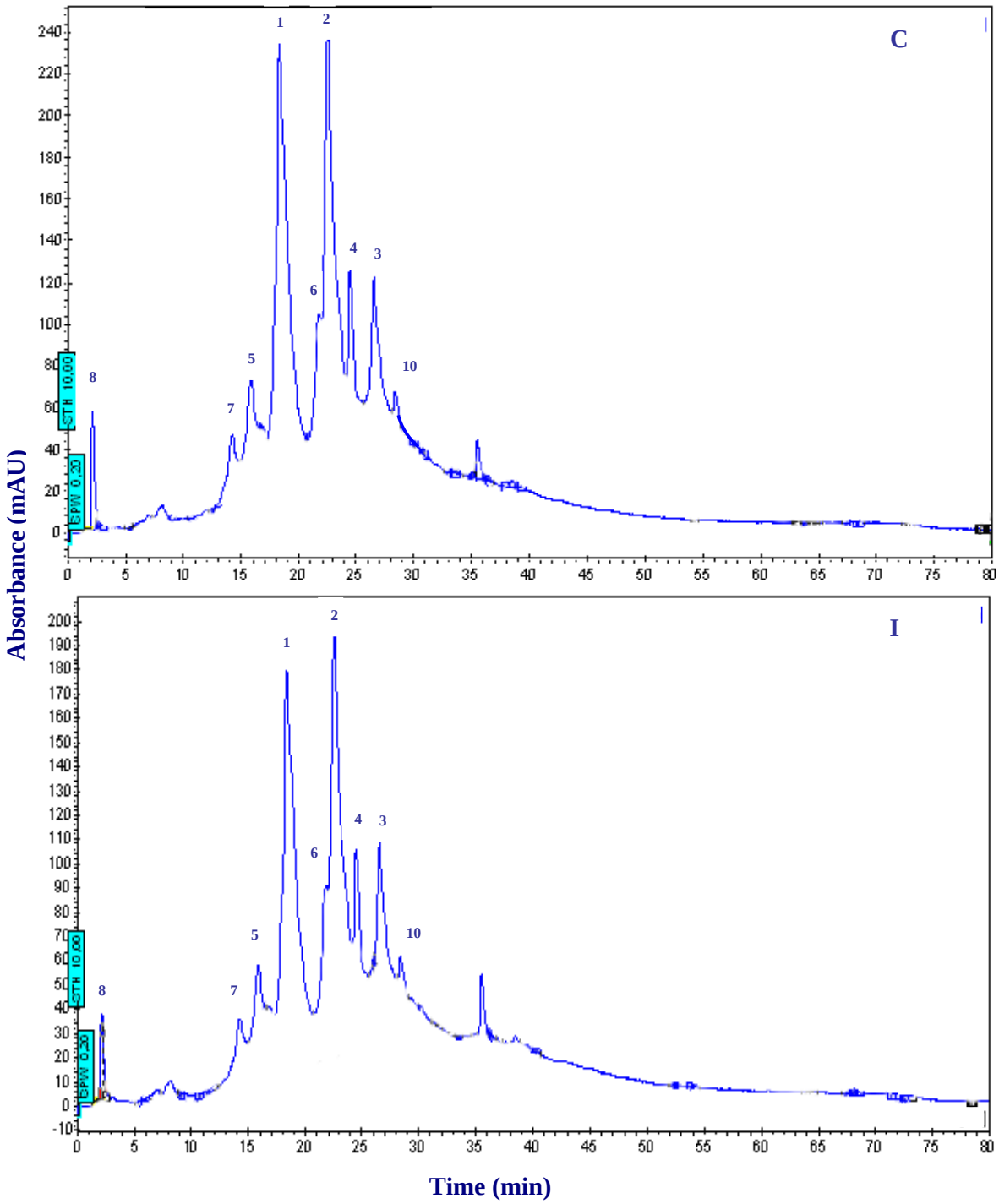


Figure (4.6) Chromatograms of methanolic extract of un-irradiated (C) and 15 KGy-irradiated (I) *Cymbopogon citratus* leaves at wave length 370 nm.

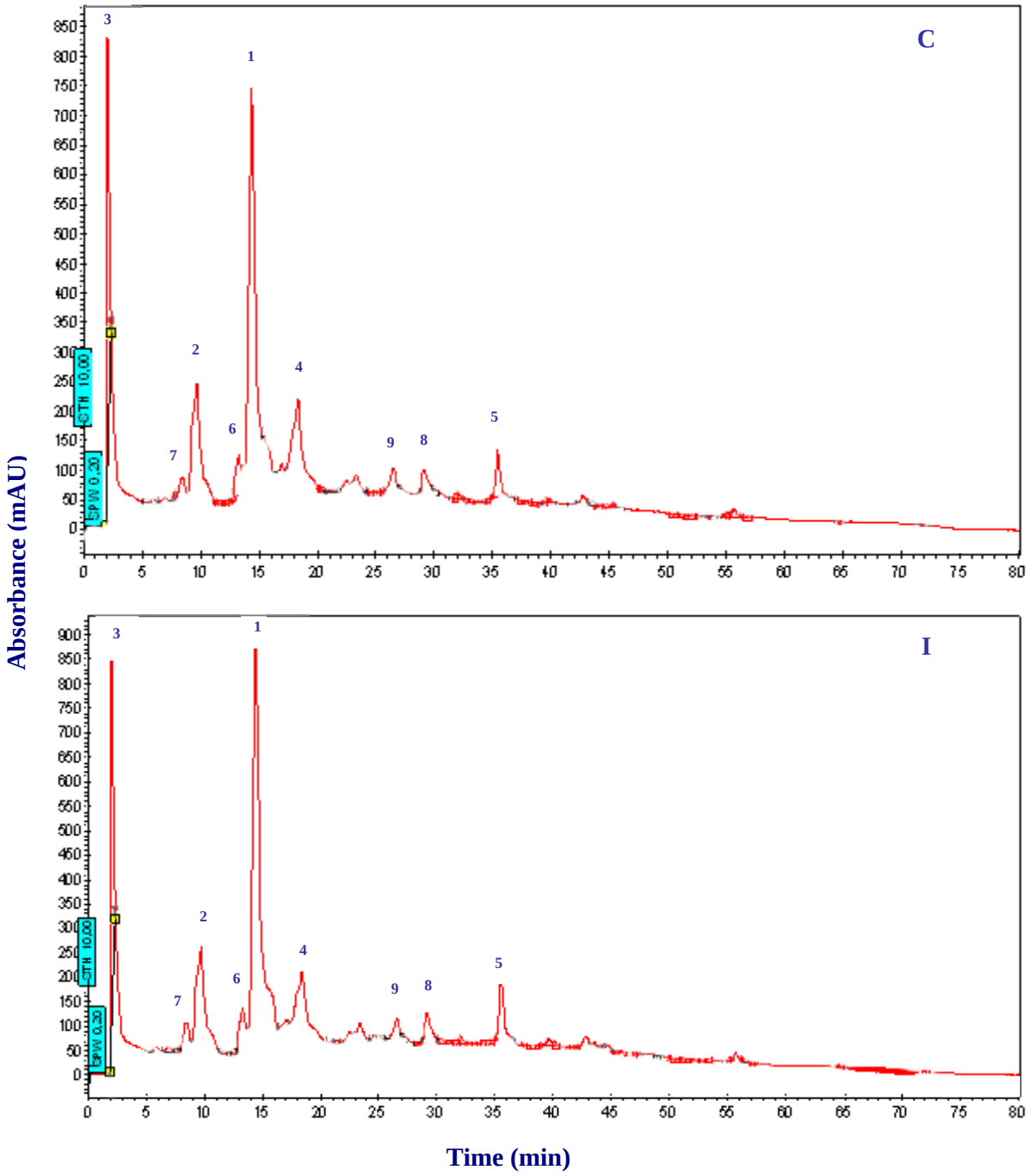


Figure (4.7) Chromatograms of methanolic extract of un-irradiated (C) and 15 KGy-irradiated (I) *Cymbopogon schoenanthus* L. leaves at wave length 280 nm.

