Original Research Article

Properties and Effect of Fresh Concentrated Extract of Garlic on Different Bacteria and Fungi

ABSTRACT

Aims: Study some characteristics of fresh concentrated extract of garlic (FCEG) and analyze its effect on the growth of different bacteria and fungi.
Place and Duration of Study: Sample: Faculty of Chemical Sciences. Autonomous University of San Luís Potosí, S.L.P., between march and November 2020.
Methodology: To obtain the FCEG, 15 heads of raw garlic, previously peeled, were ground in a mortar and the suspension obtained was filtered through gauze, pressing to obtain a greater amount of filtrate, and kept covered at 4°C. For the study of some of their characteristics, the yeast *Candida albicans* was used. Petri dishes containing Sabouraud dextrose agar were inoculated with 1 x 10^6 yeasts/mL and 50 µL of FCEG, and were incubated at 28°C for 5 days, comparing growth with respect to a control without FCEG (all inhibition experiments described below were performed with the same protocol), while for the study of antifungal properties analyzed the effect of the extract on different strains of bacteria, yeasts, and fungi.
Results: Some of the factors that modify antifungal activity of FCEG are dilution, incubation temperature, protein concentration, half-life, capping and uncapping of tubes, and treatment with activated carbon. Regarding the growth inhibition analyses, it was found that all the species analyzed were susceptible to FCEG, among which the following stand out: the fungi *Histoplasma capsulatum*, the yeasts *Cryptococcus neoformans* and *C. albicans*, as well as different species of Dermatophytes, *Aspergillus,* and bacteria. **Conclusion:** FCEG shows a good antimicrobial effect against a wide variety of bacteria, yeast, and fungal species, which makes its application in medical therapy and agriculture possible, as well as being cheap, easy to obtain and does not cause side effects, although more studies are required for its therapeutic application.

Keywords: Garlic, properties, antimicrobial effect, bacteria, yeast, fungi

1. INTRODUCTION

Antimicrobial resistance (AMR) occurs in viruses, bacteria, fungi, and parasites as a manifestation inevitability of its capacities to evolve. The decreased effectiveness of antimicrobials existing is a consequence of the very complex interaction between natural selection, environment, and patterns of use of these drugs. This has given result that AMR has become a problem of global public health: as the variety of existing pathogens that affect humans and animals have developed various mechanisms to defend against available antimicrobials [1]. AMR is acquired through genes that code for resistance to antibiotics or through mutation or alteration of some gene. These mutations can be intrinsic or spread through community's microbial cells vertically (by inheritance) or in horizontally (by using elements mobile genes and extrachromosomal plasmids). This genetic diversity among populations microbial combined with a generation time very quickly, particularly among bacteria, provides microorganisms with a response extraordinary adaptation to the selective pressure of antimicrobials [2]. Therefore, are required approaches multidisciplinary and multisectoral like One Health or other related concepts such as Planetary Health or Eco Health that involve the disciplines of human, animal, and environmental health in a context of synergy of capabilities and experience. Also, a wire is required driver who can amalgamate the efforts of the different disciplines with an intersectoral and inter-disciplinary approach to optimize these

sources allocated to AMR control, improve epidemiological surveillance, implement measures mitigation of its effects, as well as protection for the population at higher risk of acquiring multidrug-resistant infections [1, 3].

Other lines of research in antimicrobial antibiotics have focused on the use of some natural or modified plant extracts, and other natural compounds, with highly satisfactory results, such as: the effect of essential oils of oregano (*Oreganum compactum*), thyme (*Thymus vulgaris*), and rosemary (*Rosmarinus officinalis*) against *Aspergillus niger* [4], the study of the fungi toxic effects of *Allium sativum* (L) and *Ocimum gratissimum* (L.) using aqueous extraction methods on six fungal pathogens [5], a bioactive compound (trichodermin) isolated from the culture extracts of the fungus *Trichoderma brevicompactum*, and has a marked inhibitory activity on *Rhizoctonia solani* and *Botrytis cinerea* [6], the antileishmanial activity of a mixture of *Tridax procumbens* and *A. sativum* in mice [7], the bacterial effect of the extract of *Coriandrum sativum* on bacteria gastrointestinal pathogens [8], the antibacterial activity, of six essential oils and five ethanolic extracts from different Colombian plants, against Gram positive and Gram negative bacteria [9], the antifungal activity of *Euphorbia tirucalli* L against *Cryptococcus neoformans* strains [10], and the effect of different aqueous extracts of plants on the in vitro mycelial growth of phytopathogenic fungi [11].

