

Full Length Research Paper

Preliminary studies on decontamination of some dried herbal products by gamma irradiation

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Seven dried herbal products (DHP) were decontaminated using gamma radiation. The microbial loads (total viable count, TVC) of the raw and irradiated products were determined using the methods of serial dilutions and pour plate. Based on international standards for microbial load, the effective decontamination doses were determined for the DHP. The range of TVC for the DHP was 10^5 to 10^9 cfu/g. Milled roots of *Cryptolepis sanguinolenta* and milled stems and leaves of *Desmodium adscendens* had the highest counts of 8.0×10^8 and 2.0×10^9 cfu/g, respectively. Powdered seeds of *Moringa olifera* and *Grifonia simplicifolia* and the seeds of *Voacanga africanus* had relatively low TVCs of 6.4×10^5 , 6.6×10^6 and 1.3×10^6 cfu/g, respectively. Irradiation with medium doses of 2.5 to 7.5 kGy reduced microbial loads of the DHP by 3 to 6 log cycles. A dose of 10 kGy reduced the microbial load by 4 to 7 log cycles and a dose of 15 kGy eliminated viable cells from all the DHP. Effective decontamination doses for the DHP were estimated to range from 2.5 to 10.0 kGy. Decontamination using gamma irradiation can improve the microbial quality and enhance the safety of DHP for both the domestic and export markets.

Key words: Dried herbal products, gamma irradiation, microbial decontamination, microbial load.

INTRODUCTION

Traditional medicines involving the use of herbal items, animal parts and minerals have been used by mankind for thousands of years (Kaptchuk, 2000; Kloss et al., 1939; Lewis 1984; Unschuld, 1985). These medicines are indispensable to many communities due to their accessibility and affordability (Orwa, 2002). According to the World Health Organisation (1998), about 70 to 80% of the world population, particularly in the developing countries, relies on non-conventional medicines mainly of herbal sources. In Ghana, traditional medicines are widely used by approximately 60 to 70% of the population in rural areas (Botwe, 1999). The Centre for Scientific

Research into Plant Medicine has identified approximately 1,000 medicinal plants in Ghana, 40 of which are used in the treatments of 33 diseases such as asthma, malaria, jaundice, typhoid fever, diabetes, hypertension and anaemia (The Centre for Scientific Research into Plant Medicine (CSRPM), 2000).

In recent times, the use of herbal products has increased globally due to their effectiveness, low toxicity and minimal side effects (Fang and Wu, 1998). The economic benefit of herbal products is immense. Over-the-counter sales of herbal medicines is reported to be more than US\$ 5 billion world-wide and the rural economy

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dealing with these products is also worth an estimated amount of US \$ 600 million (Anon, 2001).

Despite the huge economic potential and health benefits, there is now growing concern about the purity and quality of herbal products in most countries. The products undergo long periods of crude storage and are frequently contaminated with microorganisms due to a lack of professional expertise on the part of traditional healers, collectors or producers. Quite high bacterial populations and the presence of potential toxigenic fungi have been reported for some local herbal teas (Owusu and Odamtten, 1999). Elsewhere, microbiological deterioration has been detected in herbal products in Nigeria (Okunlola et al., 2007) and dehydrated ginseng in Korea (Kwon, 1991; Lee, 1989; Fang and Wu, 1998).

In order to meet internationally accepted standards, herbal products should have acceptable microbial quality. Traditionally, sterilisation techniques using steam, methyl bromide, ethylene oxide and phosphine have been used to decontaminate herbal products (IAEA, 2008; European Pharmacopoeia, 2002; United States Pharmacopoeia, 1995). Continual use of these techniques has been limited in many respects due to their inherent drawbacks. Steam sterilisation leads to loss of volatile and/or thermo-sensitive components, methyl bromide depletes the ozone layer and ethylene oxide as well as phosphine are now considered as carcinogens and environmental toxicants (Jacobs, 1995; Boess and Boegl, 1996; Ahmed, 1991). The use of gamma radiation in the decontamination of dehydrated materials such as herbs and spices is well documented (Eiss, 2001; IAEA, 1992; Farkas, 1988; ASTM, 1998). Studies in many countries have reported the potential of ionizing radiation in reducing microbial loads of a variety of herbal medicines to acceptable levels (Hilmy et al., 1981; Migdal and Owczarczyk, 1998; Fang and Wu, 1998). There is therefore the need to explore the use of gamma irradiation as a decontaminating method by the herbal products industry in Ghana. This could improve the hygienic quality and enhance the suitability of the products for both the local and international markets.

The objective of this study was to investigate the use of gamma irradiation in the decontamination of some local dried herbal products.

