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OPTIMIZATION OF GAMMA IRRADIATION DOSE FOR MAS COTEK AS RAW MATERIAL FOR PHYTOPHARMACEUTICAL PRODUCT

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ABSTRACT

Microbial contamination in final product prone to occur in herbal raw material due to the presence of microorganisms in the environment. This may cause a shorter shelf life of the products and lead to undesirable effects on consumer. Mas Cotek, scientifically known as Ficus deltoidea is being utilised as a medication for several medical conditions throughout the Malay Archipelago. This study was conducted to identify the optimum gamma irradiation dose for sterilization of Mas Cotek raw material in order to achieve the acceptable microbiological limits without affecting the quality of raw materials. Besides, the content of vitexin in Mas Cotek with different gamma irradiation dose were also evaluated using Ultra High Performance Liquid Chromatography (UHPLC). Controlled exposure of gamma irradiation (0 kGy, 3 Gy, 6 kGy, 9 kGy and 12 kGy) were exposed to the raw material. Moreover, the specified dose is expected to reduce the bioburden to the desired level concurrently minimizing the effect on the product. Upon irradiation process, the raw materials were extracted and assessment such as antioxidant status, phenolic content, total anti-microbial content, and total yeast content were done. The results shown that, there were significant different in phytochemical component and antioxidant activity status in accordance to difference doses of gamma irradiation. Total aerobic microbial count (TAMC) and total yeast microbial count (TYMC) also showed a significantly reduced in irradiated raw material at higher dose. The vitexin content were found varies depending on the gamma irradiation doses respectively. In conclusion, gamma irradiation on raw material of Mas Cotek will decreases the number of microbial burdens. However, gamma irradiation with different dose also have varies impact on the content of vitexin in Mas Cotek.

Keywords: Mas Cotek, Gamma Irradiation, Phytochemical, Antioxidant Activity.

INTRODUCTION

Herbal medicine usage has expanded globally in recent years due to their efficacy, low toxicity, and few adverse effects (Adu-Gyamfi et al., 2014). The world health organisation (WHO) has claimed that almost 74% of 119 plant-originated medicines are being utilised in modern medicine (Banik et al., 2020). Since the use of herbal medicine grows, there is a serious concern on the microbial contamination that prone to occur at any step of harvest, storage, or herbal medicine preparation (Alijaniha et al., 2021). To support the medical herb industries, medicinal herb preservation management is required to minimise mould and bacterial contamination. Thus, to ensure the availability of hygienic medicinal herbs for the consumer, a treatment is required to minimise bacterial, mould, and yeast contamination (Katrin et al., 2011). Decontamination techniques include steam treatment, microwave heating, and radiofrequency heat therapy. Electron beams, X-rays, and gamma ray's irradiation has also been utilised. Numerous investigations on the effectiveness and efficiency of various approaches have been undertaken (Molnár et al., 2018).

Gamma irradiation is getting prominence as among the most promising and extensively utilised ways of decontaminating foods and herbal materials (Ernawati et al., 2021). Gamma irradiation exposes the target material to light packets (photons) that are so energetic (gamma rays) that they destroy the DNA strands found in microorganisms. Consequently, the impacted bacteria are unable to proliferate and enhance the shelf life of perishable goods in storage packaging (Hazekamp, 2016; Katrin et al., 2011). Gamma irradiation is favoured over other techniques of decontamination as it effectively destroys bacteria while leaving no chemical residues, allowing it hygienic and environmentally friendly. In addition, water radiolysis can be triggered by gamma irradiation, generates reactive oxygen species (ROS). Free radicals, particularly •OH, predominantly damage DNA and other macromolecules, resulting in microorganism destruction. However, these free radicals can influence the plant's ROS and antioxidant levels, degrade or alter bioactive components, and drive phenolic compound accumulation. Nevertheless, the effect of gamma irradiation on phenolic compounds and antioxidant activity will be dose dependent (Ernawati et al., 2021).