Another line of research in antimicrobial antibiotics has focused on the use of garlic, to which it is common to attribute a wide variety of properties, among which are: antifungal and antileishmanial activity [4, 7], the treatment of infections that does not affect the central nervous system [12], the in vitro anticoagulant effect of garlic extract on the blood coagulation cascade [13], evaluate the relative antidiabetic potential of garlic aqueous [14], and other medicinal properties such as: thorax, asthma, and skin diseases, blood disorder, hypertension, heart attack, antioxidant, etc., [15]. Regarding the antimicrobial antifungal properties of garlic, there are a wide variety of studies, among which are: the antifungal activity of some essential oils and their major phenolic components against *A. niger* [4], the in vitro effects of garlic (*A. sativum* L.) on fungal pathogens isolated from rotted *Cassava* roots [5], their antimycotic activity against *Trichophyton rubrum* [16, 17], *Alternaria tenuissima* [18], some Dermatophytes and *Candida albicans* [19, 20], *Histoplasma capsulatum* var. *capsulatum* [21], *Moniliophthora roreri* [22], *Aspergillus parasiticus y A. niger* [23], and the effect of garlic in the treatment of Saprolegniasis in rainbow trout (*Oncorhynchus mykiss*). Saprolegnia was long considered a fungus, but recently by consensus a new kingdom was created, thus separating it from the fungi kingdom and inserting it into the new kingdom: the Chromista Kingdom, which is much closer to algae than to fungi [24].

Too was studied their effect antimicrobial like against *Escherichia coli, Staphylococcus aureus,* and *A. niger* [25, 26,27, and 28], *Streptococcus mutans* [29], molds, yeasts, coliforms, and *E. coli* [30] on the Gram positive bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* and other Gram negative bacteria like *E. coli* and *Shigella dysinteriae* [31]. The information consulted supports the health benefits associated with garlic consumption. The crushing of garlic bulbs allows obtaining alliin, which turns into allicin due to enzymatic oxidation. This compound has a fundamental role in the garlic's medicinal properties, and the garlic plant's activity depends on its ability to produce allicin. Preclinical studies have shown the improvement of the immune system, and specific proteins associated with its immunostimulant effect have been identified. In clinical studies, supplements with garlic derivatives have been administered to patients, and a decrease in the incidence of influenza and acute respiratory diseases has been observed [32]. But there are few reports of its effect against environmental contaminating fungi, and of some of its properties such as: its half-life, storage temperature, the effect of different dilutions, and the minimum inhibitory concentration of the FCEG. Therefore, the objective of this work was to analyze some characteristics of FCEG, and their effect on the growth of different bacteria and fungi, in order, for in the future, obtain a product that is competitive with the common antimycotics used in the market.

2. MATERIAL AND METHODS

2.1Obtaining of fresh extract of garlic (FCEG)

To obtain the FCEG, 15 heads of raw garlic (from the Republic market in the City of San Luis Potosí, México), previously peeled, are placed in an extractor and ground to obtain a garlic suspension, which is filtered through gauze, pressing the extract for better performance. Subsequently, it is transferred to an amber bottle and stored at 4°C.

2.2Analysis of the properties of FCEG

For the study of the properties of the FCEG, the dimorphic fungus yeastlike *C. albicans* was used, due to its ease in quantifying the number of yeasts, and its rapid growth in the medium used: Sabouraud Dextrose Agar (SDA).

2.2.1Effect of different FCEG dilutions on the growth of *C. albicans*

100 µL of the FCEG are token, and diluted with different volumes of sterile saline solution (SS) at 0.85% (w/v), according to the following Table:

Table 1. Dilution of the FCEG with saline solution

Dilution	FCEG (µL)	Saline solution (µL)
Concentrate	50	0
1:10	100	900
1.100	10	990
1.1000	10	9990

Subsequently, aliquots of 50 µL are taken from each of the dilutions to be analyzed, and the antifungal effect is analyzed at 1 x 10^6 yeast/mL of *C. albicans*, incubating at 28°C, for 5 days in SDA, comparing growth with respect to a control without FCEG.

