Evaluation of antimicrobial activity of extracts of chamomile (*Matricaria recutita* L.) obtained from irradiated and non-irradiated samples

E.G. Solidônio[1,2,3], **Y.S. Burle**[1], **P.A. Silva**[1], **M.C.V. Vicalvi**[1,3], **I.F.A.C. de Souza**[1], **G.R. Silva**[1], **K.X.F.R. Senna**[1] and **W. Colaço**[3]

[1] Department of antibiotics, Federal University de Pernambuco, Av. Prof. Artur de Sá, S/N. Cidade Universitária, CEP: 50740-520, Recife-PE, Brazil
[2] Integrated College of Pernambuco, Avenue Caxangá, 4477 - Várzea, Recife - PE, 50740-000, Recife-PE, Brazil
[3] Department of Nuclear Energy, Federal University de Pernambuco, Av. Prof. Luiz Freire, 1000, Cidade Universitária, CEP: 50740-000. Recife-PE, Brazil

The objective of this study was to evaluate antimicrobial activity through the disc diffusion test of chamomile extracts obtained from irradiated and non-irradiated samples. Three brands of chamomile tea sachets marketed in Recife-PE were used. The material was subjected to gamma irradiation in doses of 6 kGy, 8 kGy and 10 kGy at a dose rate of 4.07 $kGyh^{-1}$. In the extraction, cyclohexane solvent, ethyl acetate and ethanol were used. A total of 36 extracts were generated; 12 extracts of each brand. The antimicrobial activity of the extracts was evaluated by the diffusion method in paper disc. Growth inhibition was observed of *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Candida albicans.* It was noticeable activity against Gram negative strains (*Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*). Chamomile has potential to be used in the production of antifungal and antibacterial. There was no significant effect of irradiation of the samples in the antimicrobial activity of the extracts.

Keywords: Antimicrobials; Radiation; Bacillus; Staphylococci; Streptococci; Yeasts

1. Introduction

Known, since ancient times, by the Egyptian, Greeks and Romans for their medicinal, cosmetic, decorative and aromatic properties, chamomile is a herbaceous plant native to from Europe and northern Asia, extensively cultivated in Hungary, Romania, Bulgaria, Germany, Greece and Egypt. Consumption in Iran has a long history in folk medicine. It is recognized as an official drug in 26 countries and is on the U.S. Pharmacopoeia and the British list. It is one of the herbs most commonly used by Bolivian and Peruvian immigrants living in London, is also much used in the countries of origin. In Brazil, is in the National List of Medicinal Plants of Interest to SUS - RENISUS [1, 2, 3, 4, 5, 6].

Chamomile is widely used in traditional medicine due to analgesic properties, anti-allergic, antispasmodic, antibacterial, anti-inflammatory, sedative, healing, anti-proliferative cancer cells, cytotoxic against cancer cells [7, 8, 9, 10, 4, 11, 12, 13, 14].

The essential oil is mentioned as a bactericide against gram positive microorganisms such as *Staphylococcus duwus* and a fungicide against *Candida albicans* [1]. The mouthwash with chamomile was considered satisfactory in reducing gingival inflammation, showing great performance in reducing bacterial plaque index [15].

With the great development of medicine and chemistry after the Second World War, synthetic drugs were gradually replacing the use of traditional medicine. However, in the last few years has been occurring a revaluation of these products by the industry, increased by the increased use of the population that seeks options to allopathic medicines aiming at better quality of life [16].

The drug resistance of human pathogens is a major theme reported in both developed and developing countries. On the European continent the consumption of over one ton daily of antibiotics has led to a large increase in resistance of bacterial populations, thus causing a serious public health problem [17]. The search for new antimicrobial substances from natural sources, including herbal products, has gained importance in pharmaceutical companies and scientific research [18].

International trade of herbal products is a major force in the global economy, there are over 1,000 companies producing medicines from plants, with annual revenues exceeding U.S. $ 60 billion in USA [19].

With the increased consumption, also grows the responsibility of regulatory agencies and the manufacturers, aiming to be guaranteed the quality and the therapeutic efficacy [20]. The treatment of food with ionizing radiation is a known method to improve the safety of a variety of products and extend the period of validity. In particular, irradiation is an effective way to eliminate or reduce pathogenic and spoilage microorganisms [21, 22, 23, 24].