MATERIALS AND METHODS

Samples

Seven samples of dried herbal products used for the study were obtained from products submitted by some pharmaceutical companies and herbal products enterprises for contract irradiation at the Radiation Technology Centre (RTC) of Ghana Atomic Energy Commission. The samples were selected based on their widespread use in treating ailments such as malaria, fevers,

cancers, hypertension, depression, asthma and rheumatism (CSRPM, 2000). Selection was also based on their potential for export and therefore the consequent need to improve their microbiological quality to meet international standards.

Milled roots of *Cryptolepis sanguinolenta*;
Milled leaves of *Lippia multiflora*;
Milled stems and leaves of *Desmodium adscendens*;
Powdered seeds of *Moringa olifera*;
Powdered seeds of *Grifonia simplicifolia*;
Seeds of *Grifonia simplicifolia*;
Seeds of *Voacanga africanus*.

Irradiation

Ten grammes of each product was packaged in a polyethylene pouch and sealed using a heat sealer (Heat Sealer, Desk Type: 300 m/m, Taiwan). The pouches were treated with medium irradiation doses of 2.5, 5.0, 7.5 and high irradiation doses of 10.0, 12.5 and 15.0 kGy at the RTC of Ghana Atomic Energy Commission using a Co^{60} source (SLL-515, Hungary) at a dose rate of 2.55 kGy/hr in air. The absorbed dose was confirmed by Fricke's dosimetry.

Determination of microbial load

After irradiation, both controls and irradiated products were analysed for their microbial load using the methods of serial dilution and pour plate. Five grammes of each sample was added to 45 ml peptone water (1% peptone + 0.5% NaCl) and placed on a mechanical shaker (Junior Orbital Shaker, Lab-line Instruments, USA) for 15 min. The mixture was then allowed to settle for about 5 min to allow coarse material to settle down. Microbial load determination was carried out on the supernatant by estimating the total viable counts (TVC) on Plate count agar (Oxoid, UK) at 36°C for 48 h using a colony counter (Stuart Scientific, UK) according to the methodology of APHA (1976). The TVC gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mold in the samples. For each product, the average of three estimations of duplicate plating was carried out, the mean microbial load and the range of the microbial load was determined.

RESULTS

As shown in Table 1, the range of microbial load (TVC) for the raw or unirradiated dried herbal products was 6.4×10^5 to 2.0×10^9 cfu/g. Milled roots of *C. sanguinolenta* and milled leaves and stems of *D. adscendens* had high counts of 8.0×10^8 and 2.0×10^9 cfu/g, respectively. Powdered seeds of *M. olifera* had a relatively low mean count of 6.4×10^5 with a range of 4.0×10^4 to 8.6×10^5 .

Irradiation with medium doses from 2.5 to 7.5 kGy reduced mean microbial loads of the dried herbal products by 3 to 6 log cycles (Table 2). A dose of 10 kGy reduced mean microbial load of all the herbal products by 4 to 7 log cycles (Table 3). In the case of powdered seeds of *M. olifera* and *G. simplicifolia* as well as whole seeds of *G. simplicifolia* and *V. africanus*, an irradiation

Table 1. Mean and range of microbial loads of dried herbal product samples.

| Dried Herbal Product | ^a Mean microbial load | ^a Range of microbial load |
|--------------------------------------------------------|----------------------------------|------------------------------------------|
| Milled roots of <i>Cryptolepis sanguinolenta</i> | ^b 8.0×10 ⁸ | 7.4×10 ⁸ –9.1×10 ⁸ |
| Milled leaves of <i>Lippia multiflora</i> | 1.0×10 ⁶ | 4.0×10 ⁴ –3.0×10 ⁶ |
| Milled stems and leaves of <i>Desmodium adscendens</i> | 2.0×10 ⁹ | 1.3×10 ⁸ –2.8×10 ⁹ |
| Powdered seeds of <i>Moringa olifera</i> | 6.4×10 ⁵ | 4.0×10 ⁴ –8.6×10 ⁵ |
| Powdered seeds of <i>Grifonia simplicifolia</i> | 6.6×10 ⁶ | 5.0×10 ⁴ –9.0×10 ⁵ |
| Seeds of <i>Grifonia simplicifolia</i> | 1.1×10 ⁷ | 4.0×10 ⁵ –1.2×10 ⁷ |
| Seeds of <i>Voacanga africanus</i> | 1.3×10 ⁶ | 1.0×10 ⁵ –1.4×10 ⁶ |

^aTotal viable cells (cfu/g); ^bvalues are means of triplicate samples.