Ficus deltoidea also commonly known as Mas Cotek, is being utilised as a medicine besides being marketed and produced as capsules, tea, and tonic tea across Malaysia. Mas Cotek has been claimed to have beneficial pharmacological applications as an anti-diabetic, anti-inflammatory, antinociceptive, anti-melanogenic, antiphotoaging, antioxidant, antiulcerogenic, and antibacterial agent (Bunawan et al., 2014). Previous phytochemical study revealed that this plant contains a wide range of secondary metabolites, including saponins, flavonoids, tannins, polyphenols, triterpenoids, and proanthocyanins (Akmalazura et al., 2020). Thus, the aim of this study is to evaluate the optimal gamma irradiation dose for sterilisation of Mas Cotek raw materials in order to meet approved microbiological limits without compromising raw material quality. Specifically, antioxidant capacities, total microbial content, yeast content and phenolic content besides vitexin content will be determined upon the gamma irradiation.

MATERIALS AND METHOD

Plant collection and authentication

The fresh Mas Cotek were harvested from Sungai Tengi Selatan, Selangor, Malaysia. The plant taxonomist from the Institute of Bioscience (IBS), Universiti Putra Malaysia authenticated the sample identification with a voucher specimen (SK1467/07).

Gamma irradiation and plant extract preparation

Mas Cotek was washed with running water and dried at 50^{O}C. The samples were then pulverised before being subjected to dosages of 0, 3, 6, 9, and 12 kGy of Cobalt-60 radiation. The sample was then freeze dried at -80^{O}C to -20^{O}C overnight for 12 hours after being subjected to an accelerated solvent extraction (ASE) for extraction at 60^{O}C for 20 minutes.

Total phenolic content (TPC)

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of the Mas Cotek extracts. The double distilled water was used to dilute the test sample. The 0.1mL of Folin-Ciocalteu reagent was added to 0.2mL of 2% sodium carbonate (Na_2CO_3) and incubated for 3 hours. The absorbance was measured at 760nm against blank (Yang et al., 2011).

Total flavonoid content (TFC)

The total flavonoid concentration (TFC) of the Mas Cotek extract was assessed using the aluminium chloride colorimetric ($AlCl_3$) method. The test samples diluted in ethanol solution was mixed with 150µL of 2% $AlCl_3$ in 96 well plates and incubated for 15 minutes at room temperature. The absorbance was measured at 435nm against blank (Yang et al., 2011).

DPPH free radical scavenging

Direct hydrogen donation to the DPPH radical was utilized to quantify hydrogen-donating activity of Mas Cotek extract. The reaction mixtures in the 96-well plates were made up of the sample diluted in ethanol (100µL) and ethanol-dissolved DPPH radical (100 µL, 0.2 mM). After shaking, the mixture was allowed to stand in the dark for 15 minutes. The absorbance was then measured at 517 nm in comparison to a blank (Yang et al., 2011).

Microbial burden test

To count the microorganisms in Mas Cotek, TAMC and TYMC were undertaken. Using the surface spread plate method, 50µL of diluted samples were individually plated on tryptic soy agar (TSA) for TAMC and Sabauroud dextrose sugar (SDA) for TYMC and incubated at 37^{O}C for 24 hours. After incubation, the distinct colonies that developed on the agar plates were counted and represented as CFU/g, or colony forming units per gramme. The colony counts were recorded as the average of three separate samples, and the plating was carried out in triplicate.

Vitexin content analysis

Samples were weighed at 20 g and soaked in 200 mL of water and sonicated for 1 hour at 80ºC. The mixture was then filtered using vacuum filtration with 0.2 µm membrane filter. The filtered extract was dried using personal evaporator with HPLC mode. Vitexin content was analysed using Ultra High Performance Liquid Chromatography (UHPLC).

Statistical analysis

All the findings were presented as mean ± SD for a specific number of observations. The Statistical Package for the Social Sciences (SPSS) software was used to conduct the statistical analysis. The significant difference level was set at $p<0.05$.

RESULTS

The therapeutic plants like herbs and spices are frequently highly contaminated with bacteria, fungus, moulds, and yeasts (Khawory et al., 2020). Both herbs and spices can quickly deteriorate items that influence health and claimed a huge concern on safety issues related to herbal medicines besides having an impact on the economy if left untreated (Hadiati et al., 2021; Khawory et al., 2020). The application of ionizing radiation such as gamma rays, which have been investigated for their efficacy in decontamination and their effects on the herbal ingredients and biological activities, is one of the most contentious approaches (Alijaniha et al., 2021). Mas Cotek has a long history of utilization in Malay traditional medicine to treat conditions including ulcers, wounds, and rheumatism as well as to promote a healthy pregnancy and treat diabetes (Bunawan et al., 2014). It is apparent that evaluating safety and its possible effects on the biological activity of natural products such as Mas

Cotek is of utmost importance given the widespread use of gamma irradiation as an effective way to disinfect and lengthen the shelf life of herbal items and consumables (Alijaniha et al., 2021).