Irradiation causes certain changes in the molecules, the main changes are: excitation or ionization leading to the formation of electrically charged species (ions) by means of losing an electron, these are the direct products of radiolysis; production of fragments with unpaired electrons (free radicals), which also result from direct radiolysis; cleavage of chemical bonds of molecules of the food; and production of new stable molecules called indirect radiolysis products as a result of the reorganization of chemical bonds or reactions of the highly reactive products [25, 26, 27, 28].

Thus, the aim of this study was to evaluate antimicrobial activity through the diffusion test of chamomile extracts obtained from irradiated and non-irradiated disc.

2. Material and methods

2.1 Selection of samples

Three brands of chamomile tea sachets marketed in Recife-PE were used. The samples were separated into groups: control (samples without any treatment), irradiated with a dose of 6 kGy, 8 kGy and 10 kGy.

2.2 Irradiation of the samples

The samples were transferred to the radiator in the original packaging, or packed in boxes properly identified with corresponding doses. The material was subjected to gamma irradiation in doses of 6kGy, 8kGy and 10kGy in irradiating with cobalt-60 source Gammacell 220 Excel - (MDS Nordion, Ottawa, Canada) at a dose rate of 4.07 $kGyh^{-1}$. The procedure was performed at the Department of Nuclear Energy, Federal University of Pernambuco (UFPE).

2.3 Obtaining extracts

20g samples were weighed initially and it was added 200 ml of cyclohexane being set in static maceration. After 24 hours of contact, at ambient temperature the mixture was filtered, and the tea powder returned to the Erlenmeyer, adding 200 mL of ethyl acetate, leaving at rest for 24 hours at the ambient temperature. The mixture was filtered and the process repeated twice. On the fourth day, there were added 200 ml of ethanol, leaving at rest for 24 hours at the ambient temperature, the mixture was filtered and the process repeated twice. At the end of eight days, 200ml of the cyclohexane extract were obtained, 600 mL of the ethyl acetate extract and 600 mL of ethanol extract. After this period, the extracts were evaporated to dryness under pressure. A total of 36 extracts were generated; 12 extracts of each brand.

2.4 Analysis of the antimicrobial activity of the extracts

The antimicrobial activity of the extracts was evaluated by the diffusion method in paper disc [29]. Disks of 6 mm diameter were impregnated with 10 μL of the extract. The disks were impregnated with a concentration of 2000 μg. Standardized suspensions of strains of micro-organisms test listed below, with 24 hours of cultivation were prepared according to the tube 0.5 McFarland which corresponds to approximately 10^8 CFU (Colony Forming Units). The micro-organisms were sown on media contained in a petri dish with the aid of a sterile swab. The disks were placed on to the surface of seeded media with the micro-organisms test. The plates were incubated at 37°C for a period of 24h. The reading of the results was performed by measuring the diameters of inhibition formed around the discs. The tests were performed in triplicate and the results expressed as the arithmetic mean of three replicates.

Eight micro-organisms were tested: *Staphylococcus aureus* UFPEDA 01; *Micrococcus luteus* UFPEDA 06; *Bacillus subtilis* UFPEDA 16; *Enterococcus faecalis* UFPEDA 138; *Escherichia coli* UFPEDA 224; *Pseudomonas aeruginosa* UFPEDA 39; *Serratia marcescens* UFPEDA 398; *Candida albicans* UFPEDA 1007.

3. Results

The results of the antimicrobial activity of the extracts in cyclohexane based on the arithmetic mean and the standard deviation calculation of inhibition zones are shown in table 1. *Staphylococcus aureus* and *Micrococcus luteus* were inhibited by all the extracts tested, with a mean of inhibition zones in millimeters between 11 (± 0) and 8.66 (± 0.57) *Staphylococcus aureus* and to *Micrococcus luteus* between 14 (± 0) and 11 (± 0). *Candida albicans* was only inhibited by extracts from irradiated from the mark 1. Other micro-organisms tested were not inhibited by extracts from cyclohexane.

The data of the antimicrobial activity of the extracts in ethyl acetate showed that *Micrococcus luteus* was inhibited by all the extracts tested. *Staphylococcus aureus* and *Candida albicans* were inhibited by all extracts except obtained from samples irradiated with 10 kGy of the brand 3. *Bacillus subtilis* was inhibited by extracts from the mark 2 and 3: by the control and irradiated with 6 kGy and 8 kGy. There was also inhibition of *Enterococcus faecalis* by all extracts of brands 1 and 2. Activity against Gram negative strains was no observed (*Pseudomonas aeruginosa, Escherichia coli e Serratia marcescens*) (table 2).