Table 2. Mean and range of microbial load of dried herbal products after gamma irradiation at medium doses.

| Dried herbal product | ^a Microbial load | | |
|--------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
| | 2.5 kGy | 5.0 kGy | 7.5 kGy |
| Milled roots of <i>Cryptolepis sanguinolenta</i> | ^b 9.0×10 ^{6c} (8.5×10 ⁵ –9.5×10 ⁶) | 1.8×10 ⁵ (5.0×10 ⁵ –1.5×10 ⁶) | 1.0×10 ⁴ (7.0×10 ³ –1.2×10 ⁴) |
| Milled leaves of <i>Lippia multiflora</i> | 2.0×10 ⁵ (9.0×10 ⁴ –3.0×10 ⁵) | 5.0×10 ⁴ (9.0×10 ³ –9.5×10 ⁴) | 4.0×10 ³ (8.×10 ² –6.0×10 ³) |
| Milled stems and leaves of <i>Desmodium adscendens</i> | 6.0×10 ⁷ (5.0×10 ⁶ –9.5×10 ⁷) | 5.0×10 ⁶ (2.5×10 ⁶ –8.0×10 ⁶) | 4.0×10 ⁵ (9.0×10 ³ –2.0×10 ⁵) |
| Powdered seeds of <i>Moringa olifera</i> | 1.3×10 ⁴ (1.0×10 ³ –2.0×10 ⁴) | 3.5×10 ³ (2.5×10 ³ –6.0×10 ³) | 3.6×10 ² (<10–6.0×10 ²) |
| Powdered seeds of <i>Grifonia simplicifolia</i> | 3.7×10 ³ (2.0×10 ³ –3.5×10 ³) | 3.0×10 ² (<10–4.0×10 ²) | ^d <10 |
| Seeds of <i>Grifonia simplicifolia</i> | 1.6×10 ⁵ (9.0×10 ³ –8.0×10 ⁵) | 7.5×10 ³ (5.5×10 ³ –9.0×10 ³) | 1.8×10 ² (<10–3.0×10 ²) |
| Seeds of <i>Voacanga africanus</i> | 2.2×10 ³ (1.6×10 ² –3.0×10 ³) | 1.0×10 ² (<10–3.2×10 ²) | <10 |

^aTotal viable cells (cfu/g); ^b values are means of triplicate samples; ^c range of microbial load, ^dViable cells not detected, limit of detection = 10 cfu/g.

dose of 10 kGy completely eliminated all viable cells from the samples. With the exception of milled roots of *C. sanguinolenta* and milled leaves of *D. adscendens*, a dose of 12.5 kGy completely eliminated all viable cells from the dried herbal products. A dose of 15 kGy eliminated viable cells from all the dried herbal products.

Generally, irradiation of all the samples of dried herbal products with medium and high doses

resulted in approximately 3 to 7 log cycles reduction in the microbial loads (TVC) as compared to those of the control or unirradiated samples.

DISCUSSION

One of the biggest challenges of the herbal pro-

ducts industry is the lack of standardization in production protocols, resulting in products of varying qualities. This is largely due to the fact that the quality of herbal products is usually dependent on the professional expertise of the traditional healers, collectors or producers of the products. Effective drying, storage and packaging are critical stages in production protocols which determine the quality of the dried herbal products.

Table 3. Mean and range of microbial load of dried herbal products after irradiation at high doses.

| Dried herbal product | ^a Microbial load | | |
|--------------------------------------------------------|-------------------------------------------------------------------------------------------|------------------------------------------------|------------------|
| | 10.0 kGy | 12.5 kGy | 15.0 kGy |
| Milled roots of <i>Cryptolepis sanguinolenta</i> | ^b 4.0×10 ³ ^c (2.6×10 ² –5.4×10 ³) | 1.0×10 ² (<10–1.6×10 ²) | ^d <10 |
| Milled leaves of <i>Lippia multiflora</i> | 4.0×10 ² (5.0×10 ² –3.0×10 ²) | <10 | <10 |
| Milled stems and leaves of <i>Desmodium adscendens</i> | 2.0×10 ³ (5.0×10 ² – 3.5×10 ³) | 2.0×10 ² (<10–3.0×10 ²) | <10 |
| Powdered seeds of <i>Moringa olifera</i> | <10 | <10 | <10 |
| Powdered seeds of <i>Grifonia simplicifolia</i> | <10 | <10 | <10 |
| Seeds of <i>Grifonia simplicifolia</i> | <10 | <10 | <10 |
| Seeds of <i>Voacanga africanus</i> | <10 | <10 | <10 |

^aTotal viable cells (cfu/g); ^b values are means of triplicate samples; ^c range of microbial load. ^dViable cells not detected, limit of detection = 10 cfu/g.