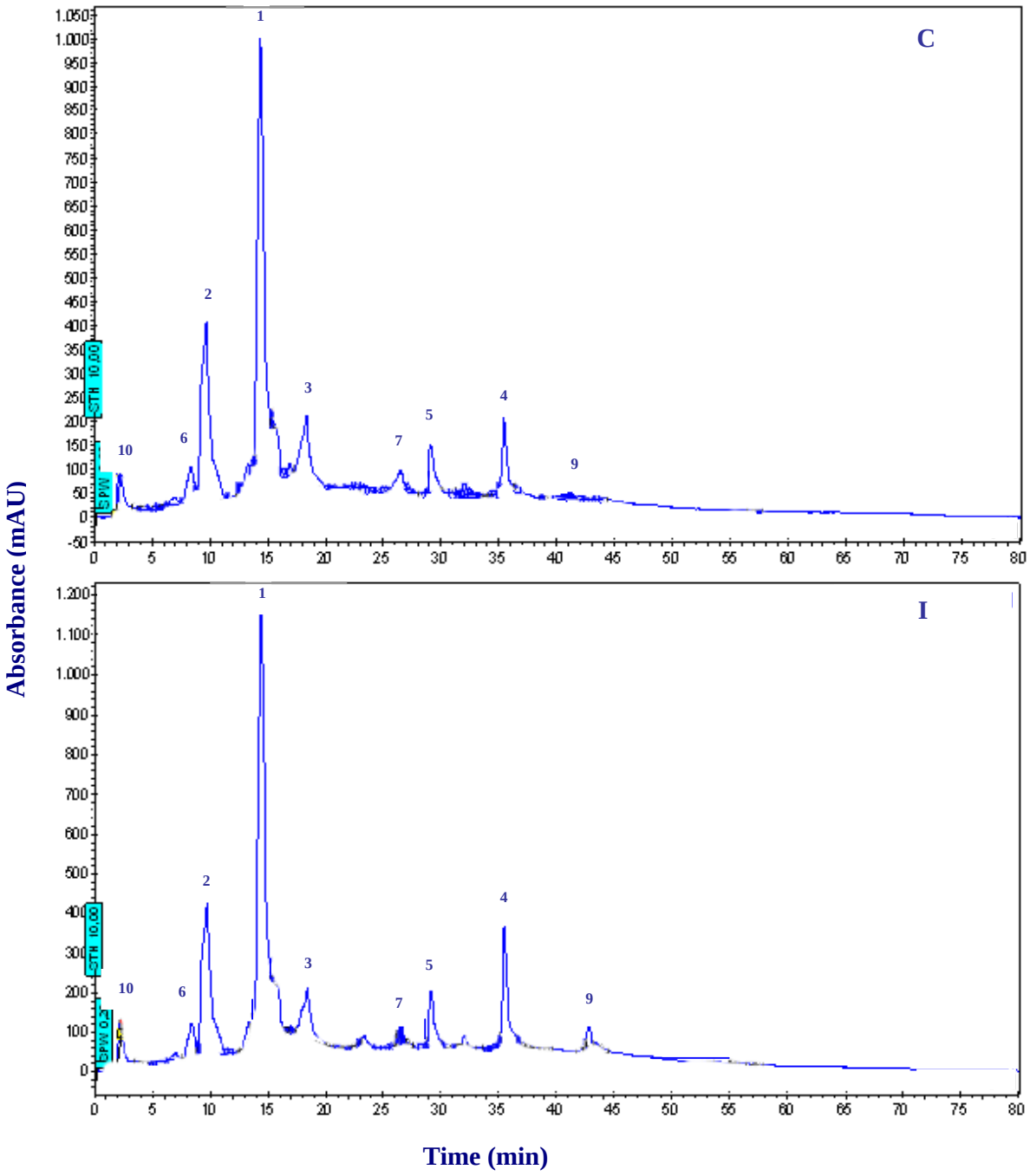


Figure (4.8) Chromatograms of methanolic extract of un-irradiated (C) and 15 KGy-irradiated (I) *Cymbopogon schoenanthus* L. leaves at wave length 320 nm.

4.4 Influence of irradiation at 15 KGY dose on the tannin content

Appendix (2) represents the analysis of variance of mean tannin content (mg/L catechin) of nine plant species irradiated with 15 KGy gamma rays. The maximum reduction was observed in *Cymbopogon citratus* from 1338.6 mg/L catechin to 119.4, indicating that it has been reduced by 91.1% followed by *Cymbopogon schoenanthus* L. from 1144.9 to 225.7 (80.3%), *Cassia senna* L. (leaves) from 570.4 to 417.8 (26.7%), *Acacia nilotica* L. from 667.3 to 490.8 (26.4%) and *Hibiscus sabdariffa* L. from 572.4 to 435.1 (24%).

On the other hand, irradiation with 15 KGy increased the tannin content in *Brassica nigra* L. Koch from 324.1 to 810.4 followed by *Trigonella foenum-graecum* L. from 339.4 to 430.8, *Lepidium sativum* L. from 317.1 to 395.1 and *Cassia senna* L. (pods) from 449.2 to 509.2 (Table 12).

Table (12): Tannin content (mg/L catechin) of nine plant species irradiated with gamma rays.

Species	**Dose KGy**	
	0	**15**
Acacia nilotica L.	667.26bc	490.78cd
		(73.6)
Trigonella foenum-graecum L.	339.41d	430.78cd
		(126.9)
Hibiscus sabdariffa L.	572.35bcd	435.1cd
		(76)
Cassia senna L. (leaves)	570.39bcd	417.84cd
		(73.3)
Cassia senna L. (pods)	449.22c	509.22bcd
		(113.4)
Cymbopogon schoenanthus L.	1144.90a	225.69d
		(19.7)
Cymbopogon citratus	1338.63a	119.41de
		(8.9)
Lepidium sativum L.	317.06d	395.10cd
		(124.6)
Brassica nigra L. Koch	324.12d	810.39b
		(250)

Means with the same following letter in a column or a row are not significantly different, according to Duncan's Multiple Range test.

4.5 Influence of irradiation at 15 KGy dose on the total phenol content

Table (13) represents the effect of irradiation with 15 KGy on the total phenol content (g/L Gallic acid equivalents) of nine plant species.
No significant differences were observed. However, it caused slight increase at phenol content in *Brassica nigra* L. Koch from 16.18 to 16.19 (0.1%) followed by *Cassia senna* L. (pods) from 7.5 to 7.6 (1.3%). The maximum increase was observed in *Cassia senna* L. (leaves) from 7.5 to 12.8 (about 70%) followed by Lepidium *sativum* L. from 15.7 to 19.7 (25.6%) and *Cymbopogon schoenanthus* L. from 10.3 to 12.9 (24.9%).

On the other hand irradiation with 15 KGy reduced the phenol content of *Trigonella foenum-graecum* L. (up to 4.1%) followed by *Hibiscus sabdariffa* L. (5.1%) and *Acacia nilotica* L. (14%). The maximum reduction was observed in *Cymbopogon citratus* from 19.10 to 13.10 (33%). The dose and species interaction had a significant effect (appendix 3).

Table (13): Phenol content (g/L Gallic acid equivalents) of nine plant species irradiated with gamma rays.

Species	**Dose (KGy)**	
	0	**15**
Acacia nilotica L.	15.09c	13.12d (86.9)
Brassica nigra L. Koch	16.18b	16.19b (100.1)
Cassia senna L. (leaves)	7.53f	12.8d (170)
Cassia senna L. (pods)	7.5f	7.61f (101.5)
Cymbopogon citratus	19.10a	13.10d (66.5)
Cymbopogon schoenanthus L.	10.39e	12.98d (124.9)
Hibiscus sabdariffa L.	7.94f	7.5f (94.9)
Lepidium sativum L.	15.72b	19.73a (125.6)
Trigonella foenum-graecum L.	7.79f	7.47f (95.9)

Means with the same following letter in a column or a row are not significantly different, according to Duncan's Multiple Range test.

4.6 Influence of irradiation at 15 KGy dose on the antioxidant activity

Table (14) shows the DPPH scavenging activity changes of the irradiated and non-irradiated plant species. Irradiation at 15 KGy resulted in a slight increase in the DPPH radical-scavenging ability of the extracts of Lepidium sativum (19.34%), Cymbopogon schoenanthus L. (9.22%) and Trigonella foenum-graecum L. (3.25%), which was found to be in-significant when compared to the non-irradiated.

Some species showed in-significant decrease, Cassia senna L. pods (18.04%), Cassia senna leaves (16.57%), Brassica nigra (8.81%), Hibiscus sabdariffa (7.16%) and Cymbopogon citratus (2.3%). Irradiation did not have any effect on the antioxidant potential of Acacia nilotica L.

For all the control samples, the scavenging activity was found to increase with concentrations between 1 and 80 µM (Table 15). After irradiation at 15 KGy, the pattern of change in scavenging activity as a function of concentration was similar in all extracts to that in the control samples. However, the concentration had significant effect on the activity (appendix 4).

The highest activity was found in Acacia nilotica L. followed by Cymbopogon citratus and Lepidium sativum while the lowest activity was found in Cassia senna pods (Table 14).

Regression equations were prepared from the concentrations of the extracts and percentage inhibition of free radical for the plant species, to calculate the extracts concentration needed to cause 50% inhibition (EC_{50}) (figs. 4.9, …, 4.17).

Table (17) represents the EC_{50} for the nine plant species in the range of 30-37 µM. In general the EC_{50} for herbal raw material was found to be 34.47 µM.

Table (14): The DPPH activity of nine plant species irradiated with gamma rays (dose and species interaction).

Species	**Dose KGy**	
	0	**15**
Acacia nilotica L. (A)	82.28a	82.25a
		(0.04)
Brassica nigra L. Koch (D)	51.32cd	46.8d
		(8.81%)
Lepidium sativum L (K)	51.25cd	61.16b
		(19.34)
Cymbopogon citratus (F)	57.3bc	55.97bc
		(2.3)
Cymbopogon schoenanthus L. (G)	48.58d	53.06cd
		(9.22)
Hibiscus sabdariffa L. (I)	42.59de	39.45cf
		(7.16)
Trigonella foenum-graecum L.(P)	33.52f	34.61fg
		(3.25)
Cassia senna L.(leaves) (EL)	36.14efg	30.15gh
		(16.57)
Cassia senna L. (pods) (EP)	30.99gh	25.4h
		(18.04)

Means with the same following letter in a column or a row for dose×species interaction are not significantly different, according to Duncan's Multiple Range test.

Table (15): The DPPH activity of nine plant species irradiated with gamma rays (dose and concentration interaction)

Conc. (µM)	Dose KGy	
	0	**15**
1	16.5e	15.67e
2	19.61e	17.47e
5	32.58d	38.29d
10	50.78c	52.16c
20	68.77b	66.41b
40	75.68a	72.63ab
80	73.92ab	70.92ab

Means with the same following letter in a column or a row for dose×conc. interaction are not significantly different, according to Duncan's Multiple Range test.

Table (16): The DPPH activity of nine plant species irradiated with gamma rays (species, dose and concentration interaction)

conc. (µM)	A	F	G	K	D	P	EP	EL	I
1	84.84 abc	11.5 hi	4.49 i	12.05 hi	11.98 hi	1.6 i	1.19 i	3.21 i	13.9 h
2	84.75 abc	22.9 g	9.92 i	16.27 h	14.05 h	1.02 i	3.81 i	3.66 i	10.5 hi
5	82.56 bc	61.05 d	37.94 f	46.47 e	34.89 f	7.39 i	11.63 hi	12.47 hi	24.53 g
10	86.19 abc	76.33 bcd	58.16 d	69.19 d	48.31 e	25.09 g	26.51 g	35.16 f	38.27 f
20	81.28 bc	72.24 cd	83.67 abc	84.09 abc	77.46 bcd	47.63 e	42.34 e	60.21 d	59.37 d
40	81.29 bc	75.82 bcd	80.52 Bcd	83.74 abc	77.3 bcd	77.62 bcd	54.21 e	64.54 d	72.37 c
80	74.97 bcd	76.61 bcd	82.53 bc	81.63 bc	79.43 bcd	77.92 bcd	57.66 d	52.78 e	68.21 d

Means with the same following letter in a column or a row for species×conc. interaction are not significantly different, according to Duncan's Multiple Range test.