2.2.2Effect of different FCEG concentrations on the growth of *C. albicans*

Different FCEG concentrations are token (0-50 µL=0-1.0 mg/mL of protein) and are diluted with different volumes of sterile saline solution (SS) to the 0.85% (w/v) and were added 1 x 10^6 yeast/mL of *C. albicans*, incubating at 28°C, for 5 days in SDA, comparing growth with respect to a control without FCEG.

2.2.3Effect of FCEG (50 µL= 1.0 mg/mL of protein) on the growth of different concentrations of *C. albicans*

To different *C. albicans* concentrations (1 x 10^6 to 10 x 10^6 yeast/mL) spread in Petri dishes, they were added with 50 µL of FCEG, incubating at 28°C, for 5 days in SDA, comparing growth with respect to a control without FCEG.

2.2.4Half-life of antifungal activity of FCEG to 4°C

The FCEG in covered containers was incubated at 4°C for 60 days, to determine the time in which it loses its antifungal properties, it was analyzed by sowing every 5 days 1 x 10^6 yeast/mL of *C. albicans* in SDA in the presence of 50 µL of FCEG, incubating at 28°C, for 5 days, comparing growth with respect to a control without FCEG.

2.2.5Effect of tube capping on the antifungal activity of FCEG at 4°C and 28°C

The FECG is aliquoted by duplicate in volumes of 5 mL in 8 mL test tubes and incubated uncovered at 4°C and 28°C for 24 hours, taking aliquots of 50 µL from each tube every 4 hours, and added to Petri dishes containing 1 x 10^6 yeast/mL of *C. albicans,* to determine the effect antifungal seeded in SDA at 28°C for 5 days.

2.2.6Effect of the temperature on the antifungal activity of FCEG

The FECG is aliquoted by duplicate in volumes of 5 mL in 8 mL test tubes and incubated at 60°C for 60 minutes, taking aliquots of 50 µL from each tube every 20 minutes, and added to Petri dishes containing 1 x 10^6 yeast/mL of *C. albicans,* to determine the effect antifungal seeded in SDA at 28°C for 5 days.

2.2.7Effect of adsorption in activated carbon on the antifungal activity of FCEG

5 g of activated carbon (CAGR, ground and grain) were placed in 125 mL Erlenmeyer flasks, and 20 mL of FCEG were added, incubating them at 28°C for 6 days under constant agitation (100 rpm). Subsequently, aliquots of 50 µL were taken every 24 hours, and added to Petri dishes containing 1 x 10^6 yeast/mL of *C. albicans,* to determine the effect antifungal seeded in SDA at 28ºC for 5 days.

2.3Effect of FCEG on Lee medium filamentation for *Candida albicans*

Resuspend a pellet of a young colony suspected of *C. albicans* in 0.5 mL of Lee's medium and 50 µL of FCEG, were incubate at 37°C for 2½ to 3 hours. A fresh examination is performed to observe the development of the germ tube. This test is positive for *C. albicans* and *Candida dubliniensis* if a germ tube of approximately 5 to 15 µm long from the yeast, should not present constriction at the point of origin. The germ tube is like a hand mirror. After 3 hours of incubation, all *Candida* species can form tubes germ cells, except *Candida glabrata* [33].

2.4Determination of the antifungal effect of FCEG on the growth of different fungi

Samples of the different species of fungi (obtained from Laboratory of Experimental Mycology/FCQ/UASLP) to be analyzed were taken and resuspended in 1 mL of SS. Subsequently, 50 µL aliquots of each suspension of the different fungi were taken and inoculated in petri dishes containing SDA, and 50 µL of the FCEG were added, spreading with a glass rod in the form of a triangle, and they were incubated at 28°C for 7 days, and the growth was compared with the controls of the different fungi seeded without FCEG.

2.5Determination of the antimicrobial effect of FCEG on the growth of different bacteria

Samples of the different species of bacteria (obtained from Laboratory of Microbiology/FCQ/UASLP) to be analyzed were taken and resuspended in 10 mL of McFarland solution. Subsequently, 50 µL aliquots of each suspension of the different bacteria were taken and planted in petri dishes containing SDA, and 50 µL of the FCEG were added, spreading with a glass rod in the form of a triangle, and they were incubated at 35°C for 24-48 hours, and the growth based on the optimum density obtained from McFarland turbidity standard was compared with the controls of the different bacteria seeded without FCEG [34].

