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Original Article

Effect of gamma radiation on the antibacterial activity of *Syzygium aromaticum*

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ABSTRACT

Irradiation is known as an effective method for decontamination and preservation of chemical and nutritional properties of various medicinal herbs and spices. This study was undertaken to evaluate the influence of irradiation dose (10kGy) on the antibacterial potency of *Syzygium aromaticum* (clove), plant known for its various biological properties notably its powerful antimicrobial power. *Syzygium aromaticum* (*S. aromaticum*) extract, prepared by maceration in methanol, was characterized by phytochemical analysis and colorimetric determination of chemical compounds. The antibacterial potential was also evaluated against this extract by observing growth inhibition zones. The result of gamma radiation effect on the clove, showed the preservation of main chemical constituents, with a significant increase in the content of total polyphenols and flavonoids. The methanolic extract of *S. aromaticum* revealed an inhibitory effect on the resistant strains, which improved significantly under the gamma radiation effect. These results seem very encouraging and suggest using gamma radiation as procedure to decontaminate the medicinal herbs and spices and improve the composition of extracts in bioactive molecules as well as their biological activities.

1. Introduction

The growing demand for a healthier diet and the use of natural products in the prevention and treatment of health problems have led to an intensive search for bioactive compounds of plant origin.

The use of plants has been a common practice across generations due to their sensory and preservative properties [1]. However, their use also covers the field of medicine knowing that the majority of these medicinal plants contain bioactive substances, which are precursors for the synthesis of conventional drugs, used for preventive and therapeutic purposes.

The natural contamination of plants by microorganisms (bacteria and fungi), parasites and insects during the processes of growth, harvesting, storage and drying, poses a threat not only to the plant material but also to public health, which makes the application of effective techniques for their elimination absolutely essential. Irradiation known as a safe and effective method has been commonly used for decontamination and preservation of chemical and nutritional properties of various food matrices including medicinal herbs and spices. It is currently approved by national legislations in more than 55 countries worldwide [2]. This study was undertaken to assess the influence of the irradiation dose on the antibacterial power of S. aromaticum (clove), a plant chosen for its various biological properties [3] in particular its powerful antimicrobial power.

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2. Materials and Methods

2.1 Preparation of samples for gamma irradiation

The flower buds of *S. aromaticum* were crushed, packaged in sachets and labeled before irradiating them with gamma radiation, using Cobalt 60 as a source.

2.2 Preparation of clove extract

Clove extract was prepared by maceration in methanol.The filtrate obtained was subsequently freeze-dried and stored at 4° C. until its use.

2.3 Qualitative analysis

The main secondary metabolites were evaluated by qualitative analysis based on color and/or precipitation reactions [4, 5]. Salkowski test was used for terpenoids and steroids, alkaline reagent test for flavonoids and quinones, ferric chloride test for phenols, Wagner's reagent for alkaloids, UV and foam test, for coumarins and saponins respectively.

2.4 Assay of total polyphenols and flavonoids

The dosage of total polyphenols and flavonoids was carried out, by colorimetric method, adapted to 96-well microplates as described by Wang et al. [6] with slight modifications, using Follin-ciocalteun and aluminum trichloride reagents respectively. The content of these chemical compounds in the irradiated and non-irradiated extracts was expressed in milligram equivalents of gallic acid (total polyphenols) or quercetin (flavonoids) per gram of freeze-dried extract.

2.5 Evaluation of antibacterial activity

2.5.1. Preparation of bacterial suspensions

The antibacterial activity of the *S. aromaticum* extract against three pathogenic strains, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E.coli*) and Methicillin-susceptible *Staphylococcus aureus* (*S. aureus*), was determined by the method of diffusion on "Mueller-Hinton" agar medium, which promotes the satisfactory growth of most pathogens. The strains were cultured for 24 h at 37°C in order to obtain a young culture and well-isolated colonies. Bacterial suspensions in sterile physiological water were prepared from the colonies whose opacity was adjusted to 0.5 McFarland (optical density between 0.08 to 0.1, read at 625nm).