[(A) *Acacia nilotica* L., (D) *Brassica nigra* L. Koch, (K) *Lepidium sativum* L**,** (F) *Cymbopogon citratus*, (G) *Cymbopogon schoenanthus* L., (I) *Hibiscus sabdariffa* L., (P) *Trigonella foenum-graecum* L., (EL) *Cassia senna* L.(leaves), (EP) *Cassia senna* L. (pods)]

Table (17): Extraction concentration (µM) for 50% inhibition of free radical in nine plant species

Herbal raw material	**EC_{50} (µM)**
Acacia nilotica L. (pods)	37.57
Brassica nigra L. Koch (seeds)	32.82
Lepidium sativum L (seeds)	33.61
Cymbopogon citratus (leaves)	30.64
Cymbopogon schoenanthus L. (leaves)	34.28
Hibiscus sabdariffa L. (calyces)	33.24
Trigonella foenum-graecum L. (seeds)	37.32
Cassia senna L. (leaves)	33.56
Cassia senna L. (pods)	37.2
Mean	**34.47**

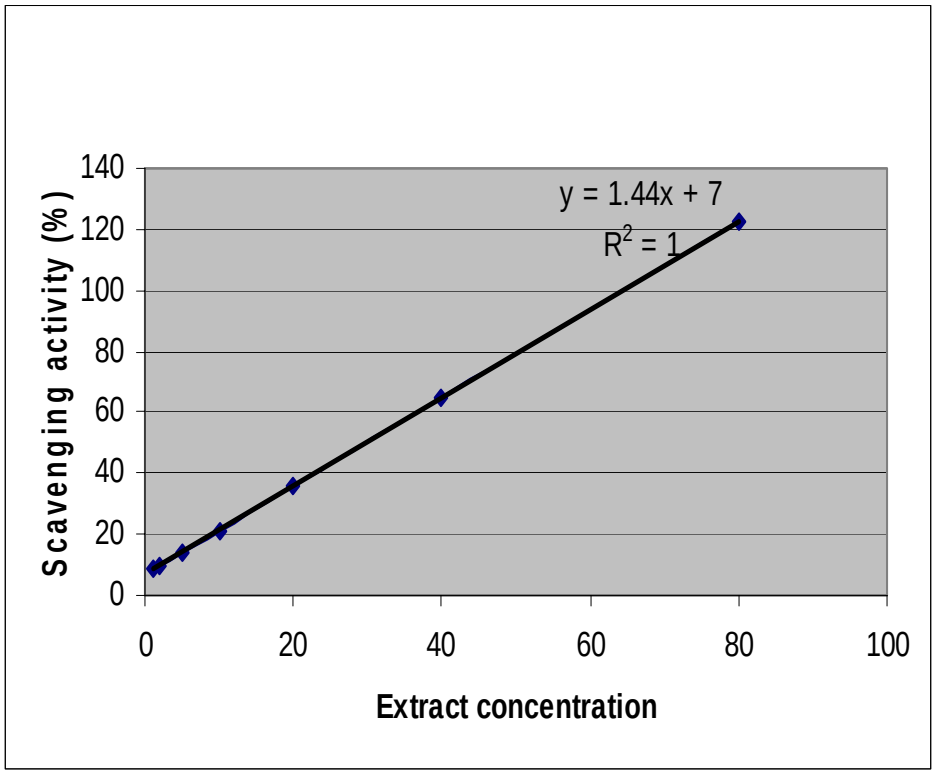


Figure (4.9) Inhibitory effects of Acacia nilotica L. (pods) extracts ranging in concentration between 1 and 80 µM.

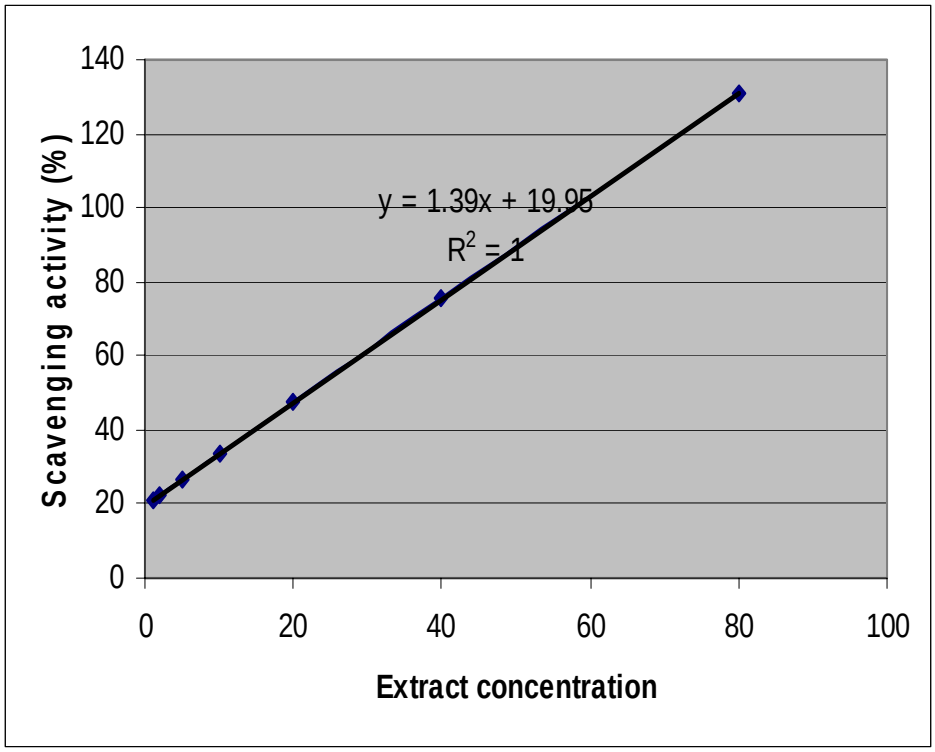


Figure (4.10) Inhibitory effects of Brassica nigra L. Koch (seeds) extracts ranging in concentration between 1 and 80 µM.

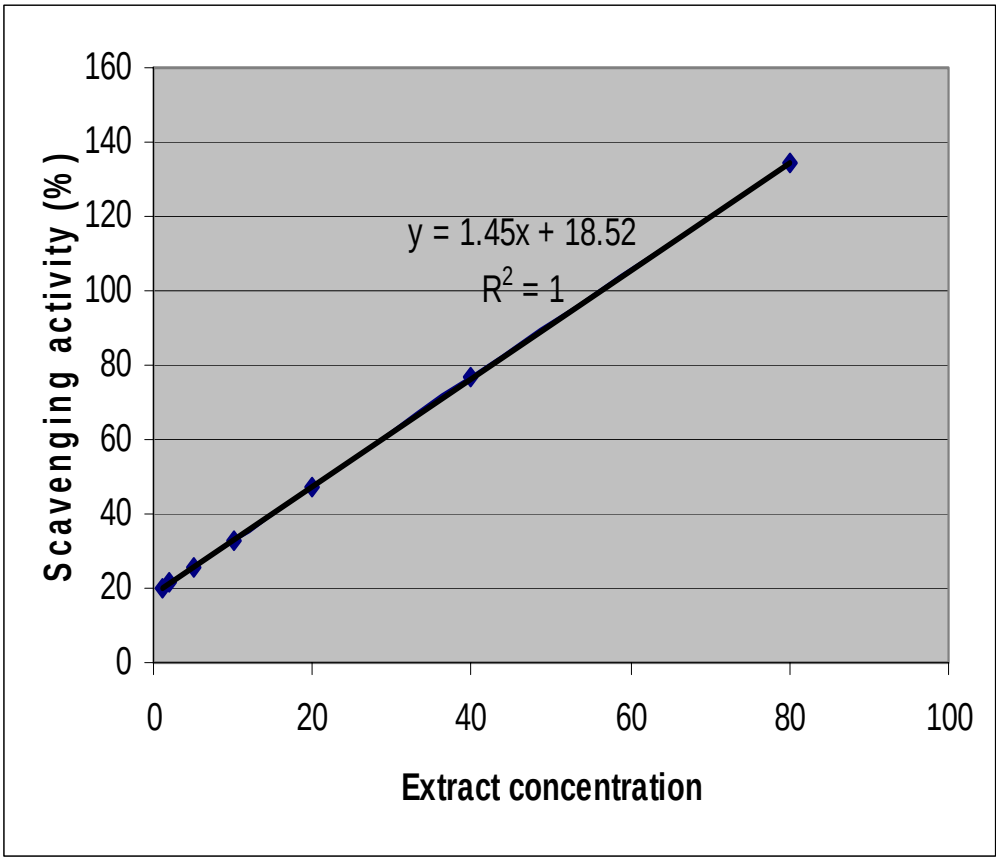


Figure (4.11) Inhibitory effects of Lepidium sativum (seeds) extracts ranging in concentration between 1 and 80 µM.