Ionizing radiation such as gamma irradiation has the power to influence alterations in plant matter and secondary compounds on both a quantitative and qualitative level (Ja&rsquo & afar, 2019). The use of gamma irradiation in herbal medicines is now governed by general food irradiation laws because there is no special legislation governing it (Hadiati et al., 2021). According to previous studies, parasites like protozoa and helminths have been rendered inactive in meat products, fresh produce, and vegetables by low doses of gamma irradiation of less than 1 kGy. In the meanwhile, fresh, frozen, and dried foods and spices have been given medium dosages of 1–10 kGy to diminish or eradicate non-viral bacteria and lengthen the product shelf life. Additionally, strong doses of 10-60 kGy have been used to sterilise or reduce the number of bacteria in foods like dry components and meals for astronauts and hospital patients (Ernawati et al., 2021).

Table 1 below demonstrated the total phenolic and flavonoid content in gamma irradiated Mas Cotek extract. The highest phenolic content, 41.74 µg GAE/mg extract and highest flavonoid content, 189.33 µg QE/mg extract were observed in Mas Cotek extract irradiated with the dose of 3kGy, respectively. There was a significant difference between the total phenolic content of non-irradiated and gamma irradiated Mas Cotek sample at irradiation doses of 6 and 9 kGy. In addition, there was significant different between the total flavonoid content of non-irradiated and gamma irradiated Mas Cotek sample at all irradiation doses.

Table 1: Total phenolic and flavonoid content in non-irradiated and gamma irradiated Mas Cotek sample at different irradiation doses (mean ± SD).

Irradiation Doses (kGy)	**Total Phenolic Content µg GAE/mg Extract**	**Total Flavonoid Content µg QE/mg Extract**
0	39.74 ± 0.23[a]	182.67 ± 0.74[a]
3	41.74 ± 1.26[a]	189.33 ± 1.24[b]
6	33.74 ± 1.12[b]	179.33 ± 0.87[c]
9	35.06 ± 0.80[b]	169.33 ± 0.34[d]
12	39.74 ± 0.70[a]	176.00 ± 0.94[e]

[a,b,c,d,e]: $p<0.05$ as compared between different gamma irradiation doses.

Since phenolic compounds are recognised as natural antioxidants, they have significant effects on lipid peroxidation inhibition, carcinogenesis inhibition, antibacterial activity, direct capillary constriction, naturally occurring phytohormones, and ascorbic acid stability. Based on this study, there were no significant difference of phenolic content between the Mas Cotek irradiated at multiple doses of gamma irradiation and non-irradiated samples. It is hypothesised that the release of phenolic compounds from glycosidic components and the breakdown of larger phenolic compounds into smaller ones may be responsible for the rise in total phenolics content brought on by gamma irradiation (Harrison & Were, 2007). Besides, according to Taheri et al., (2014), acute gamma irradiation at the dose of 20Gy showed higher content of total phenolic content in *Curcuma alismatifolia* leaves. Based on the previous studies, irradiation causes increased in total phenolic content of *Carulluma tuberculate*, almond skin, *Nigella sativa*, *Thymus vulgaris*, *Glycyrrhiza glabra*, pistachio green hull, clove, Curcuma alismatifolia, nutmeg, and soybean. However, it is also found that certain plants had lost their overall phenolic content upon gamma irradiation (Hadiati et al., 2021). Thus, the environmental factor, type and portions of the plant, the composition of the phenolic content, solvent and extraction techniques utilised, storage conditions, and the irradiation dose may all have a significant influences on how irradiation impacts total phenolic content (Hadiati et al., 2021; Harrison & Were, 2007; Khattak et al., 2008).