The microbial loads of the dry herbal products recorded in this study were generally high and had a wide range (10⁵ to 10⁹ cfu/g). The observed differences in microbial loads recorded in this study could be due to factors such as differences in indigenous microflora, lack of standard processing procedures, contamination and defective packaging. Although these values compare well with total bacterial counts of 10⁵ to 10⁸ cfu/g reported in herbal teas (Wilkinson and Gould, 1996), they are clearly higher than the range of 10⁴ to 10⁵ cfu/g reported for some local herbal teas (Owusu and Odamitten, 1999) and the range of 10² to 10⁴ cfu/g reported for herbal medicines in Nigeria (Okunlola et al., 2007).

International standards for herbal raw materials require that total aerobic bacteria and fungi should not exceed 10⁵ and 10³ cfu/g or ml, respectively (European Pharmacopoeia, 2007; WHO, 1998). However, the microbiological criteria for local herbal materials state that TVC should be less than 10⁶ cfu/g (Ghana Standards, 1997). Since herbal raw materials are utilized in a manner similar to raw food, their microbial criteria should be of a higher standard to ensure a safety margin for these products. Significantly, all the dried herbal products assessed in this study did not meet the criteria of the international and local standards in terms of their microbial loads with the exception of the powdered seeds of *M. olifera*.

Fortunately, decontaminating the products using gamma radiation helped in meeting the required standards. Irradiation is one of the few processes that allow the attainment of high standards for foods and medical products through the destruction of bacteria and other microorganisms. The primary mechanism by which radiation kills microorganisms is by splitting water molecules into hydrogen (H⁺), hydroxyl (OH[•]) and oxygen (O^{2•}) radicals. These radicals react with and destroy or deactivate microbial components such as DNA, proteins and cell membranes (Niemira and Sommers, 2006; IAEA,

1982). Irradiation has the capacity to improve the hygienic quality and extend the shelf-life of various products thus enhancing their competitiveness on both domestic and export markets. From the results, the effective irradiation doses for reducing microbial loads of the samples to acceptable international standards were estimated as:

1. Powdered seeds of *G. simplicifolia* and *M. olifera* and seeds of *V. africanus* 2.5 kGy.
2. Milled leaves of *L. multiflora* and whole seeds of *G. Simplicifolia* 5.0 kGy.
3. Milled roots of *C. Sanguinolenta* 7.5 kGy.
4. Milled stems and leaves of *D. Adscendens* 10.0 kGy.

In comparison with the control or unirradiated samples, results of this study has shown that irradiation of the dried herbal products with medium to high doses considerably reduced their microbial loads to acceptable national and international standards. However, a wide range of effective irradiation doses (2.5 to 10.0 kGy) was required for decontamination of the dried herbal products to acceptable standards. This observation might be due to non-uniformity of the microbial loads and that underlines the need for good manufacturing practices (GMPs) in production protocols to ensure low microbial load of products and subsequently the use of low (effective) irradiation doses. It is noteworthy that 15 kGy eliminated all contaminating microflora in all the dried herbal products. A careful examination of the results further indicates that while a dose of 12.5 kGy reduced the microbial loads of all the samples to < 10³ cfu/g, 10 kGy reduced the microbial loads to < 10⁴ cfu/g. In a related study, irradiation doses between 7.5 to 10.0 kGy reduced the microbial load of a range of herbal tea and products to less than 10³ cfu/g without any significant changes to the quantity and composition of volatile oils (Farkas, 1988). Studies by Migdal and Owczarczyk (1998) also

indicated a dose of 10 kGy effectively decreased microbial load of raw herbal products by 6 log cycles to 8.6×10 cfu/g. Similarly, a dose of 2 to 3 kGy reduced mycoflora population in some local herbal teas by 4 to 5 log cycles (Owusu and Odamtten, 1999).

Ghana, as a tropical country, has a rich biodiversity including 3,600 plant species of which over 2,900 are indigenous (MES, 2002). The country therefore has a high potential for production of herbal products considering the fact that an estimated 33 and 27% of drugs worldwide are derived, respectively from higher plants and lower plants/microbes (Essegbey, 2002). However, for the country to break into the global market for dry herbal products, it is important to improve and modernize the herbal medicine industry. Production of high quality herbal products through the use of irradiation could guarantee the country a huge export market just as in most Asian countries.

It is recommended that future studies should investigate the radiosensitivities of the different bacterial and fungal isolates of dried herbal products with the view to their elimination with low irradiation doses. Additionally, the impact of irradiation on the active phytochemicals of dry herbal products should be investigated.

Conclusion

The microbial load of dried herbal products is quite high probably due to deficiencies in production protocols. It is important to integrate GMP into production protocols of dried herbal products so as to generally improve their quality. Gamma irradiation effectively reduced the microbial loads of dried herbal products to acceptable national and international standards. The utilization of gamma irradiation by the herbal products industry could improve quality and guarantee access to lucrative global markets.

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