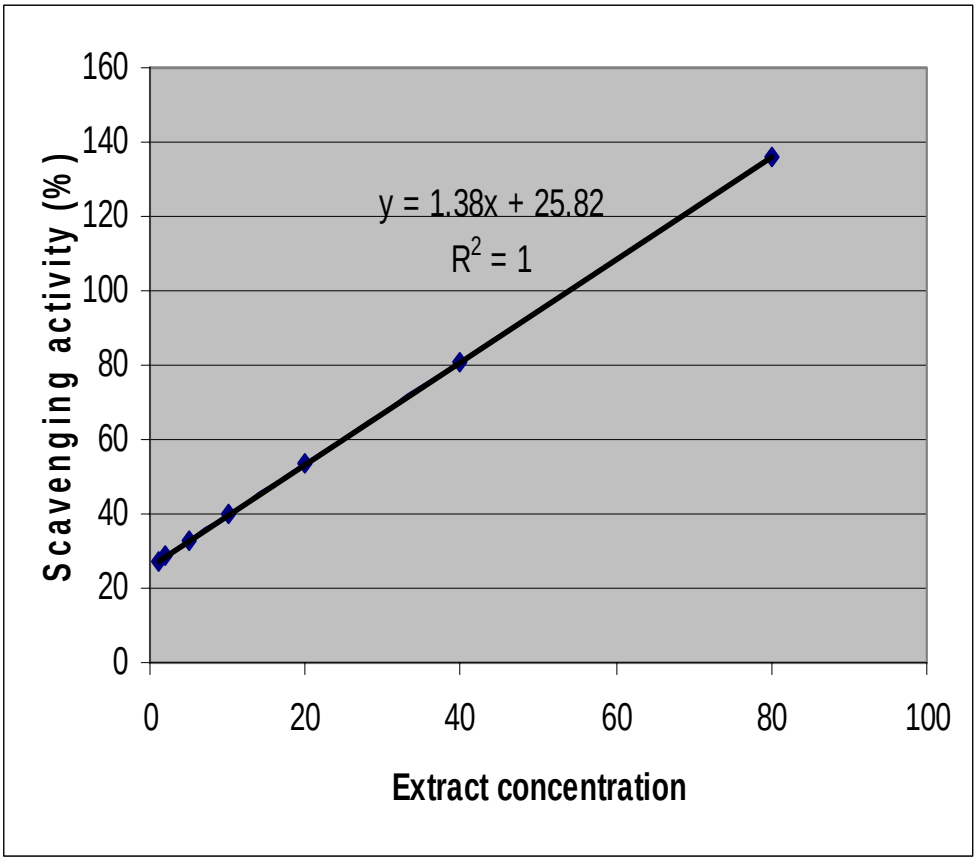


Figure (4.12) Inhibitory effects of Cymbopogon citratus (leaves) extracts ranging in concentration between 1 and 80 µM.

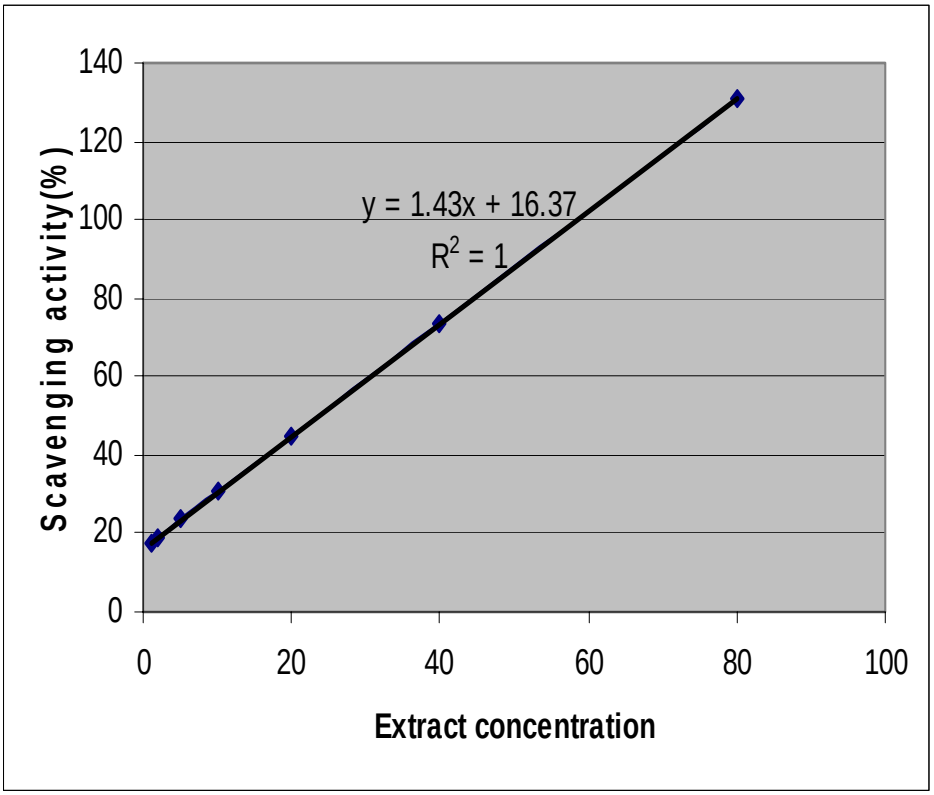


Figure (4.13) Inhibitory effects of Cymbopogon schoenanthus L. (leaves) extracts ranging in concentration between 1 and 80 µM.

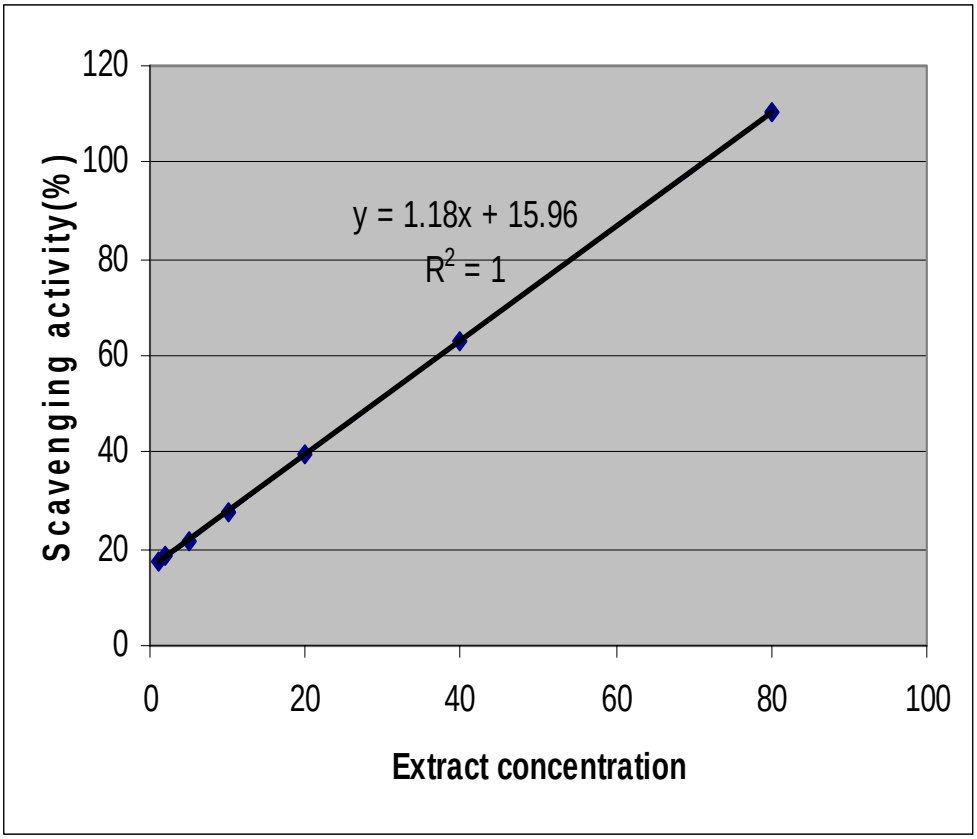


Figure (4.14) Inhibitory effects of Hibiscus sabdariffa L. (calyces) extracts ranging in concentration between 1 and 80 µM.

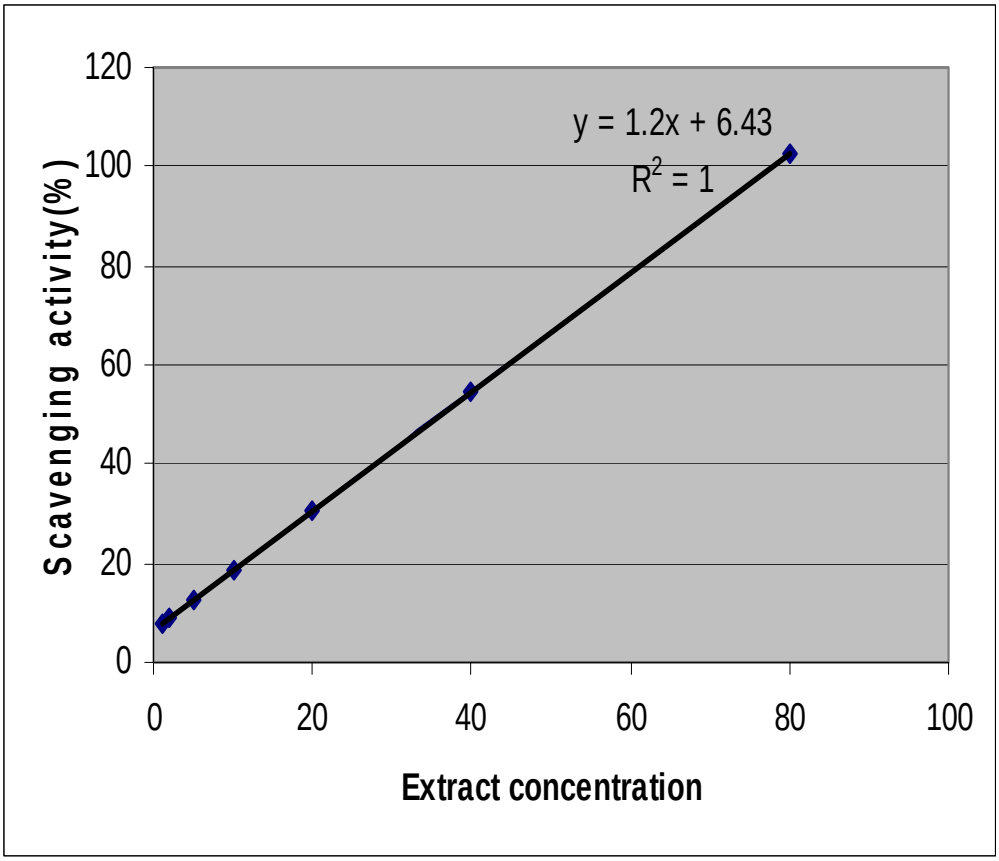


Figure (4.15) Inhibitory effects of Trigonella foenum-graecum L. (seeds) extracts ranging in concentration between 1 and 80 µM.