2.6Determination of Protein

This was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard [35].

3. RESULTS AND DISCUSSION

3.1Analysis of some properties of FCEG

In this work, some properties of the antifungal activity of FCEG were analyzed, such as: half-life, temperature, effect of different dilutions, minimum inhibitory concentration, etc., using the fungus like yeast *C. albicans* as standard, obtaining the following results summarized in Table 2:

Table 2. Analysis of some properties of the FCEG.

Properties	Results	Figure in text
Dilution	Any dilution inhibits its antifungal properties	1

Minimum inhibitory concentration	40 and 50 µL (0.8 and 1 mg/mL of protein)	2
Effect of the growth of different Concentrations of *C. albicans*.	50 µL inhibit the growth of the yeast in different percentage	3
Half-life of the activity	55 and 60 days at 4°C in covered containers	4
Storage in uncovered containers	Activity is lost in 24 hours at 4°C and 28°C, and in 60 minutes at 60°C	5, 6
Partial purification	The treatment eliminates the antifungal activity at 6 days a 28°C.	7
Effect on the germ tube induction	Significant inhibition	8

In relation to these parameters, there are few related reports, like: for evaluate the antimould activity of oregano, thyme, rosemary, and clove essential oils and some of their main constituents: eugenol, carvacrol and thymol against *A. niger,*

and all the investigated agents showed no inhibitory effect on this fungus, which grow at concentrations lower than 10% (v/v) [4], for the lyophilized extract of garlic diluted to 10%, the minimum inhibitory concentration was 500 ug/mL for *T. mentagrophytes, T. rubrum* and *Microsporum canis* [19], the antifungal activities of fresh garlic (*A. sativum*) and ginger (*Z. officinale*) on the growth of three known pathogenic fungi were investigated. The test organisms were *Aspergillus* spp., Penicillium spp., and *C. albicans*. Under the conditions analyzed the more concentrated extracts (100 g/50 mL) inhibited the fungi growth more than the less concentrated extracts (100 g/100 mL). The actions of these extracts in these organisms were more on garlic. Between the two extracts used, the best in terms of inhibition is garlic followed by ginger [20]. The ajoene inhibits the development of *H. capsulatum* in mycelial phase at concentrations of 1.25 to 4.3 g/mL [21]. Too was evaluate the effect of the garlic crude extract on the in vitro development of *A. parasiticus* and *A. niger* to control the occurrence of these fungi on crops and other foods. The effect of the extract on the development of the fungi was evaluated by measuring of inhibition zones, puncture, colony forming units (CFU) and determination of minimum inhibitory concentration (MIC). The interaction of the garlic crude extract with the fungi was observed using visible light microscopy. The garlic crude extract induced inhibition zones of 12 mm in *A. parasiticus* and 15.5 mm for *A. niger*, it also inhibited growth in 13 and 46.8% respectively. The CMI for *A. parasiticus* was found to be the 1:2 dilution (50 µL of crude extract) and the 1:32 dilution (3.12 µL of crude extract) for *A. niger*. The treatment also inhibited the production of mycelium and sporulation of the two fungi species [23]. On the other hand, was analyzed the in vitro sensitivity of an isolate of *T. rubrum* and an isolation of *T. mentagrophytes* against ajoene, finding that this compound was able to inhibit the growth of both isolates showing a minimum inhibitory concentration (MIC) of 60 µg/mL and a concentration minimum fungicide (CMF) of 75 µg/mL [36]. The extract concentration also affects the antimicrobial activity, *Allium* extract concentrated (100%) have more lethal effect, followed by 75, 50, and 25% the least effective, obtained from McFarland turbidity methodology, and *Pseudomonas aeruginosa* showed resistance against extracts of both *A. sativum* and *Allium tuberosum*, with no zone of inhibition. The highest antimicrobial activity of *A. tuberosum* was noticed against *S. aureus* and *B. subtilis* with 43.9 and 40.7-mm zone of inhibition using 100% extract, respectively, followed by 75, 50, and 25%. The least antimicrobial activity was noticed for *Enterococcus faecalis* with zone of inhibition of 20.07 mm in 100% extract, whereas *P. aeruginosa* showed resistance against all concentrations evaluated [37]. Bernaldez and Vicencio (2021), determined the antibacterial activity of soap from garlic extract using the paper-disc method and Kirby-Bauer antibacterial sensitivity test against *S. aureus* and *E. coli*, and to determine the physical properties of garlic soap and the presence of saponin through phytochemical screening. Garlic soap showed antibacterial activity against *E. coli* and *S. aureus*. Mean zone of inhibition was numerically higher in plate extract obtained using garlic soap (14.70 mm-18 mm), compared to commercial soap [38], and was studied the antibacterial activity of the ethanolic extracts of *A. sativum* "Garlic" on *S. aureus* ATCC 25923 cultures. Ethyl alcohol of 96° was used for the extraction of the metabolites of the plants and concentrations at 100%, 75% and 50% were prepared, and the antibacterial activity was demonstrated by the method of disc diffusion in agar or Kirby Bauer, the ethanolic extracts of the *A. sativum* “Garlic” leaf presented inhibition halos of 20,433 mm, 17,126 and 10,659 for the concentrations of 100%, 75% and 50% respectively [39]. With respect to stability, we found that it is completely lost between 55 and 60 days after obtaining it at 4°C in covered containers, and evaluating the suitability of five different garlic cultivars for the processing of unsalted garlic paste, chopped fried garlic, and fried sliced garlic, the concentration of allicin in the products was evaluated immediately after processing and at 45-day intervals during 180 days of storage, and the amount of allicin lost during the process to obtain paste for the different varieties was less than 9.5%, and it reached a maximum loss of 22% for the commercial varieties during storage (180 days) [40].

