

Effects of Cobalt-60 Gamma on Microbial Elimination and Phytochemical
Constituents in *Orthosiphon aristatus* (Misai Kucing) (Blume) Miq.
(Kesan Gamma Kobalt-60 terhadap Penghapusan Mikrob dan Konstituen Fitokimia pada *Orthosiphon aristatus*
(Misai Kucing) (Blume) Miq.)

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ABSTRACT

Medicinal plants are used for various purposes, however, the presence of microorganisms in them is the main safety risk. The study aimed to evaluate the effects of gamma irradiation on microbial contaminants and phytochemical constituents of *Orthosiphon aristatus* (Blume) Miq. The plant was irradiated using doses of 3, 6, 9, and 12 kGy and the microbial contamination was assessed using phenotypic and genotypic analyses. The qualitative screening using chemical tests was performed to identify the presence of important phytochemical constituents including alkaloids, saponins, flavonoids, tannins, steroids and triterpenes. Results showed that the total microbial counts in *O. aristatus* were significantly reduced ($P < 0.05$) following irradiations at 3- and 6 kGy. Pathogenic bacteria were not detected in *O. aristatus* after irradiation at 6 kGy while the phytochemical constituents were conserved. In conclusion, gamma irradiation has significantly reduced and eliminated microbial contaminants and preserved the phytochemical constituents of *O. aristatus*. This study highlights the use of a low and specific dose, 6 kGy that is effective to reduce and eliminate microbial contaminants in *O. aristatus*.

Keywords: Cobalt-60; gamma irradiation; medicinal plants; microbial contaminants; *Orthosiphon aristatus*; phytochemical constituents

ABSTRAK

Tumbuhan ubatan telah digunakan untuk pelbagai tujuan, namun, kandungan mikroorganisma dalam tumbuhan tersebut merupakan risiko keselamatan utama. Kajian ini bertujuan untuk menilai kesan sinaran gamma terhadap kandungan mikrob dan kandungan fitokimia dalam *Orthosiphon aristatus* (Blume) Miq. Blume (Miq.). Tumbuhan tersebut telah didedahkan kepada sinaran gamma menggunakan dos iaitu 3, 6, 9 dan 12 kGy dan kandungan mikrob telah dinilai menggunakan analisis fenotip dan genotip. Ujian kualitatif menggunakan ujian kimia telah dijalankan untuk menentukan kandungan fitokimia utama termasuklah alkaloid, saponin, flavonoid, tannin, steroid dan triterpin. Keputusan menunjukkan bahawa jumlah mikrob dalam *O. aristatus* telah berkurang secara ketara ($P < 0.05$) selepas penyinaran pada 3- dan 6-kGy. Bakteria patogen tidak dikesan dalam *O. aristatus* selepas penyinaran pada 6 kGy, sementara kandungan fitokimianya dapat dikekalkan. Sebagai kesimpulan, penyinaran gamma telah dapat mengurangkan dan menghapuskan kandungan mikrob dan mengekalkan kandungan fitokimia dalam *O. aristatus*. Kajian ini memfokuskan penggunaan dos yang rendah dan khusus iaitu 6 kGy yang berkesan untuk mengurangkan dan menghapuskan kandungan mikrob dalam *O. aristatus*.

Kata kunci: Kandungan fitokimia; kandungan mikrob; kobalt-60; *Orthosiphon aristatus*; sinaran gamma; tumbuhan ubatan

INTRODUCTION

Medicinal plants are widely used for medicinal purposes against various diseases, with 80% of the worldwide population still relying on these herbal medicines for their healthcare needs (Jamshidi-Kia, Lorigooini & Amini-Khoei 2018). They are relevant in healthcare sectors across many regions including Europe, South America, Asia, and Southeast Asia and have gained increasing attention due to their antimicrobial, pharmacological and chemical properties (Abdel-Wahab et al. 2011; Ncube et al. 2008). As they are rich in bioactive constituents, this leads them to be commercialized not only as alternative medicines but also as food supplements, cosmetics, flavour enhancers and fragrances (Mohd-Hafizudin et al. 2019; Selvamohan, Ramadas & Kishore 2012).