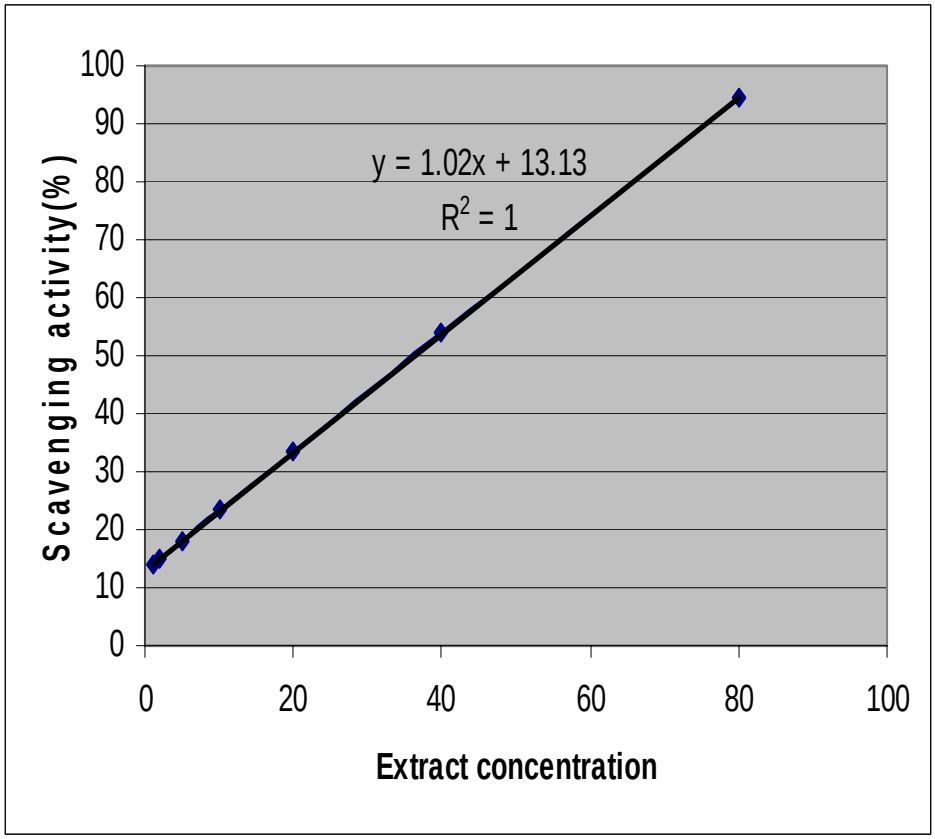


Figure (4.16) Inhibitory effects of Cassia senna (leaves) extracts ranging in concentration between 1 and 80 µM.

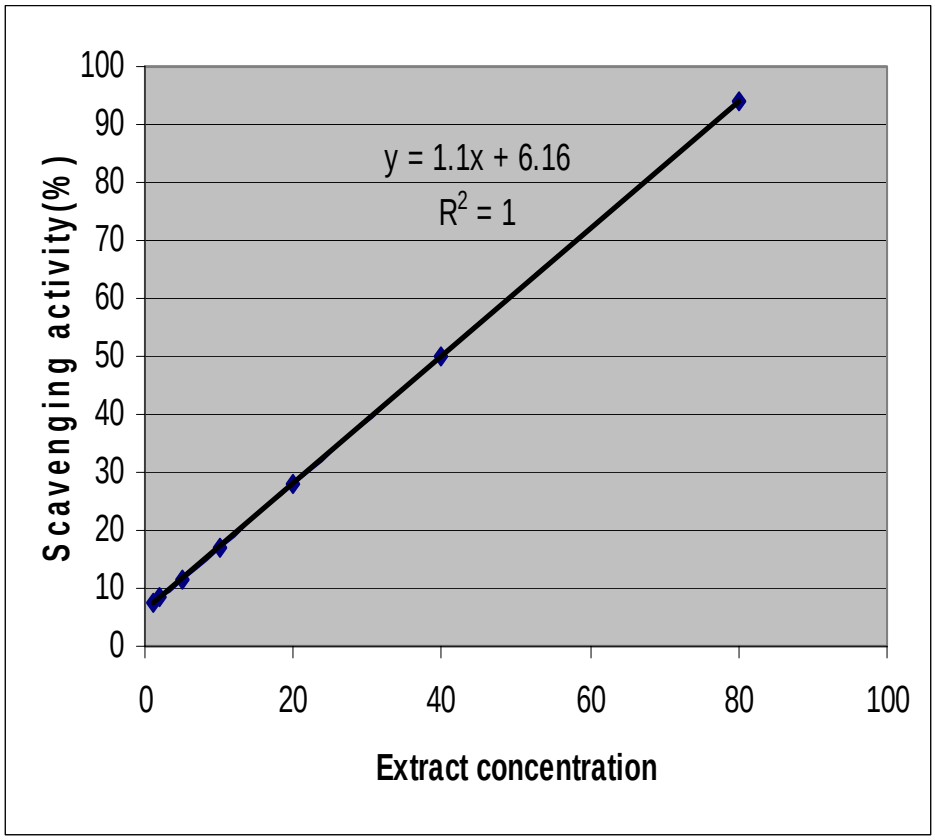


Figure (4.17) Inhibitory effects of Cassia senna (pods) extracts ranging in concentration between 1 and 80 µM.

CHAPTER FIVE

CHAPTER FIVE

DISCUSSION

The results of the bacterial count indicated that the non-irradiated samples of *Trigonella foenum-graecum* L., *Cassia senna* (pods), *Cassia senna* (leaves), *Acacia nilotica L., Brassica nigra* L. Koch, *Lepidium sativum* L., *Cymbopogon citratus and Hibiscus sabdariffa* L. were highly contaminated with bacteria at the levels of 1.44×10^6, 3.0×10^5, 1.2×10^5, 7.0×10^4, 4.0×10^4, 3.5×10^4, 3.0×10^4, 1.3×10^4 CFU/g, respectively. These values exceeded the level of 1.0×10^4 CFU/g reported by WHO (1998) as the maximum permissible total count level. The sample of *Cymbopogon schoenanthus* L. showed a lower count of bacteria (9×10^3 CFU/g), which did not exceeded the accepted value.

The high contamination level could be attributed to the high natural micro flora of the herbs as well as the general conditions during their cultivation, harvesting, drying, handling, processing, storage, distribution and sales. However, it was reported that the microbial status of dried herbal material is not so much caused by secondary contamination during processing, but it is primarily due to the fact that plants have their own microbial flora (Abou Donia, 2008).

The samples irradiated with 5, 10 and 15 KGy of gamma radiation had significantly lower bacterial counts than the non-irradiated (control). The killing effect of radiation can be attributed to the ionization of water which results in forming highly reactive radicals such as H, OH etc. These free radicals split carbon bonds of macromolecules such as DNA in

living organisms, thereby killing the organisms (Abdel-Khalek, 2008). The highest sensitivity to gamma rays at 5 KGy was observed in *Trigonella foenum-graecum* L. (up to 99%) and *Acacia nilotica L.* (97.4%). These findings were in agreement with that of Toofanian and Stegeman (1988) who reported that radiation doses of 6-8 KGy can effectively eliminate bacteria in fenugreek. Swailam and Abdullah (2002) found that radiation dose of 5 and 10 KGy reduced the total bacterial counts of garad by about 49.9 and 80.9%, respectively. At 15 KGy dose *Hibiscus sabdariffa* L. and *Cymbopogon citratus* showed complete absence of microorganisms. Abdel-Karem *et al.* (2002) reported that irradiation at dose level of 5 KGy reduced the total bacterial count of karkade by 97.5%, while complete elimination was achieved at dose level 7 KGy.

The effect of gamma irradiation on the microbial load of *Cassia senna* (pods and leaves), *Cymbopogon citratus, Brassica nigra* L. Koch, *Lepidium sativum* L. and *Cymbopogon schoenanthus* L. was not reported in the literature, but decrease in microbial load of other plant materials following irradiation was reported by several researchers. Mishra *et al.* (2004) conducted a study on the radiation treatment of fresh ginger pieces and reported that a radiation dose of 5 KGy was best utilized for the shelf life extension for a period of more than two months, maintaining superior microbiological quality. Also, a dose of 5KGy was shown to reduce the aerobic populations of aniseeds to an acceptable level (Al-Bachir, 2007). However, gamma radiation at a dose greater than 10 KGy may be required to achieve commercial sterility (i.e. a total aerobic plate counts of <10 per gram), according to IAEA (1992). Chaudry *et al.* (2004) found that a dose of 2 KGy is sufficient to maintain the textural and sensorial

quality, as well as the reduction of bioload of minimally processed carrots for 14 days at 15°C. Wen *et al.* (2006) reported that gamma irradiation at 14 KGy can be a potential method as the cold decontamination for lycium fruit to improve the hygienic quality and to prolong the shelf life. Chattak *et al.* (2009) suggested that chopped lotus rhizomes should be treated with a dose of 4 KGy to keep them microbiologically acceptable for 12 days. Oh *et al.* (2003) studied the irradiation of domestic and imported spices in Korea, and indicated that the effect of gamma irradiation on the microbial population of spices is dependent on the irradiation dose and the type and initial number of microorganisms. They also reported that the irradiation effect on bacterial reduction is relatively linear. Kim *et al.* (2000) found that gamma irradiation at 5–10 KGy inactivated contaminating microorganisms in twenty-one kinds of Korean medicinal herb. Also, Arici *et al.* (2007) reported that a dose of 10 KGy reduced the bacterial count of black cumin to the undetectable limit. Regarding the LD_{50}, significant differences were detected among the nine plant species (fig.4.1). Although the different plant species had different microbial loads, they recorded the same LD_{50} value. A good example is where the microbial load was 700 and 14400 CFU/g on species *Acacia nilotica* and *Trigonella foenum-graecum* L., respectively. The LD_{50} for these two plant species was almost the same, 3.51 KGy for *Acacia nilotica* and 3.41 KGy for *Trigonella foenum-graecum* L. This may indicate that the different evaluated plant species carry different combinations of microorganisms. The high LD_{50} value estimated for *Cymbopogon schoenanthus* L. indicates that the microorganism (s) carried on this plant species were the least sensitive to the applied irradiation treatment. The same pattern was observed for LD_{75} (fig. 4.2), in which the plant species

followed the same arrangement detected in the LD_{50}. According to literature data, enterobacteria are relatively sensitive to irradiation and, in most cases, a dose of about 5 KGy is sufficient for their elimination (Farkas, 1998). Also, Abdel-Khalek (2008) reported that a dose of 5 KGy could be successfully used to reduce the total mesophilic bacteria to the permissible level recommended by WHO. Aziz *et al.* (1997) reported that the bacterial radio-resistance is proportional to the concentration of total lipids in the bacterial cell and can be increased considerably by increasing the number of carbon-carbon double bonds in the membrane lipids.