Flavonoid is a significant group of natural products and they are a group of secondary plant metabolites with a polyphenolic structure that are prevalent in vegetables and fruits. Similar to phenolic content, flavonoid content in natural and herbal medicines can be altered significant either by increasing or decreasing upon gamma irradiation (Hadiati et al., 2021). This is because, irradiation may contribute to making molecules more extractable, but it may also cause the breakdown of some less stable chemicals (Pereira et al., 2016). In addition, gamma radiation may interact with atoms and molecules to produce free radicals that have the power to alter crucial plant cell components. Depending on the irradiation dose, it has been shown that these radicals have an impact on the morphology, anatomy, biochemistry, and physiology of plants. The consequences include modifications to the plant's cellular structure and metabolism, such as thylakoid membrane dilatation, altered photosynthesis, altered antioxidant defences, and enhanced phenolic chemicals (Kavitha et al., 2015). According to research by Khatun et al., (2012), the total flavonoids content in bitter gourd increased significantly with increasing radiation dosage as compared to control sample. The increase in total flavonoid may influences the phenylalanine ammonialyase activity, which is one of the significant enzymes responsible in the production of phenolic compounds in plant tissue.

The DPPH scavenging activity of the gamma irradiated Mas Cotek extract irradiated with multiple doses were presented in Figure 1 in form of percentage of DPPH radical inhibition. Based on the result obtained, the highest DPPH radical inhibition with 54.91% was observed in Mas Cotek irradiated at dose of 6kGy, whereas lowest DPPH radical inhibition was obtained at the irradiation dose of 9kGy at 50.75%. This study signified that there was no significant difference between percentage of DPPH radical inhibition among Mas Cotek sample irradiated at different doses. However, there were significant difference between percentage of DPPH radical inhibition of Mas Cotek sample irradiated at different doses and ascorbic acid.

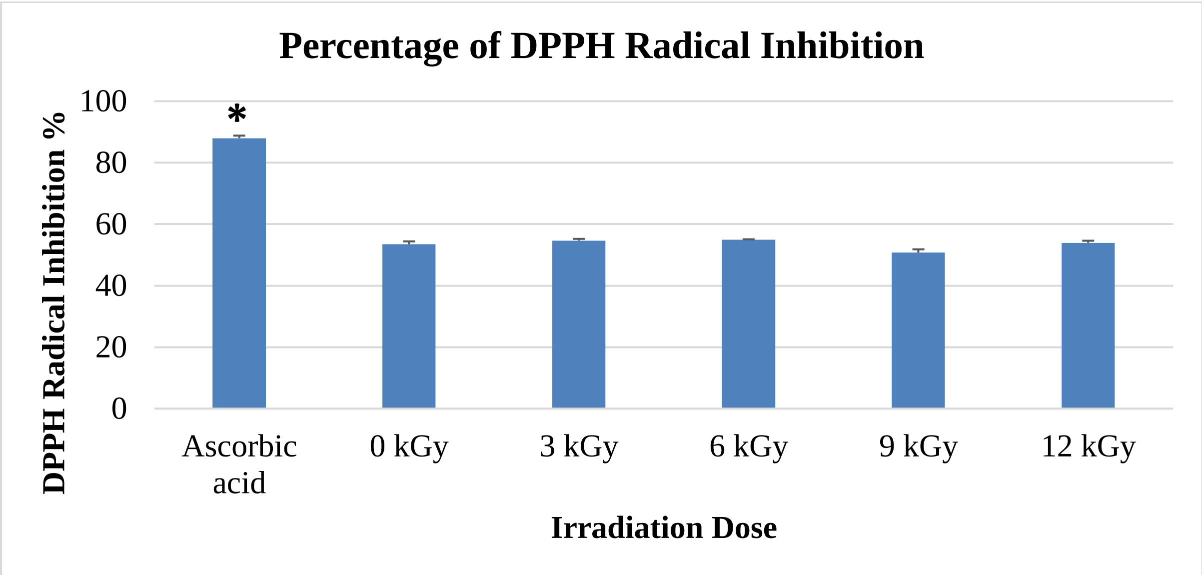

Figure 1: Effect of gamma irradiation dose on the DPPH scavenging activity of Mas Cotek and ascorbic acid (mean ± SD). *: showed a significant highest ($P<0.05$) compared to gamma irradiation dose on the DPPH scavenging activity of Mas Cotek.