C. albicans is a dimorphic fungus, which is in the form of yeast when it is in the saprophytic state, while in the parasitic state, it forms filaments (hyphae and pseudohyphae) of variable length. This capacity is related to the pathogenicity of this yeast fungus, in addition to various metabolic products, as well as some components of the cell wall are involved in the mechanisms of pathogenicity by this species [41]. The transition from yeast to hyphae is one of the virulence attributes that enable to *C. albicans* to invade tissues. It has been found that the growth of filamentous form has advantages over yeast in penetrating the cell or tissue, and although the hypha may be suitable to open the gap between tissue barriers, thanks because its tip is the site of secretion of enzymes capable of degrading proteins, lipids, and other cellular components, it facilitates their infiltration into solid and woven substrates [42]. There are three findings that support the hypothesis of that filamentation is required for virulence by this fungus: 1. Filament formation is stimulated at 37ºC in the presence of serum, with neutral pH. 2. The newly formed filaments (called tubes germ cells) are more adherent to cells mammals than yeasts and adherence is the requirement for tissue penetration. 3. Yeasts captured by macrophages produce filaments and can lyse them, therefore, the formation of filaments is a form to evade host defense mechanisms [43, 44]. And finally, in this work, the FCEG significantly inhibits germ tube induction in *C. albicans*, which can be very important and effective for the treatment of diseases related to *C. albicans*, but more studies are required to determine the role of germination inhibition, in addition to the fact that in the literature we did not find related reports.

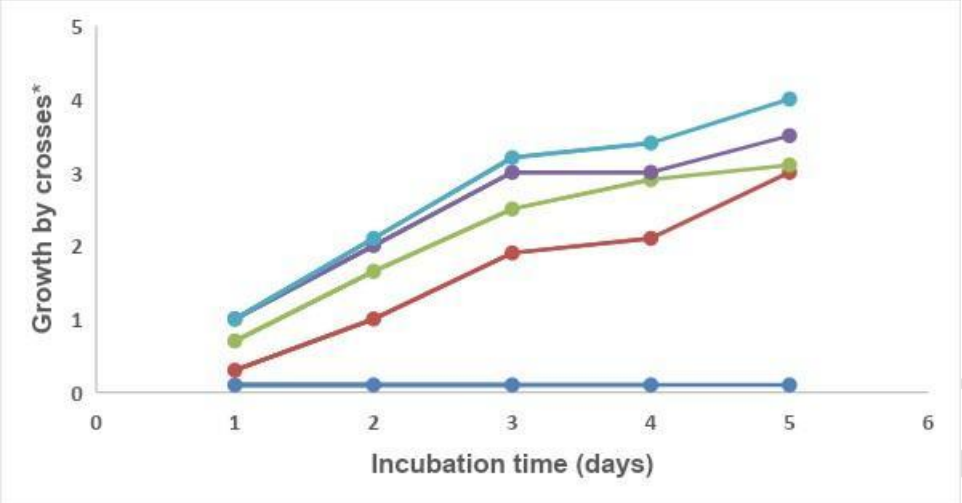


Fig. 1. Effect of different FCEG dilutions on the growth of *C. albicans.* 1 x 10^6 yeast/mL. 28°C. 5 days of incubation. ._____. (without dilution), ._____. (1:10), ._____. (1:100), ._____. (1:1000), ._____. (1:10000).***+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms,++++ 10 cms.**