The maximum reduction in tannin content (mg/L catechin) due to irradiation with 15 KGy was observed in *Cymbopogon citratus* , where it has been reduced by 91.1% , followed by *Cymbopogon schoenanthus* L. (80.3%), *Cassia senna* L. leaves (26.7%), *Acacia nilotica* L. (26.4%) and *Hibiscus sabdariffa* L. (24%). This reduction in the tannin contents is very favorable, since this anti-nutritional factor has the capacity for decreasing protein digestibility. When this anti-nutritional factor is found at the proportion of 5:1 tannin/protein, all protein is precipitated due to the tannin action (Toledo *et al.*, 2007). The reduction is probably due to chemical degradation by the action of free radicals produced by the irradiation. Villavicencio *et al.* (2000), Brigide and Canniatti-Brazaca (2006) found that gamma radiation promoted reduction in the tannin contents as the radiation dose increased.

On the other hand, irradiation with 15 KGy increased the tannin content in *Brassica nigra* L. Koch from 324.1 to 810.4 followed by *Trigonella foenum-graecum* L. from 339.4 to 430.8, *Lepidium sativum* L. from 317.1 to 395.1 and *Cassia senna* L. (pods) from 449.2 to 509.2. The

differences in effect may be attributed to the different phenolic compounds present in the herbs. Hydrolysable tannins, which may be more susceptible to gamma-irradiation compared to the condensed tannin, may explain the increase in tannin contents. Harrison and Were (2007) reported an increase in tannin contents of irradiated clove and nutmeg that have appreciable amounts of hydrolysable tannins compared to the condensed tannins present in cinnamon and other spices.

Irradiation with 15 KGy caused slight increase in phenol content in *Brassica nigra* L. Koch (0.1%) followed by *Cassia senna* L. (pods) (1.3%). However, the maximum increase was about 70% and was observed in *Cassia senna* L. (leaves) followed by *Lepidium sativum* L. (25.6%) and *Cymbopogon schoenanthus* L. (24.9%). On the other hand irradiation with 15 KGy reduced the phenol content of *Trigonella foenum-graecum* L. by 4.1% followed by *Hibiscus sabdariffa* L. (5.1%) and *Acacia nilotica* L. (14%). The maximum reduction was 33% and was observed in *Cymbopogon citratus*. The effect of gamma irradiation on the phenolic content of the plants under study has not been investigated previously, but similar observations on other biological materials were reported.

Adamo *et al.* (2004) reported increase in phenol content for irradiated samples of truffles at the dose level in the 1.0-1.5 KGy and proposed that the destructive process of oxidation and gamma radiation were capable of breaking the chemical bonds of poly phenols, thereby releasing soluble phenols of low molecular weight. Harrison and Were (2007) found increase in total phenolic content of irradiated almond skin extract as compared to that of the control at irradiation levels of 4 KGy

and above. Similarly, Huang and Mau (2007) reported a higher content of phenolics in irradiated than in non-irradiated mushrooms. The dose of 8 KGy promoted an increase in the content of total phenolic compounds in soybean grains, while, a decrease at doses of 2 and 4 KGy, was observed by Toledo *et al.* (2007). Ahn *et al.* (2005) found that gamma irradiation at 1 KGy or above significantly reduced the phenolic contents in the cut Chinese cabbage. In contrast, Mishra *et al.* (2006) described that no significant effect was observed in total phenolics in irradiated tea leaves at 5 KGy.

Irradiation at 15 KGy resulted in a slight increase in the DPPH radical-scavenging ability of the extracts of *Lepidium sativum* (19.34%), *Cymbopogon schoenanthus* L. (9.22%) and *Trigonella foenum-graecum* L. (3.25%) , which was found to be non significant when compared to the non-irradiated.

Some species showed a non-significant decrease, *Cassia senna* L. pods (18.04%), *Cassia senna* leaves (16.57%), *Brassica nigra* (8.81%), *Hibiscus sabdariffa* (7.16%) and *Cymbopogon citratus* (2.3%). Irradiation did not have any effect on the antioxidant potential of *Acacia nilotica* L. Variyar *et al.* (2004) found that in soybean with 0.5-5 KGy of irradiation the content of glycosidic conjugates decreased and that of aglycones increased with increased radiation dose. Accordingly, gamma-irradiation might degrade some antioxidant components or decompose some components into antioxidant components. However, the differences in the effect of gamma irradiation on the free radical scavenging activity in the different plant species may be due to the differences in chemical composition and other characteristics (Chattak *et al.*, 2009).

The highest activity was found in *Acacia nilotica* L. followed by *Cymbopogon citratus* and *Lepidium sativum* while the lowest activity was found in *Cassia senna* pods. In plant tissues many phenolic compounds are potential antioxidants, flavonoids, tannins and lignin precursors may act as ROS (reactive oxygen species) scavenging compounds. It has been reported that observed increase in total phenolics was beneficial for antioxidant properties of soybean seeds due to polymerization of phenolic constituents and also cross linking and fragmentation (Stajner *et al.*, 2007). In the present results, the comparatively high scavenging activity can be correlated to the higher phenolic content. This agrees with reports of Yang *et al.* (2007), who demonstrated a positive correlation between scavenging activity and phenolic contents. In contrast, Zielinski and Kozlowska (2000) indicated negative correlation.

Research studies showed different results for the effect of gamma irradiation on antioxidant properties of plant materials. Chattak *et al.* (2008) showed that gamma irradiation enhanced the scavenging activity of *Nigella sativa*. Harrison and Were (2007) also, found that gamma irradiation of almond skins above 4 KGy in trial 1 and 12 KGy in trial 2, enhanced the antioxidant activity. Likewise, Variyar *et al.* (2004) reported that the scavenging ability of soybean on DPPH radicals increased with the increase in radiation doses in the range 0.5 to 5 KGy. On the other hand, Suhaj *et al.* (2007) reported significant decrease in DPPH radical scavenging activity of black pepper irradiated at doses 5, 7.5, 10, 20 and 30 KGy. Lampart-Szcapa *et al.* (2003) found that lupin rhizome extracts showed decrease in antioxidant activity at doses of 1, 5 and 10 KGy. Ahn *et al.* (2005) also reported that the scavenging ability of Chinese cabbage was reduced after irradiation at 2 KGy. In contrast,

Mishra *et al.* (2006) showed that the free radical scavenging activity of tea was not affected due to radiation treatment at 1, 2, 5 and 10 KGy. Murica *et al.* (2004) evaluated the effect of gamma irradiation on antioxidant properties of seven desert spices (anise, cinnamon, ginger, licorice, mint, nutmeg and vanilla), they found that the irradiated spices at 1, 3, 5 and 10 KGy did not show significant differences of antioxidant activity with respect to the non irradiated. According to a Huang and Mau (2006) report, irradiation of freeze-dried mushrooms at doses between 2.5 and 20 KGy did not show significant modifications in their scavenging activity. Byun *et al.* (2002) observed no significant changes in the scavenging abilities of non-irradiated and irradiated Chungkookjang and Doenjang at 5, 10 and 20 KGy.

The results indicated that no differences in the RF values were noted among the irradiated and un-irradiated samples of herbal material, in the qualitative analysis of flavonoids and glycosides by thin layer chromatography (TLC), as was observed in plate1 and 2. However, obvious quantitative changes were noted in some of the chemical constituents of herbs as affected by gamma irradiation. Breitfellner *et al.* (2002) studied the effect of gamma irradiation on flavonoids in strawberries and reported that, in hydrolyzed samples four phenolic acids (gallic acid, 4-hydroxybenzoic acid, ρ-coumaric acid and caffeic acid) were identified and five flavonoids were detected in non hydrolyzed samples [(+)-catechin, (-)-epicatechin, kaempferol-3-glucoside, quercetin-3-glucoside and quercetin-3-galactoside]. The concentration of 4-hydroxybenzoic acid increased and that of catechins and kaempferol-3-glucoside decreased as irradiation dose increased, whereas those of quercetin-3-glucoside remained unchanged up to a dose of 6 KGy.

Chromatographic analysis of the different studied herbal extracts indicated that although some changes were observed, there were no significant differences in the chemical constitution of the herbs between the irradiated and un-irradiated samples. The response of compounds to irradiation was found to be variable. While the content of several compounds increased after gamma irradiation, the content of some major compounds decreased. Variation in the relative proportion of some of the constituents upon irradiation as observed in the present study could presumably be due to the radiation sensitivity of these compounds at the dose employed (Gyawali *et al.*, 2008).

The results indicated that total elimination of some compounds was observed. Bhat *et al.* (2007) reported that some compounds such as phytic acid in velvet bean seeds (*Mucuna pruriens*) was completely eliminated on exposure to dose of 15 KGy. Decrease or elimination of phytic acid could be probably due to the degradation of phytate to lower inositol phosphates and inositol, by the action of free radicals generated during irradiation. Another possible mode of phytate loss could have been through cleavage of the phytate ring itself (Duodo *et al.*, 1999). Similarly, treatment of broad bean seeds with gamma irradiation reduced the level of phytic acid compared to controls (Al-Kaisey *et al.*, 2003).