In Europe, medicinal plants such as *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) are widely commercialized as food flavours and health supplements (CBI, 2019). Similarly, Southeast Asia shows an increasing trend of medicinal plant commercialization into health supplements and herbal drinks (Narayanaswamy & Ismail 2015). Malaysia on the other hand, has several commonly commercialized medicinal plants including *Orthosiphon aristatus* (Misai Kucing), *Labisia pumila* (Kacip Fatimah), *Eurycoma longifolia* (Tongkat Ali), *Ficus deltoidea* (Mas Cotek) and *Clinacanthus nutans* (Belalai Gajah) (Ahmad et al. 2015). These plants are available in the market as energy drinks, anti-ageing creams, whitening serums, wound-healing gels, and herbal teas (Mohd-Hafizudin et al. 2019).

One of the plants, *O. aristatus* (Blume) Miq. which is popular among the European local community as Cat's whiskers and *Misai Kucing* in Malaysia is an herbaceous plant from the Lamiaceae family and widely distributed in Southeast Asia and Australia (Chung et al. 2020). This plant consists of white or purple flowers, leaves, stems, and roots. Among these, the leaves are mostly utilized and have been used to treat diabetes, kidney stones, nephritis, arteriosclerosis, rheumatism, hypertension, menstrual disorder, tonsillitis, epilepsy, edema, fever, hepatitis, and jaundice (Abdullah et al. 2020). The healing properties of *O. aristatus* are attributable to the existence of phytochemical contents such as phenolics, saponins, terpenoids, caffeic acid, sinensetin and eupatorin which are known to possess anti-inflammatory, antimicrobial, antioxidant, antihypertensive, and antidiabetic (Abdullah et al. 2020;

George et al. 2015). Thus far, clinical investigations on the effectiveness of *O. aristatus* in treating diseases are somehow limited, however, *in-vivo* studies showed that aqueous extracts of *O. aristatus* at 200 - 1000 mg/kg exhibited hypoglycemic and antihyperglycemic effects where it reduced the plasma glucose levels and stimulate the production of insulin (Ashraf, Sultan & Adam 2018). Apart from that, the methanol extract of *O. aristatus* possessed a significant anti-inflammatory and analgesic activity after 3 to 5 hours of oral administration at 500 - 1000 mg/kg dose (Abdullah et al. 2020). Overall, *O. aristatus* extracts are unlikely to cause toxicity with average doses of 200 - 5000 mg/kg/day (Abdullah et al. 2020; Yam et al. 2013).

Despite societies' claims that herbal-based products are safer and more effective, outbreaks of foodborne diseases and health problems such as Salmonellosis, gastroenteritis and infectious diarrhoea have been linked to the consumption of contaminated herbs or their products. According to prior reports, several medicinal plants contained high levels of microbial contaminants ($> 10^8$ CFU/g) and were contaminated with pathogens such as *Salmonella* spp., *Escherichia coli*, *Bacillus cereus*, *Shigella* spp., and *Staphylococcus aureus* (Canadian Food Inspection Agency 2019; Fakruddin et al. 2017; Kamal et al. 2018). Here, the safety of herbal products in terms of their microbiological aspect is of utmost importance. It is essential to conduct microbiological quality tests; the Total Aerobic Microbial Counts (TAMC), Total Yeast and Mould Counts (TYMC) and test for specified microorganisms using selective media. The microbiological quality specifications must be complied in which in an herbal product, the TAMC and TYMC must not exceed 10^3 and 10^2 CFU/g, respectively, and the product must be free from pathogens such as *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella* spp. (European Pharmacopoeia 2010; WHO 2012). The products' stability must also be considered by evaluating the microbial contents for at least 6 months to ensure the quality is maintained throughout storage (NPRA 2016).

In an attempt to produce safer and higher quality herbal products, a new preservation method known as cobalt-60 gamma irradiation is introduced. This technology employs low temperatures (< 30 °C) to reduce microbial contaminants in foods, extend the shelf-life and improve food security. The International Atomic Energy Agency (IAEA), World Health Organization (WHO) and Food and Agricultural Organization (FAO) have confirmed that food irradiation does not give rise to

any toxicological, microbiological or nutritional hazards (Maherani et al. 2016; Morehouse & Komolprasert 2004). To date, gamma irradiation up to a maximum dosage of 10 kGy is applicable in 60 countries to decontaminate various types of foods such as herbs, spices, vegetables, fruits and meats (FDA 2016; Khawory et al. 2020). The Food and Drug Administration (FDA) has also approved a range of doses below 30 kGy to be used on various herbs and spices, though this is subject to the countries' authority. In Malaysia for instance, gamma irradiation doses of less than 15 kGy are suggested to be applied for the elimination of spoilage microorganisms and to extend the shelf life of food (Malaysian Standard 2005).