2.5.2. Paper disc technique

The assay was carried out by spreading the human pathogenic bacteria on the entire surface of the medium (Mueller-Hinton) using a sterile swab. The discs impregnated with extracts (prepared in Diméthylsulfoxyde (DMSO)), were placed on the surface of this medium, then left in diffusion for 20 min before incubating at 37° C for 16 to 18 h. DMSO and Colistin were used as negative and positive control, respectively. For each sample, three replicates per concentration were considered.

3. Results and Discussion

3.1 Extraction yield

The methanolic extraction yield of *S. aromaticum* showed an increase after irradiation, reaching a significant rate of 28.23% compared to that of the untreated sample, which was 24.15%.

A significant increase in the dry weight of the extract is probably due to the effect of radiation on the structure of the plant tissue and to the degradation of certain insoluble high molecular weight compounds into solvent-soluble compounds [7, 8].

The extractability of these compounds also depends on the solvent used, knowing that high yields have been obtained with methanol and ethanol [9].

3.2. Qualitative and quantitative analysis

Phytochemical analysis of irradiated and non-irradiated *S. aromaticum* extracts was performed to define the presence or absence of secondary metabolites. The result of this analysis highlighted certain molecules (Table 1), namely flavonoids, gallic tannins, quinones, coumarins, saponins and terpenoids, except alkaloids and catechetical tannins which were absent in our samples. These molecules are among the essential bioactive constituents of medicinal plants, conferring them several pharmacological properties.

It was found that irradiating this plant at 10 kGy had no effect on the chemical compounds tested, as they were still present in the irradiated sample. This reflects the ability of gamma radiation to preserve the chemical properties of plants [2, 10].

The most interesting, the quantitative assay showed after irradiation, a significant increase ($p<0.0001$) in the content of total polyphenols (438.1±4.95 vs 136.6±4.34 (mgGAE/g)) and flavonoids (19.44±0.32 vs 8.563±0.39 (mgEQ/g)).

Table 1. Illustration of qualitative assay results

Test	Description	Results
Flavonoids	Appearance of red color	Positive
Catechetical tannins	No appearance of red color	Negative
Gallic tannins	Appearance black blue color.	Positive
Quinones	Appearance of reddish yellow color	Positive
Total Polyphenols	Appearance color black, greenish	Positive
Coumarins	Fluorescence under UV lamp	Positive
Alkaloids	No appearance of a precipitate, Wagner reagent	Negative
	No appearance of a precipitate, Mayer reagent	Negative
Saponins	Appearance of persistent foam	Positive
Terpenoids	Appearance of 2 phases + a layer in the interphase.	Positive

Several authors [11, 12] have reported changes in the content of phenolic compounds, under the effect of gamma radiation.

By examining the quantitative variation in phenolic acid of five spices irradiated at 10 kGy, Varyar [13] also observed a significant change in the distribution of these compounds, particularly for nutmeg and clove.

Our results are in agreement with the findings of these authors who observed after irradiation, an increase in the content of total phenolic acids compared to that of non-irradiated cloves. On the contrary, these changes could not be proven by Suhaj and Horvathova [14] who found unchangeable contents.
This increase in the content of phenolic compounds is probably due to the direct and indirect effect of gamma radiation, capable of degrading molecules of complex forms such as lignins and hydrolysable tannins into simpler forms [15]. In addition, the presence of these polymeric molecules in appreciable quantities in the clove [13] could explain the increased content of phenolic compounds.

Another probable explanation is that related to the cleavage of the glycosidic bonds of certain molecules, which release phenolic compounds, or to a conformational change in the structure of the molecules [16]. In fact, the changes in phytochemical content, produced under the effect of gamma radiation, are related to several factors, including the type of radiation, the dose applied, the exposure time and even the water content. The latter is the cause of the indirect effects of gamma radiation, induced by free radicals that are produced following the radiolysis of water molecules [17, 18].

The study of the antibacterial power of irradiated and non-irradiated *S. aromaticum* extracts showed a positive effect against the strains tested, with a variable degree of sensitivity (Table 2). No effect was exerted by DMSO, used as a negative control.

According to Table 1, a positive effect of the extracts was observed against Gram- strains, *P. aeruginosa* and particularly *E. coli* that showed significant sensitivity with a diameter of 17 mm at 300 mg/ml. This result seems interesting knowing that the Gram- have an outer envelope, which gives them resistance, making the permeability of the bioactive molecules difficult. As for Gram+, better activity was obtained against Methicillin-susceptible *Staphylococcus aureus* (MSSA).