The increase in the quantity of some constituents as affected by gamma irradiation may be attributed to the fact that some natural chemical constituents are released from their precursors due to the degradation resulting from irradiation. Harrison and Were (2007) and Adamo *et al. (*2004) reported that phenols are increased by gamma irradiation due to the release of phenolic compounds from glycosidic

components and the degradation of polyphenolic compounds into soluble phenols as well as other small metabolites.

Significant quantitative changes were noted by (Variyar *et al.*, 1998) in some of the phenolic acids in clove and nutmeg upon irradiation with 10 KGY dose. They reported that the content of gallic and syringic acids in irradiated clove was increased, whereas that of ρ-coumaric, ferulic and synapic acids decreased to approximately half that in the control spice and that of caffeic and gentisic acids remained unchanged. In the case of irradiated nutmeg, except for protocatechuic acid and ρ-coumaric acid, which remained unchanged, the content of other phenolic acids showed wide variations compared to that of control samples.

Variyar *et al.* (2004) reported radiation-induced breakdown of glycosides resulting in release of free isoflavones and demonstrated radiation stability of genistein and diadzein. Whereas the content of genistein increased with radiation dose, that of diadzein showed an initial increase at a dose of 0.5 KGy and then decreased at higher doses. Degradation of diadzein beyond 0.5 KGy could thus be assumed.

Elevation of some compounds by gamma irradiation may be attributed to their higher extractability. Some reports indicate that irradiation decreases tannins in bean seeds (Villavicencio *et al.*, 2000). Such differences may be attributed to the differential response and variability in the genetic constituents (strains and varieties). Doses of 7.0 and 10 KGy significantly reduced the tannin content of Shahalla sorghum variety but not that of Hemaira variety (Siddhuraju *et al.*, 2002).

Fan and Gates (2001) found that irradiation of orange juice reduced the concentration of acyclic monoterpenes such as geranial, neral, myrcene and linalool 1 and 7 days after irradiation. The concentrations of monocyclic monoterpenes such as α-pinene, were not influenced by irradiation. Valencene, a bicyclic sesquiterpene was also resistant to irradiation. It appears that the ring structure of terpenes protect these compounds from degradation induced by gamma irradiation.

Also, it was reported that the content of several volatile compounds was increased after γ-irradiation of licorice. In comparison to non-irradiated licorice, 10 KGy dose of γ-irradiation induced the maximum level of total yield of volatile compounds such as 2-ethoxy-1-propanol, ethyl acetate, hexanol, hexanol, [E]-2-tetradecenal, γ-nanolactone, ρ-cymen-8-ol, acetic acid, 2-pentylfuran and α-terpineol (Gyawali *et al.*, 2008).

Gyawali *et al.* (2006) reported that though the content of majority of volatile compounds of dried Welsh onion was increased after different doses of gamma irradiation (1, 3, 5, 10 and 20 KGy), the content of major sulphur-containing compounds such as 1-propanethiol, 5-methyl thiazole, propenyl propyl disulfide, dipropyl disulfide and 3,5-diethyl-1,2,4-trithiolane was decreased after the process. But γ-irradiation up to 20 KGy did not bring qualitative change in the volatile constituents of dried Welsh onion. Sulphur-containing compounds such as dimethyl disulfide, dimethyl trisulfide, methyl propyl disulfide and methyl propyl trisulfide were highly increased by irradiation at 10 KGy. Also, Kim *et al.* (2004) obtained similar results with the same compounds in anchovy sauce at the same dose of gamma irradiation. Lai *et al.* (1994) studied dry shiitake

irradiated from 1 to 10 KGy and concluded that γ-irradiation reduced the content of S-containing compounds.

In a study on nutmeg, Variyar *et al.*, (1998) found that the essential oil constituents showed clear quantitative differences upon gamma irradiation. Thus the content of α-terpeniol, 1-terpinene-4-ol and myristicin was increased in irradiated nutmeg, while that of sabinene, β-pinene and elimicin decreased in comparison to the control sample. Less significant changes in the content of some of the essential oil constituents between the control and irradiated samples were noted in clove and cardamom.

Seo *et al.* (2007) showed that among the essential oils constituents of *Angelica gigas* Nakai, oxygenated terpenes such as α-eudesmol, β-eudesmol and verbenone were increased after irradiation, but their proportions were variable in a dose dependent manner.

The results of the effect of gamma irradiation on the microbiological and chemical quality of the plants under study further support the notion that gamma irradiation process is chemically inert, and could be used for the sterilization of medicinal herbs. Thus, irradiation improves the microbiological safety and maintaining or even enhancing the antioxidant activity. It may emerge as one of the important techniques for preserving or improving the nutritional or pharmaceutical quality in the Sudan.

CHAPTER SIX

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

- Eight medicinal plants were used in this investigation. The total bacterial count indicated that the non-irradiated samples were highly contaminated with bacteria. The sample of *Cymbopogon schoenanthus* L. showed a lower count of bacteria (9×10^3 CFU/g), which did not exceed the acceptable level. There was a significant reduction on the bacterial load in the irradiated samples compared to the un-irradiated.
- No major changes occurred in the metabolite patterns after irradiation and that, therefore, this method for microbial decontamination is suitable for the selected plant species.
- The results indicated that the high dose of 15 KGy of gamma irradiation was the most suitable dose for microbial decontamination of the tested plants. Only *Cymbopogon citratus and Hibiscus sabdariffa* L. achieved commercial sterility (i.e. a total aerobic plate counts of <10 cfu/g).

6.2 Recommendations

- The use of irradiation treatment for microbial decontamination of medicinal plants is to be recommended.
- For most plants tested, doses higher than 15 KGY are recommended for commercial sterility.
- Further investigations to elucidate the effect of gamma irradiation on the pure compounds of the plants are needed.
- Further investigations to elucidate the effect of gamma irradiation on the other biological activities are needed.

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APPENDIX

Appendix (1): Analysis of variance of total bacterial load (cfu×10^2) on nine medicinal plant species irradiated with different doses of gamma ray.

Source	D.F.	Sum of squares	Mean of squares	F-value	F-tabulated 5%	1%
Treatment	35	616843244.3	17624092.69			
Dose	3	101506922.6	33835640.87	2549.22**	2.76	4.1
Species	8	131844239.8	16480529.98	1241.66**	2.10	2.79
Dose species interaction	24	647180561.5	26965856.73	2031.64**	1.70	2.1
Error	72	955651.66	13272.93972			

Ns: non-significant

*, **: significant at 5% and 1%, respectively.

Appendix (2): Analysis of variance of tannin content of nine plant species irradiated with gamma rays.

Source	**D. F.**	**Sum of squares**	**Mean of squares**	**F-value**	**F-tabulated**	
					5%	**1%**
Treatment	17	4728642.4	262702.4			
Dose	1	595025.9	595025.9	17.30**	4.12	7.3
Species	8	740152.17	92519	2.69*	2.22	3.11
Dose×species interaction	8	4873768.7	609221.1	17.71**	2.22	3.11
Error	36	1238156	34393.2			
Total	53	5966798.5				

Ns: non-significant

*, **: significant at 5% and 1%, respectively.

Appendix (3): Analysis of variance of total phenol content of nine plant species irradiated with gamma rays.

Source	**D. F.**	**Sum of squares**	**Mean of squares**	**F-value**	**F-tabulated**	
					5%	**1%**
Treatment	17	954.23	56.13			
Dose	1	1.2	1.2	13.95**	4.12	7.3
Species	8	806.91	100.9	1173.26**	2.22	3.11
Dose species interaction	8	146.12	18.26	212.33**	2.22	3.11
Error	36	3.1	0.086			
Total	53	5966798.5				

Ns: non-significant

*, **: significant at 5% and 1%, respectively.

Appendix (4): Analysis of variance of mean DPPH activity of nine plant species irradiated with gamma rays.

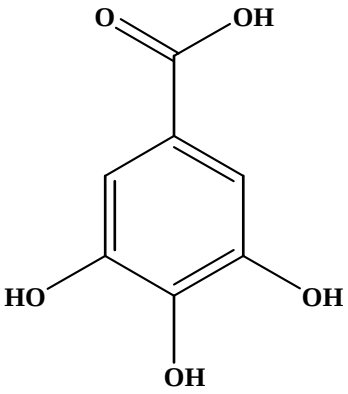
Source	D. F.	Sum of squares	Mean of squares	F-value	F-tabulated	
					5%	1%
Treatment	125	347965.1				
Dose	1	45.15	45.15	0.35 ns	3.81	6.63
Species	8	91612.05	11451.51	89.17 **	1.94	2.51
Concent.	6	201647.06	33607.84	261.68 **	2.11	2.80
Dose×sp. interaction	8	256307.9	32038.49	249.46 **	1.94	2.51
Dose×Conc. Interaction	6	146272.89	24378.82	189.82 **	2.11	2.80
Sp.×Con. interaction	48	54705.99	1139.71	8.87 **	1.53	1.77
Dose×sp.×C. interaction	48	54660.84	1138.77	8.87 **	1.53	1.77
Error	252	32365	128.43			
Total	377	380330.1				

- ns: non-significant

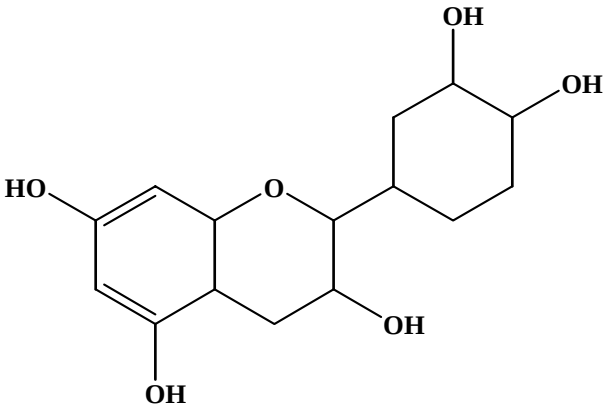
- *, **: significant at 0.05 and 0.01 levels of significance, respectively.