The implementation of gamma irradiation in the food industry, however, has raised some concerns, especially on the cost. About USD3 million is needed for the application of food sterilization. The costs depend on the applied dose, irradiation design and dose uniformity, for instance, USD15 and USD250 are needed for a low- and high-dose application, respectively (Kume & Todoriki, 2013). Currently, Asian countries have more than 100 000 tons of irradiated foods, while Malaysia has about 785 tons in 2010 and

these numbers are estimated to increase over the years (Kume & Todoriki 2013). Undoubtedly, irradiation can give many advantages, especially in food quality and safety, therefore the countries that widely use this sterilization technique should consider cost-effective procedures including low irradiation doses. In this regard, the effectiveness of cobalt-60 gamma irradiation to reduce microorganisms and conserve its phytochemical constituents was assessed for 6 months of storage.

MATERIALS AND METHODS

SAMPLES COLLECTION AND PREPARATION

Fresh *O. aristatus* (Figure 1) were collected between February and June 2018 at TKC Herbal Nursery, Pusat Pertanian Pantai, Jelebu, Negeri Sembilan, Malaysia (GPS Coordinate: 2.769781648090106, 101.99573735619676). The leaves were rinsed with distilled water and subjected to air drying for 2 h. The dried leaves were subsequently grinded into powder form and 50 g of the dried raw powder was packaged individually in capped bottles prior to gamma irradiation exposure (Figure 2) (Khawory et al. 2020).

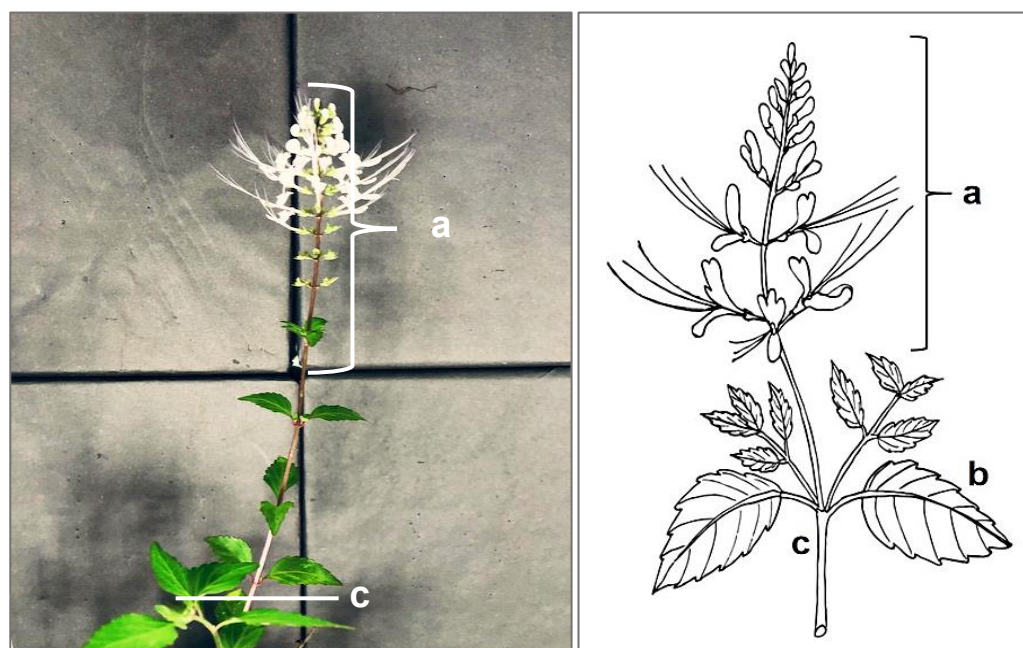


FIGURE 1. *Orthosiphon aristatus* (Blume) Miq. (Misai kucing). (a) Flowers (b) Lanceolate leaf (c) Quadrangular stem



FIGURE 2. *Orthosiphon aristatus* powder was packaged individually in a capped bottle before exposure to cobalt-60 gamma irradiation

PLANT AUTHENTICATION

Fresh *O. aristatus* were sent for plant authentication at the Institute of Bioscience (IBS), Universiti Putra Malaysia, Selangor, Malaysia and the characteristics were listed in Table 1.