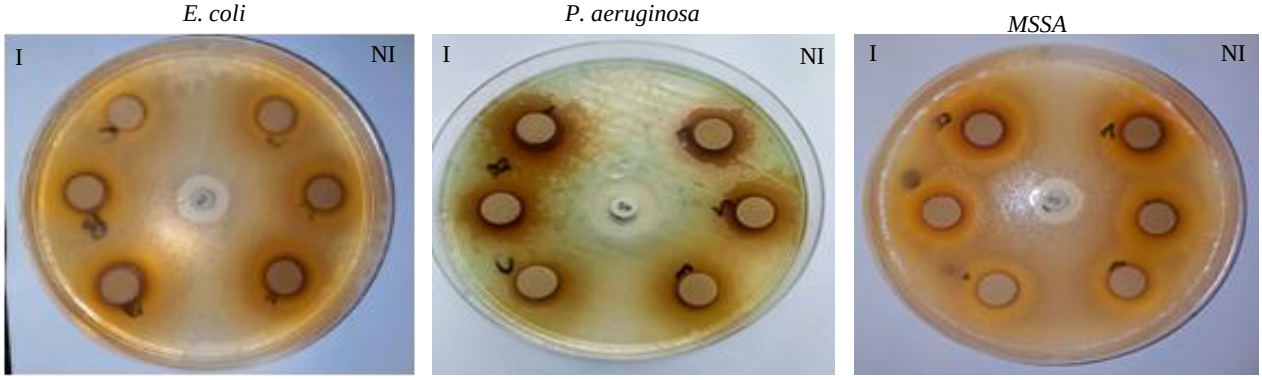
The study of the impact of gamma radiation on *S. aromaticum* revealed a significant improvement in its antibacterial power (Fig.1). Indeed, a better inhibition was obtained against MSSA, giving a diameter of 21 mm for a concentration of 200mg/ml. The inhibition zones of 17.5 and 17 mm corresponded to those of *E. coli* and *P. aeruginosa*, respectively.

Interestingly, sensitivity to low concentrations (25 and 50 mg/ml) of irradiated *Syzygium aromaticum* extracts was observed for MSSA and *E. coli*. This reflects the beneficial effect of gamma radiation.

The preservation of the antibacterial activity of plants has been demonstrated by several authors [2, 19, 20] who found no effect on the antimicrobial potency for doses of irradiation reaching 10kGy.

Table 2: Study of the antibacterial activity of *Syzygium aromaticum* extract

Sample Concentration (mg/ml) / Bacterial strains	Inhibition zone diameter (mm)										
	[25]		[50]		[100]		[200]		[300]		CST
	NI	I	NI	I	NI	I	NI	I	NI	I	
***Pseudomonas aeruginosa* ATCC 27853**	-	-	-	-	11	11	14	17	15	19	17
***Escherichia coli* ATCC 25922**	-	-	-	12,5	14.5	14,5	16	17,5	17	18	13
***Methicillin-susceptible Staphylococcus aureus (MSSA)* ATCC 25923**	-	13,5	-	17,5	15.5	19	16.5	21	17	21	9



NI: Non-irradiated Sample; I: Irradiated Sample

Fig. 1. Zones of strain growth inhibition by *Syzygium aromaticum* extracts

On the contrary, some studies have noticed an improved effect of extracts after irradiation [21], as observed in our study. This is probably linked to the increased content of total polyphenols (see the quantitative assay), in other words the existence of bioactive molecules having either a direct action on the pathogenic agent or by acting in synergy with other molecules [22] to enhance the effect. Additional analyzes would be necessary to provide more information.

4. Conclusion

The treatment of medicinal herbs and spices by gamma radiation, as a physical and non-thermal method, appears effective for not only decontamination, disinfection and shelf life but also for modifying the composition of extracts in bioactive molecules as well as their biological properties. However, the modification levels remain important to consider because they can vary depending on the product to be treated, irradiation dose delivered, and the type of radiation source employed.

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Conflict of Interest

The authors declare that they have no conflict of interest

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