Regarding antimicrobial activity of the extracts in ethanol, *Micrococcus luteus* was inhibited by all the extracts tested. There was inhibition of *Staphylococcus aureus* by extracts of the control, 8 kGy and 10 kGy of mark 2. *Bacillus subtilis* was inhibited by extracts control, 6 kGy and 10 kGy of mark 2. *Candida albicans* was inhibited by extracts of all the marks 2 and 3. No activity against the strains was observed *Pseudomonas aeruginosa, Escherichia coli* and *Serratia marcescens* and *Enterococcus faecalis* (table 3).

Table 1 Antimicrobial activity of extracts from cyclohexane against standard strains of the culture collection of the Department of Antibiotics UFPE (UFPEDA) (halos of inhibition in mm ± standard deviation).

Micro-organism/ Brands		*Staphylococcus aureus*	*Micrococcus luteus*	*Bacillus subtilis*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Serratia marcescens*	*Candida albicans*
	Control	10 (± 1)	11,66 (± 0,57)	0	0	0	0	0	0
BRAND 1	**6 kGy**	11 (± 0)	13 (± 0)	0	0	0	0	0	10,33 (± 0,57)
	8 kGy	9,66 (± 0,57)	13,33 (± 0,57)	0	0	0	0	0	9 (± 0)
	10 kGy	10,66 (± 0,57)	11 (± 0)	0	0	0	0	0	11 (± 0)
	control	10,66 (± 0,57)	11,66 (± 0,57)	0	0	0	0	0	0
BRAND 2	**6 kGy**	10 (± 0)	13 (± 0)	0	0	0	0	0	0
	8 kGy	11 (± 0)	12,33 (± 1,15)	0	0	0	0	0	0
	10 kGy	10,66 (± 1,15)	12 (± 0)	0	0	0	0	0	0
	Control	8,66 (± 0,57)	11,33 (± 0,57)	0	0	0	0	0	0
BRAND 3	**6 kGy**	8,66 (± 0,57)	14 (± 0,57)	0	0	0	0	0	0
	8 kGy	9,33 (± 0,57)	12,33 (± 0,57)	0	0	0	0	0	0
	10 kGy	9,33 (± 0,57)	14 (± 0)	0	0	0	0	0	0

4. Discussion

Bacteria are present long before human evolution and bacterial diseases probably co-evolved with each species that involuntarily it hosts [30]. Micrococcus species are isolated from human clinical samples, representing contaminants from the skin or mucous membranes, can behave as opportunistic agents in immunocompromised patients. These species have been reported to cause pneumonia, meningitis, bacteremia, sepsis [31]. One of micro-organisms most widely studied is *Staphylococcus aureus*, by being involved in a wide variety of infectious processes, the ability to develop antimicrobial resistance and represent a major cause of hospital acquired infections, contributing to increases in the rates of morbidity and mortality [32]. Highlighting the importance of the discovery of new agents against these bacteria.

Antibiotics are natural or synthetic compounds able to inhibit growth or cause death of bacteria or fungi [33]. Dučaiová *et al*. (2013) [34] highlight the phytotherapic effect of chamomile attributed to the amounts of related compounds of coumarin, which show antimicrobial activity. McKay and Blumberg (2006) [35] reported the antimicrobial activity of chamomile tea.

Chamomile oil at a concentration of 25 mg ml^{-1}, showed an antibacterial activity against Gram-positive strains *Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans and Streptococcus salivarius*, well as some fungicidal activity against *Candida albicans* [36]. Results close to those found in this study in wich was observed that inhibition *Bacillus subtilis, Staphylococcus aureus* and *Candida albicans.*

Table 2 Antimicrobial activity of ethyl acetate extracts against standard strains of the culture collection of the Department of Antibiotics UFPE (UFPEDA) (halos of inhibition in mm ± standard deviation).