EXPOSURE TO COBALT-60 GAMMA IRRADIATION AND STORAGE

The irradiation procedures were performed at the Malaysian Nuclear Agency using cobalt-60 gamma as the source and doses ranging from 3, 6, 9 and 12 kGy. Exposure to gamma irradiation was carried out at 30 °C and 1 atm pressure with a 3 kGy/h dose rate for each irradiation. In this study, gamma irradiation employs a dosimetric method of ceric-cerous dosimeter produced

by SSDL (JS 10000 IR219 MDS Nordion Inc, Canada). The non-irradiated and irradiated *O. aristatus* were then stored at room temperature (25 °C) for up to 6 months. The plant samples were subjected to microbial enumeration and bacterial identification tests every 3 months interval.

MICROBIOLOGICAL ANALYSIS OF MEDICINAL PLANT

The microbiological analyses were performed according to the Microbial Examination of Non-sterile Products and Test for Specified Microorganisms (European Pharmacopoeia 2010; NPRA 2016; WHO 2012). Other than to evaluate the microbial contents, this test was carried out to specifically determine the presence of pathogenic bacteria in non-irradiated and irradiated plant samples.

TABLE 1. Medicinal plant species

ID	Family name	Scientific name	Local name
MFI 0140/19	Lamiaceae	<i>Orthosiphon aristatus</i> (Blume) Miq.	Misai kucing

MICROBIAL ENUMERATION TESTS: TOTAL AEROBIC MICROBIAL COUNTS (TAMC) AND TOTAL YEAST AND MOULD COUNTS (TYMC)

Prior to the microbial enumeration tests, serial dilution of plant samples was conducted. A suspension of the plant sample was prepared by weighing 1 g of the *O. aristatus* dried raw powder and homogenising it with 9 mL of sodium chloride peptone broth. The mixture was then vortexed for 20 s and serially diluted with 9 mL of sterile 0.9% saline solution. Following serial dilutions of plant samples, 50 µL of each dilution was plated separately on tryptic soy agar (Oxoid, UK) for TAMC and Sabouraud dextrose agar (Oxoid, UK) for TYMC using surface spread plate technique and incubated at 37 °C for 24 h. The discrete colonies formed on agar plates were enumerated and recorded as colony-forming units per gram (CFU/g). The plating was done in triplicates and the colony counts were recorded as an average of three independent plates. The reduction of microorganisms during pre- and post-irradiation was calculated using the following formula and represented in percent of reduction (%);

Percent of reduction (%) =

$$\frac{\text{Average pre- irradiation} - \text{Average post- irradiation}}{\text{Average pre- irradiation}} \times 100\%$$

IDENTIFICATION OF SPECIFIC PATHOGENS USING SELECTIVE MEDIA

One mL of non-irradiated and irradiated plant samples suspension was pre-enriched with 9 mL of tryptic soy broth (Oxoid, UK). The mixtures were then incubated aerobically under shaking conditions (180 rpm) at 37 °C for 24 h. One mL of the inoculated tryptic soy broth was sub-cultured into 9 mL of selected enrichment broth; MacConkey broth (Oxoid, UK) and Rappaport Vasiliadis Salmonella broth (Oxoid, UK), followed by incubation at 37 °C for 24 h. Subsequently, 50 µL of the supernatant from each selective broth was sub-cultured separately on the selective media; MacConkey agar (Oxoid, UK) and Xylose, Lysine and Deoxycholate (XLD) agar (Oxoid, UK). Incubation continued at 37 °C for 24 h. The morphological characteristics of colonies grown on both MacConkey agar and XLD agar were recorded and confirmed using 16S rRNA PCR amplification and sequencing analysis.

MOLECULAR IDENTIFICATION OF BACTERIA 16S rRNA PCR AMPLIFICATION AND SEQUENCE ANALYSIS

16S rRNA PCR amplification technique (Thermal Cycler, BioRad) was performed using universal primers (Integrated DNA Technologies), 27F(5'-GTGCTGCAGA GAGTTGATCCTGGCTCAG-3') and 1492R(5'-CACGATC CTACGGGTACCTTGTTACGACTT-3'). PCR amplification was carried out with the following conditions: pre-denaturation at 95 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 1 min and extension at 72 °C for 2 min and the last step was final extension at 72 °C for 4 min. The purified PCR products were then sequenced (MyTACG Bioscience Enterprise) and analysed using ClustalW (MEGA7 Software) and National Center for Biotechnology (NCBI) database.