Appendix (5): Chemical structure of bioactive components reported in *Acacia nilotica* L.

Gallic acid

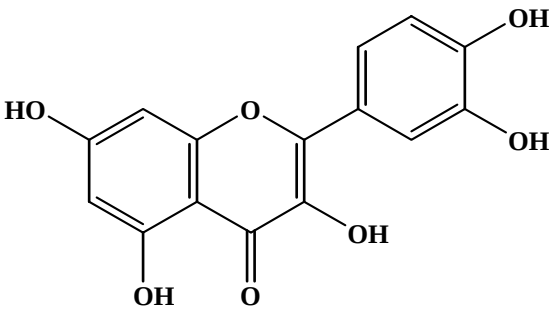


Catechin

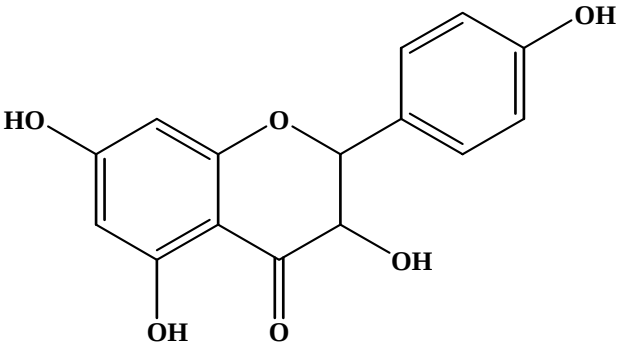


Appendix (6): Chemical structure of bioactive components reported in *Brassica nigra* Koch.

Quercetin

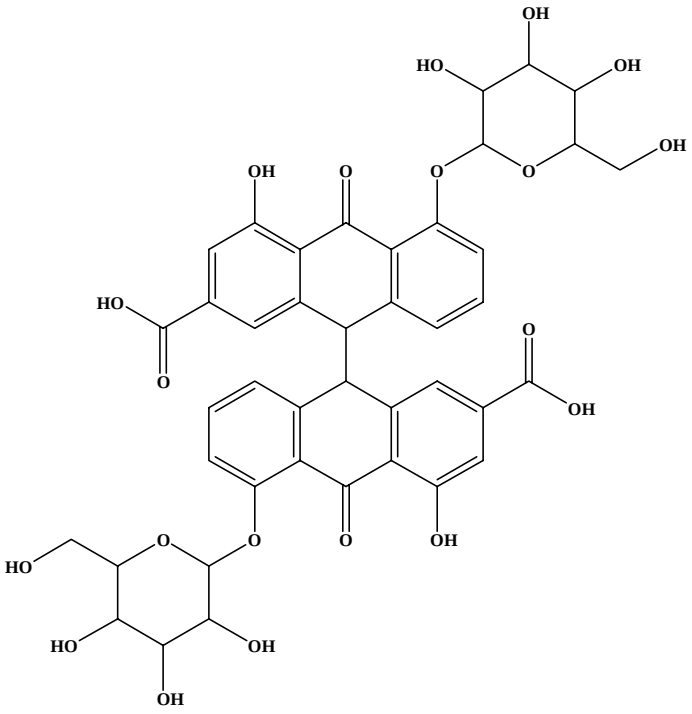


Kaempferol



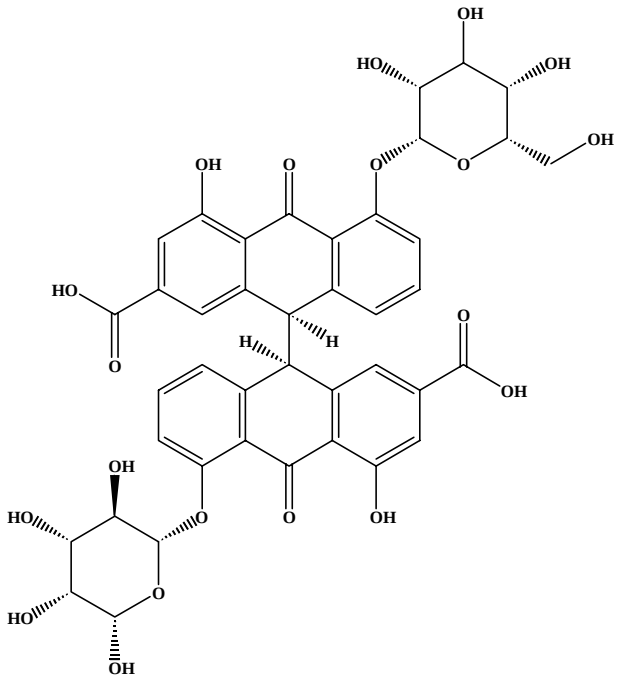
Appendix (7): Chemical structure of bioactive components reported in *Cassia senna* L.

Sennoside A



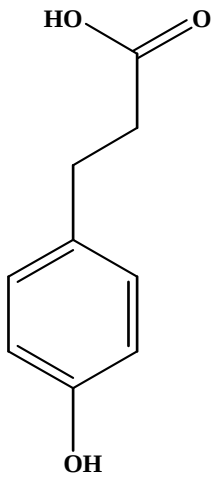
Cont. Appendix (7): Chemical structure of bioactive components reported in *Cassia senna* L.

Sennoside B

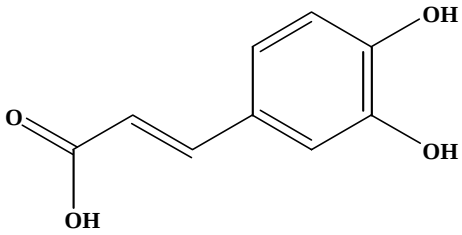


Appendix (8): Chemical structure of bioactive components reported in *Cymbopogon citratus*

p-Coumaric acid

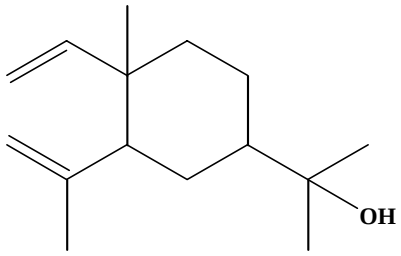


Caffeic acid

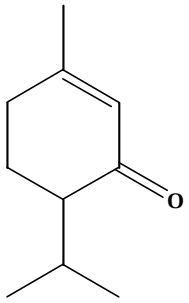


Appendix (9): Chemical structure of bioactive components reported in *Cymbopogon schoenanthus* L.

Elemol

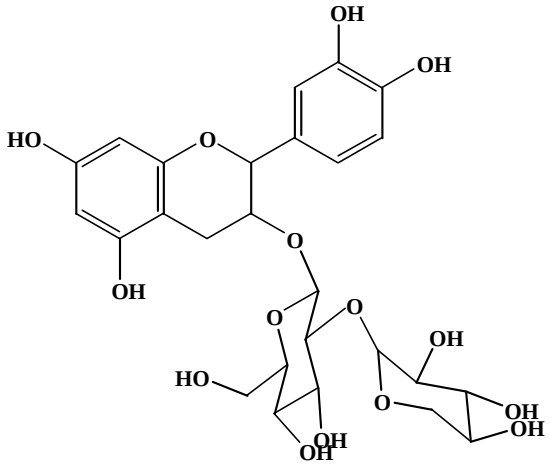


Piperitone

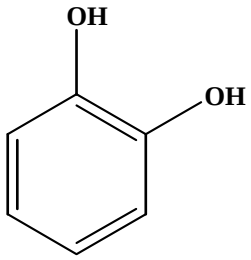


Appendix (10): Chemical structure of bioactive components reported in *Hibiscus sabdariffa*

Cyandin 3-sambubioside (Anthocyanin)

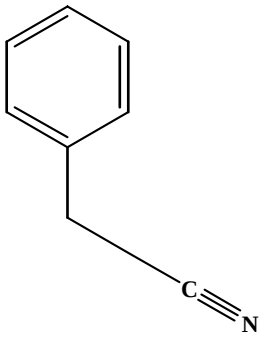


Catechol

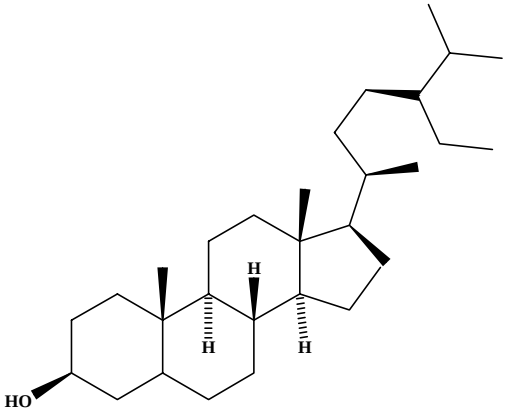


Appendix (11): Chemical structure of bioactive components reported in *Lepidium sativum* L.

Phenylacetonitrile

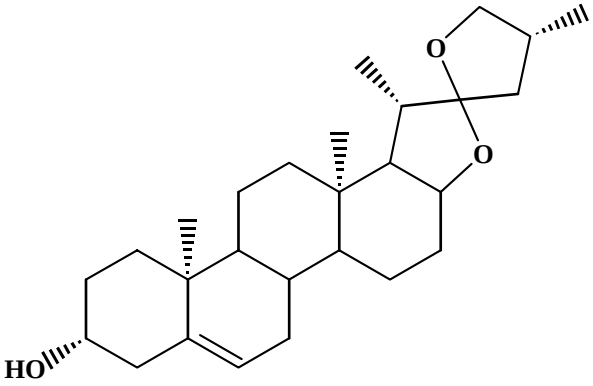


Stigmat 5-en-3



Appendix (12): Chemical structure of bioactive components reported in *Trigonella foenum- graceum* L.

Diosgenin (Saponin)



Neochlorogenic acid