Micro-organism/ Brands		***Staphylococcus aureus***	***Micrococcus luteus***	***Bacillus subtilis***	***Enterococcus faecalis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Serratia marcescens***	***Candida albicans***
	Control	9,66 (± 0,57)	15 (± 0)	0	9,66 (± 0,57)	0	0	0	9,33 (± 0,57)
BRAND 1	**6 kGy**	9 (± 0)	13,66 (± 1,15)	0	9,66 (± 0,57)	0	0	0	10,33 (± 1,15)
	8 kGy	9,33 (± 0,57)	13 (± 1)	0	9,66 (± 0,57)	0	0	0	9 (± 0)
	10 kGy	9,33 (± 0,57)	15,66 (± 0,57)	0	9,66 (± 0,57)	0	0	0	9,33 (± 0,57)
	Control	9 (± 0)	9 (± 0)	9 (± 0)	9,66 (± 0,57)	0	0	0	9,33 (± 1,52)
BRAND 2	**6 kGy**	9,33 (± 0,57)	8,33 (± 0,57)	9 (± 0)	9,66 (± 0,57)	0	0	0	9,33 (± 1,52)
	8 kGy	9 (± 0)	9 (± 1)	9 (± 0)	9,66 (± 0,57)	0	0	0	9 (± 1)
	10 kGy	9,66 (± 0,57)	8,66 (± 1,15)	0	9,66 (± 0,57)	0	0	0	8,66 (± 0,57)
	Control	9 (± 0)	12 (± 1)	8,66 (± 0,57)	0	0	0	0	10,66 (± 1,15)
BRAND 3	**6 kGy**	9 (± 0)	11,66 (± 0,57)	8 (± 0)	0	0	0	0	9 (± 1,73)
	8 kGy	9,33 (± 0,57)	13,66 (± 0,57)	8 (± 0)	0	0	0	0	9 (± 1)
	10 kGy	0	11 (± 1)	0	0	0	0	0	0

Methanol extract of chamomile showed activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Cryptococcus neoformans* [37]. The data of Coelho *et al*. (2012) [37] are close to this study, since the activity of some extracts against *Bacillus subtilis* and the yeast *Candida albicans* was observed. However differs as to *Pseudomonas aeruginosa*.

Nogueira *et al*. (2008) [38] evaluated the antimicrobial activity of the essential oil of chamomile against strains isolated from samples of external otitis, noting inhibitory activity on the growth of three strains of *Staphylococcus* and two strains of *Candida*, with inhibition zones varying from 10 to 12mm diameter. Close to those found in the study, with halos of up to 11mm for the two micro-organisms.

Aliheidari *et al*. (2013) [39] suggest that because of the greater resistance of Gram negative to chamomile essential oil is the double membrane more complex involving these microorganisms in comparison with single membrane glycoprotein of Gram positive bacteria. What could explain the lack of activity of the extracts tested at work for gram-negative bacteria.

The essential oil of flower of *M. recutita* was described as a strong inhibitor of mold growth in all 10 tested species of fungi (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Trichoderma harzianum*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*), including major pathogenic dermatophytes and opportunistic saprophytes [40] . Antifungal activity of the extracts evaluated in this study was also observed.

Table 3 Antimicrobial activity of ethanol extracts against standard strains of the culture collection of the Department of Antibiotics UFPE (UFPEDA) (halos of inhibition in mm ± standard deviation).

Micro-organism/ Brands		*Staphylococcus aureus*	*Micrococcus luteus*	*Bacillus subtilis*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Serratia marcescens*	*Candida albicans*
BRAND 1	**control**	0	12 (± 1)	0	0	0	0	0	0
	6 kGy	0	12 (± 1)	0	0	0	0	0	0
	8 kGy	0	11,33 (± 0,57)	0	0	0	0	0	0
	10 kGy	0	11,66 (± 0,57)	0	0	0	0	0	0
BRAND 2	**control**	9 (± 0)	12,66 (± 1,15)	8,33 (± 0,57)	0	0	0	0	9,33 (± 0,57)
	6 kGy	0	8,66 (± 0,57)	8,66 (± 0,57)	0	0	0	0	10 (± 1)
	8 kGy	9,66 (± 0,57)	13,66 (± 0,57)	0	0	0	0	0	10 (± 1)
	10 kGy	9 (± 0)	13 (± 0)	8,66 (± 0,57)	0	0	0	0	9 (± 0)
BRAND 3	**control**	0	14,66 (± 2,31)	0	0	0	0	0	9 (± 1)
	6 kGy	0	13 (± 1)	0	0	0	0	0	8,66 (± 0,57)
	8 kGy	0	11 (± 0)	0	0	0	0	0	9,66 (± 0,57)
	10 kGy	0	11 (± 0)	0	0	0	0	0	9,66 (± 0,57)

Chamomile can be considered a potential candidate for the commercial production of antifungal agents suitable for the treatment of dermatophytosis and other important fungal infections as well as in the treatment of diseases caused by gram -positive bacteria.

No significant effect of irradiation of the samples in the antimicrobial activity of the extracts was observed.

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