PHYTOCHEMICAL CONSTITUENTS SCREENING

Twenty-five g of non-irradiated and irradiated plant samples were transferred individually into a sterile falcon tube and sent for qualitative phytochemical constituents screening at the Forest Research Institute Malaysia (FRIM). The screening was done mainly to detect the important components in medicinal plants such as alkaloids, flavonoids, saponins, triterpenes, steroids and tannins pre- and post-irradiation.

STATISTICAL DATA ANALYSIS

The statistical difference between irradiation treatments within 6 months of storage was obtained using Multivariate Analysis of Variance (MANOVA) followed by a post-hoc test, the Tukey test in IBM SPSS Version 22 (Allen, Bennett & Heritage 2014). All data were recorded as mean CFU/g ± standard deviation (SD).

RESULTS AND DISCUSSION

INFLUENCE OF DIFFERENT IRRADIATION DOSES AND STORAGE DURATION ON THE MICROBIAL COUNTS (TAMC AND TYMC)

Medicinal plants have been used in traditional medicine for decades to cure infections, inflammations, and diseases, and recently, plant-derived medicinal products have gained attention worldwide due to their positive impacts on human health. However, contamination of medicinal plants by microorganisms such as bacteria, fungi, yeast, and mould has emerged as the main safety

risk, and it results in many health issues including bloody diarrhoea and acute gastroenteritis (Lorenzo et al. 2018). The significance of gamma irradiation is now apparent. This technology is employed to inhibit sprouting and delay ripening in fruits, disinfect insects, and reduce spoilage by eliminating the microorganisms to a point of sterility (Schottroff et al. 2021). The level of microbiological contamination in food shall not exceed 10^3 CFU/g for total aerobic microbial counts (TAMC) and 10^2 CFU/g for total yeast and mould counts (TYMC) (European Pharmacopoeia 2010; WHO 2012).

The results showed that before gamma irradiation, the microbial counts of non-irradiated *O. aristatus* showed an average of 10^3 CFU/g within 6 months of storage duration. Following exposure to the 3 kGy

irradiation dose, the total bacteria, yeast and mould were reduced by 89% to an average of 10^2 CFU/g. Further decrement by 99% was observed when exposed to a 6 kGy irradiation dose, and no microorganisms were detected when higher doses were applied (Tables 2 & 3).

Based on the data presented in Tables 2 and 3, there were fluctuations in the microbial loads of non-irradiated *O. aristatus* throughout the storage periods. The irradiated plant samples were shown to contain a constant number of microorganisms within the 6 months of storage duration. The data also highlighted that gamma irradiation is significant in reducing the number of microorganisms in *O. aristatus* and following the irradiation procedures, the medicinal plant can be maintained for up to 6 months.

TABLE 2. Effects of irradiation dosages on the total aerobic microbial counts (TAMC) of *O. aristatus* at different storage duration

Doses (kGy)	Total Aerobic Microbial Counts (TAMC) Mean CFU/g \pm SD		
	Months*		
	0	3	6
0	$9.00 \times 10^2 \pm 0.03$ ^{Ac}	$1.29 \times 10^4 \pm 0.07$ ^{Aa}	$6.12 \times 10^3 \pm 0.01$ ^{Ab}
3	$2.11 \times 10^2 \pm 0.03$ ^{Bb}	$2.27 \times 10^2 \pm 0.11$ ^{Bb}	$4.49 \times 10^2 \pm 0.11$ ^{Ba}
6	$1.80 \times 10^1 \pm 0.16$ ^{Ca}	$2.40 \times 10^1 \pm 0.21$ ^{Ca}	$3.00 \times 10^1 \pm 0.17$ ^{Cb}
9	0 ^{Da}	0 ^{Da}	0 ^{Da}
12	0 ^{Da}	0 ^{Da}	0 ^{Da}

*0 = no colony detected

Mean values within the columns followed by the same capital letters are not significantly different; mean values within the rows followed by the same small letters are not significantly different, $P < 0.05$, Tukey test.