The DPPH free radical scavenging assay was utilised to determine the antioxidant activity of both gamma irradiated and non-irradiated sample of Mas Cotek. An antioxidant assay based on electron transfer; the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical approach generates a violet solution in ethanol. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, is reduced, producing a colourless ethanol solution (Garcia et al., 2012). As a result, up to the greatest dose, gamma irradiation has no effect on the free radical scavenging capabilities of sample. Based on previous study, it is observed that radiation therapy within a dosage of 10 kGy had no effect on the free radical scavenging activity of tea (Mishra et al., 2006). Besides, the antioxidant activity of methanol extracts from an irradiated plant of *Rosmarinus officinalis* remained the same as in the control in a DPPH test (Gumus et al., 2011). The discrepancies in the effect of gamma irradiation on the free radical scavenging activity of plants might be attributed to changes in their chemical makeup, extraction solvent, and other factors (Gumus et al., 2011).

Table 2 below showed the microbial content in non-irradiated and gamma irradiated Mas Cotek. The total aerobic microbes and yeast/mould content were higher in non-irradiated Mas Cotek sample compared to the gamma irradiated Mas Cotek sample at the irradiation dose of 3kGy. However, both aerobic microbes and yeast were not detected in Mas Cotek samples that have been irradiated at the dose of 6,9 and 12 kGy.

Table 2: Microbial burden measurements in non-irradiated and gamma irradiated Mas Cotek extract at different irradiation doses.

Irradiation Doses (kGy)	**TAMC (CFU/g or CFU/mL)**	**TYMC (CFU/g or CFU/mL)**
0	7.46×10^6	5.14×10^4
3	2.39×10^2	1.86×10^2
6	NA	NA
9	NA	NA
12	NA	NA

Both the total anaerobic microbes, yeast/ fungi content reduced significantly in irradiated samples and were not detected as the gamma irradiation doses increased. Irradiation has a multitude of physical and biochemical impacts on microorganisms. Microorganisms are destroyed primarily due to the hydroxyl radicals formed within their cells react with the base and sugar moieties of DNA, causing sugar-phosphate bonds to break and lost the replication function. The chromosomal volume of an organism determines its sensitivity to irradiation. This is because, differences in irradiation sensitivity may be attributable to the capacity to repair nucleic acid damage rather than the nucleic acids intrinsic irradiation resistance. The amount of chemical change in radiation-sterilised foods is minimal and uniform *(Gamma Irradiation as a Treatment to Address Pathogens of Animal Biosecurity Concern Final Policy Review*, 2014).

Effect of gamma irradiation doses at doses 0, 3, 6, 9 and 12 kGy on the content of vitexin in Mas Cotek extract were analyzed using UHPLC system. Table 3 presented the concentration of vitexin found in Mas Cotek samples that have been subjected to gamma irradiation of different doses. Results show that the vitexin content are 9.21 ng/mL, 13.19 ng/mL, 18.91 ng/mL, 10.40 ng/mL and 9.48 ng/mL for 0 kGy, 3 kGy, 6 Gy, 9 kGy and 12 kGy gamma irradiation doses respectively. Based on this result, it was found that gamma irradiation with different doses has resulted in varying vitexin content in Mas Cotek extract. Vitexin content shows the highest yield at a dose of 6 kGy compared to other doses.

Table 3: Concentration of vitexin (ng/mL) in non-irradiated and gamma irradiated Mas Cotek sample at different irradiation doses.

Irradiation Doses (kGy)	Concentration of Vitexin (ng/mL)
0	9.21
3	13.19
6	18.90
9	10.40
12	9.48