TABLE 3. Effects of irradiation dosages on the total yeast and mould counts (TYMC) of *O. aristatus* at different storage duration

Doses (kGy)	Total Yeast and Mold Counts (TYMC) Mean CFU/g \pm SD		
	Months*		
	0	3	6
0	$1.58 \times 10^3 \pm 0.06$ ^{Ac}	$7.20 \times 10^3 \pm 0.05$ ^{Aa}	$4.65 \times 10^3 \pm 0.05$ ^{Ab}
3	$3.60 \times 10^2 \pm 0.10$ ^{Ba}	$3.30 \times 10^2 \pm 0.10$ ^{Ba}	$3.00 \times 10^2 \pm 0.10$ ^{Ba}
6	$2.48 \times 10^1 \pm 0.30$ ^{Ca}	$2.85 \times 10^1 \pm 0.25$ ^{Cb}	0 ^{Cc}
9	0 ^{Da}	0 ^{Da}	0 ^{Ca}
12	0 ^{Da}	0 ^{Da}	0 ^{Ca}

*0 = no colony detected

Mean values within the columns followed by the same capital letters are not significantly different; mean values within the rows followed by the same small letters are not significantly different, $P < 0.05$, Tukey test.

In this study, it was demonstrated that the level of microbial contaminants in non-irradiated *O. aristatus* was more than 10^3 CFU/g, which exceeded the microbiological acceptable range. Based on the guidelines from the European Pharmacopoeia (2010) and WHO (2012), the level of microbial contaminants present in non-sterile herbal products must not exceed 10^3 CFU/g for the total aerobic microbial counts (TAMC) and 10^2 CFU/g for the total yeast and mould counts (TYMC). It was found that following exposure to gamma irradiation at lower doses of 3 and 6 kGy, the microbial loads in *O. aristatus* were significantly reduced. The data highlights that a low irradiation dose can reduce the total bacterial, yeast and moulds to an acceptable level. It was also observed that exposure at 9 and 12 kGy can completely eliminate the microbial contaminants from the plant.

The findings of this study reflect that the irradiation dose is depending on the nature of the foods that need to be treated. There are different doses suggested for food sterilization, in which doses ranging from 5-30 kGy would be efficient to eliminate microorganisms in dried medicinal plants and extend their shelf-life (Mostafavi et al. 2010; Roberts 2014). Meanwhile, some studies claimed that lower doses below 15 kGy would be sufficient in decontaminating herbs and spices (Hazekamp 2016; Malaysian Standard 2005). Above all, the maximum absorbed doses allowed to be applied on all types of foods for sterilization purposes and extension of shelf life should not exceed 10 kGy (Malaysian Standard, 2005). Many studies have shown that gamma irradiation doses of less than 10 kGy can penetrate foods uniformly and reduce microbial contaminants without compromising the nutrient contents, structures and textures (Nathawat et al. 2013; Wang et al. 2019). Recent research has proven that doses of 5 to 10 kGy are effective in suppressing the growth of bacteria and fungi (Aly et al. 2022; Sajjabut et al. 2019).

In this study, it was shown that 6 kGy is the most effective dose for *O. aristatus* as the total microbial counts were reduced significantly following the exposure. The sterility of the plant can be maintained up to 6 months of storage duration and consistent with the previous studies which reported that low irradiation doses help to maintain the sterility of irradiated plants for longer storage duration (Nathawat et al. 2013).

CHARACTERIZATION OF NON-PATHOGENIC AND PATHOGENIC BACTERIAL SPECIES IN NON-IRRADIATED AND IRRADIATED *O. aristatus*

Bacteria isolated from *O. aristatus* were first identified using two selective media, MacConkey agar and Xylose,

Lysine and Deoxycholate (XLD) agar for the detection of two main foodborne pathogens, *Escherichia coli* and *Salmonella* spp, respectively. Nevertheless, it was found that the bacterial colonies that formed on both selective media were morphologically different from the controls; *E. coli* ATCC 25922 and *Salmonella typhi*. Therefore, 16S rRNA PCR amplification was used to confirm the bacterial species.

Sequence analyses showed the presence of pathogenic bacteria *Pantoea* sp., *Klebsiella* sp. and *Clostridium* sp. in non-irradiated *O. aristatus*. The presence of pathogenic strains *Bacillus cereus* and *Acinetobacter radioresistens* were eliminated when exposed to 6 kGy. Therefore, the data suggested that gamma irradiation at 6 kGy is the best dose to be applied on *O. aristatus* since no pathogenic bacteria were identified for at least up to 6 months of storage. The identified bacterial species were summarized in Table 4.

The bacterial species detected in non-irradiated *O. aristatus* belong to the family of Gammaproteobacteria and Clostridia where most of them are pathogens. *Pantoea calida* which was isolated from *O. aristatus* is a Gram-negative bacterium and is known to form host associations with plants, insects, and humans, thus it is commonly found in soil and water. Recently, *P. calida* is recognized as an opportunistic pathogen that is responsible for causing bacteraemia in humans, although information on its pathogenic mechanisms is still limited (Yamada et al. 2017). *Klebsiella* spp. was identified as pathogenic and commonly causes nosocomial infections in humans (Fan et al. 2018). Conversely, *Clostridium tertium* is a ubiquitous Gram-positive, spore-forming bacterium and it distinguishes itself from other *Clostridium* spp. as non-toxin-producing and low-virulence bacteria. *C. tertium* is commonly found in soil, animal, and human gastrointestinal tract (Cote et al. 2018; Sutton et al. 2017). A few studies, however, reported that *C. tertium* could harm humans, especially in immunocompromised individuals such as neutropenia, intraabdominal infection, enterocolitis, meningitis, septic arthritis, and pneumonia (Bhattacharyya et al. 2017).

The occurrence of pathogenic *B. cereus* and *A. radioresistens* was also observed in *O. aristatus* after irradiation at 3, 9 and 12 kGy. *Bacillus cereus* is a common food pathogen, and it is well-adapted in the gastrointestinal tracts of humans and animals (Fan et al. 2018). This bacterium can be isolated from raw or processed meats, vegetables, rice, and dried food products. The presence of *B. cereus* in irradiated *O. aristatus* may be due to its ability to produce spores

under stress conditions such as heat and radiation. The spores are highly resistant to extreme temperatures, UV light radiation and ionizing radiation (Radulovic et al. 2013). Meanwhile, *Acinetobacter radioresistens* is a Gram-negative, non-spore-forming and mostly known as radiation-resistant bacteria. *A. radioresistens* is also able to survive under UV radiation, desiccation and hydrogen peroxide. Although the pathogenicity of this bacterium remains unknown, it was reported that *A. radioresistens* had caused opportunistic infection in immunodeficiency patients and bacteraemia in humans (Zantar et al. 2015).

Even though bacteria such as *B. aryabhataii*, *B. subtilis*, and *B. megaterium* were identified in irradiated *O. aristatus*, these species are non-pathogenic and are not known to cause food poisoning. Instead, previous

studies reported that *B. aryabhataii*, *B. subtilis*, and *B. megaterium* are known to be plant-growth-promoting rhizobacteria (PGPR). The bacteria can produce specific metabolites such as indole acetic acid (IAA) and gibberellins that are beneficial for plant growth and development (Naveed, Rizwan & Sajid 2017).

This study highlights the ability of gamma irradiation at 6 kGy in eliminating pathogenic bacteria such as *B. cereus* and *Clostridium* spp. throughout the tested storage periods. Meanwhile, longer exposure to irradiation at higher doses of 9 and 12 kGy showed the presence of pathogenic bacteria including the spore-forming species. These findings underlined the importance of a minimum absorbed dose (6 kGy) to achieve the desired effects and maintain the microbial contents at an appropriate level for longer storage that is beneficial during global trade.

TABLE 4. Bacterial isolates from *O. aristatus* within 6 months of storage duration