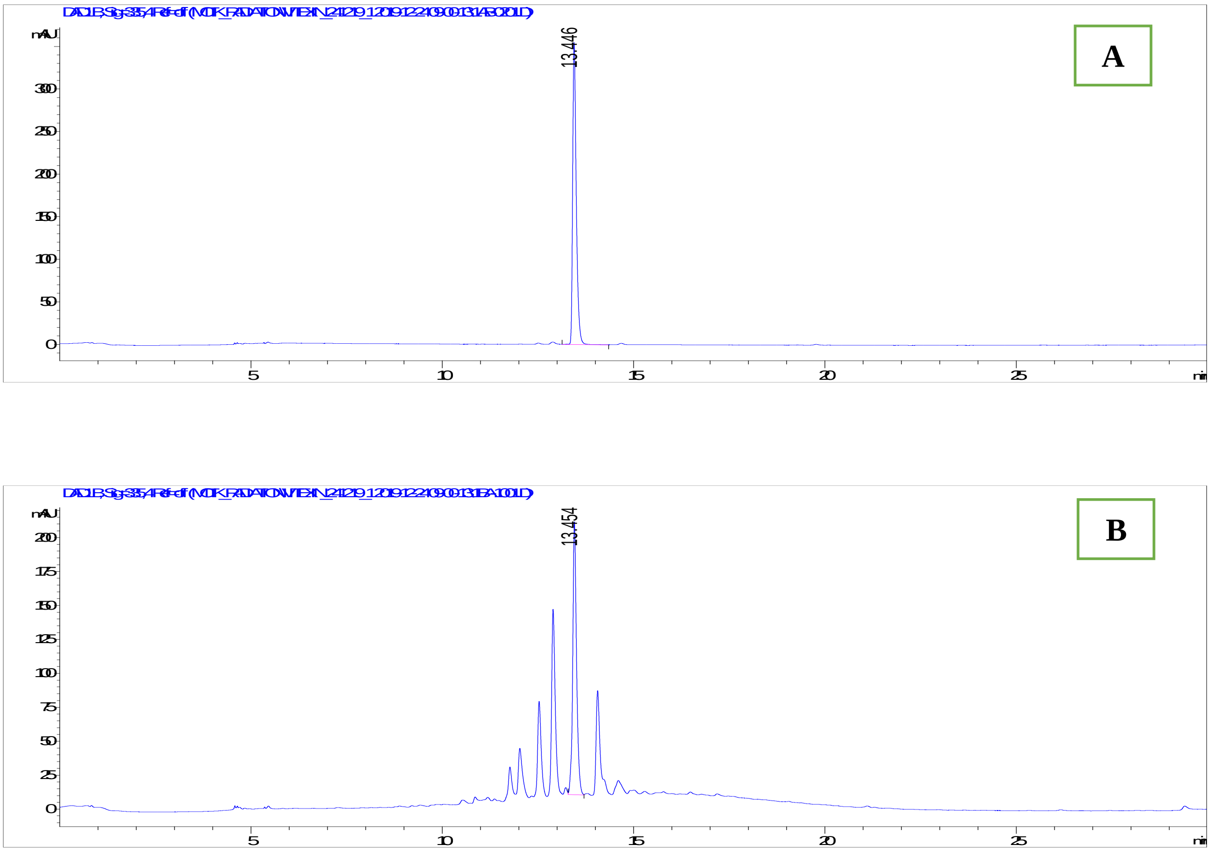

Figure 2: HPLC chromatograms of vitexin concentration (ng/mL) in non-irradiated and gamma irradiated Mas Cotek sample at different irradiation doses. (A) Standard vitexin; (B) Vitexin concentration obtained in non-irradiated and gamma irradiated Mas Cotek sample at different irradiation doses.

Besides, gamma irradiation with different doses has varying vitexin content in Mas Cotek extract. Vitexin is an apigenin flavone glycoside found in ethnomedicinal plants that has antioxidant properties against reactive oxygen species, lipid peroxidation, and other oxidative damages in a number of oxidative stress related disorders, with potential molecular and cellular pathways (Babaei et al., 2020). The varying concentration of vitexin is obtained among different doses of gamma irradiated Mas Cotek because, the micronutrients will be destroyed to varying degrees depending on their capacity to compete for primary radicals with other main components, as well as the irradiation circumstances, including dosage. The irradiation treatment has no effect on the nutritional value or digestibility of macronutrients. Certain micronutrients are vulnerable to irradiation, even though the amount depends on the food's composition as well as processing and storage circumstances (*Gamma Irradiation as a Treatment to Address Pathogens of Animal Biosecurity Concern Final Policy Review*, 2014).

CONCLUSION

Gamma irradiation, as a food processing technique performed on the Mas Cotek samples did not affect the quality of raw material and reduce the bioburden significantly. However, gamma irradiation affects the content of selected targeted compound, vitexin quantitatively. Thus, this gamma irradiation method can ensure the prolong self-life of the product being exposed at an optimum dosage. However, further studies should be perform to determine the effectiveness of medicinal properties of Mas Cotek upon irradiation and examine the radiolytic by product of irradiation in irradiated Mas Cotek samples.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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