Months	Doses (kGy)	Bacteria identity	Class	Description*
0	0	<i>Pantoea calida</i>	Gammaproteobacteria	Plant-pathogenic bacterium, cause bacteremia in humans
		<i>Klebsiella</i> sp.	Gammaproteobacteria	Cause human nosocomial infections
	3	<i>Bacillus cereus</i>	Bacilli	Pathogenic, cause food poisoning
	6	<i>Bacillus aryabhataii</i>	Bacilli	Non-pathogenic, soil bacterium, plant growth-promoting bacteria
	9	<i>Bacillus cereus</i>	Bacilli	Pathogenic, cause food poisoning
3	0		Bacteria were not detected	
		<i>Clostridium tertium</i>	Clostridia	Uncommon pathogens in humans
		<i>Bacillus cereus</i>	Bacilli	Pathogenic, cause food poisoning
		<i>Bacillus subtilis</i>	Bacilli	Non-pathogenic, soil bacterium, plant growth-promoting bacteria
	9		Bacteria were not detected	
6	12	<i>Acinetobacter radioresistens</i>	Gammaproteobacteria	Cause bacteremia in humans
	0	<i>Clostridium tertium</i>	Clostridia	Uncommon pathogens in humans
		<i>Bacillus velezensis</i>	Bacilli	Non-pathogenic, soil bacterium, plant growth-promoting bacteria
		<i>Bacillus megaterium</i>	Bacilli	Non-pathogenic, plant growth-promoting bacteria
		<i>Bacillus megaterium</i>	Bacilli	Non-pathogenic, plant growth-promoting bacteria
12		Bacteria were not detected		

*References: Nithya and Babu (2017); Fan et al. (2018)

PHYTOCHEMICAL CONSTITUENTS OF NON-IRRADIATED
AND IRRADIATED *O. aristatus*

The qualitative screening of the phytochemical constituents of *O. aristatus* showed the presence of 3 major metabolites which are saponins, tannins and steroids in both non-irradiated and irradiated plant samples as presented in Table 5. Saponins, tannins and steroids are known as antifungal, antimicrobial, antibacterial and anti-inflammatory agents (Radulovic et al. 2013). There are variations in the current reports on the effects of gamma irradiation on the production of phytochemical contents of plants. Previously, gamma irradiation at 10, 20 and 30 kGy was found to increase the level of bioactive compounds such as menthone and piperitone oxide in the medicinal plants *Thymus vulgaris* and *Menta pulegium* (Zantar et al. 2015). Nonetheless, another study reported a decrease in total phenolic and flavonoids contents in several medicinal plants such as *Amygdalus communis* (almond), *Nigella sativa* (black seeds), *Zingiber officinale* (ginger) and *Allium sativa* (garlic) (Naveed, Rizwan & Sajid 2017).

Limited studies have reported on the effects of gamma irradiation on the bioactive compounds of *O. aristatus*. Several studies showed that *O. aristatus* leaf extracts contained high levels of phenolics and flavonoids as compared to stem extracts (Chua et al. 2018). Another study showed the presence of terpenes, triterpenes, sinensetin and flavonoids in the methanolic extracts of *O. aristatus* (Chai et al. 2014). These variations could be due to the extraction procedure of the medicinal plants in which this study did not screen the phytochemical constituents in *O. aristatus* extracts but in the dried raw powder. This shows that ethanolic or methanolic extraction enhances the production of bioactive compounds in medicinal plants. Although this study did not report the level of saponins, tannins and steroids detected in *O. aristatus*, yet it provides insights that gamma irradiation conserves the bioactive compounds of medicinal plants. It is suggested that the characterization of the phytochemical constituents in *O. aristatus* can be carried out using high-performance liquid chromatography (HPLC) to provide quantitative data on the level of saponins, tannins and steroids in non-irradiated and irradiated *O. aristatus*.

TABLE 5. Phytochemical contents of non-irradiated and irradiated *O. aristatus*

Phytochemical constituents	Tests	Observation	Standard doses (kGy)*	
			0	6
Alkaloids	Mayer's reagent	No white precipitate formed	Not detected	Not detected
Saponins	Froth test	Stable froth formed	Detected	Detected
Flavonoids	Ammonia test	No yellow colour formed	Not detected	Not detected
Tannins	Ferric chloride test	Brownish-green colour formed	Detected	Detected
Steroids	Liebermann-Buchard reagent	Greenish colour formed	Detected	Detected
Triterpenes	Liebermann-Buchard reagent	No reddish color formed	Not detected	Not detected

*Standard dose was selected based on the molecular identification of bacteria from non-irradiated and irradiated *O. aristatus* up to 6 months of storage duration

CONCLUSION

In conclusion, cobalt-60 gamma irradiation is effective and efficient to reduce and eliminate microbial contaminants in *O. aristatus* and ensures the sterility and safety of the plant during longer storage periods. As a lower dose is recommended, therefore it could save cost and production time which are useful during international trade.

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