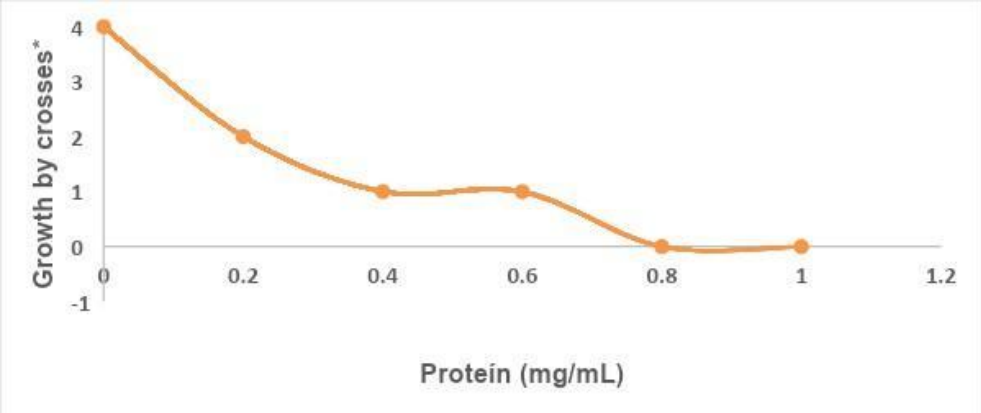


Fig. 2. Effect of different FCEG concentrations (mg(mL) on the growth of *C. albicans.* 1 x 10^6 yeast/mL. 28°C. 5 days of incubation. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

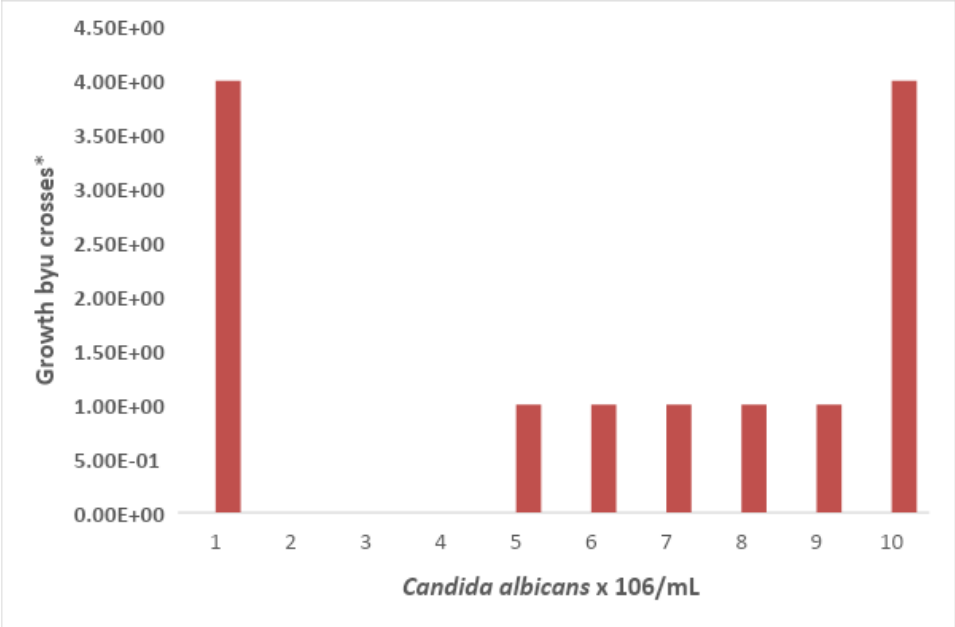


Fig. 3. Effect of FCEG (1 mg/mL) on the growth of different *C. albicans* concentrations 28°C. 5 days of incubation. 1,10 controls without FCEG. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

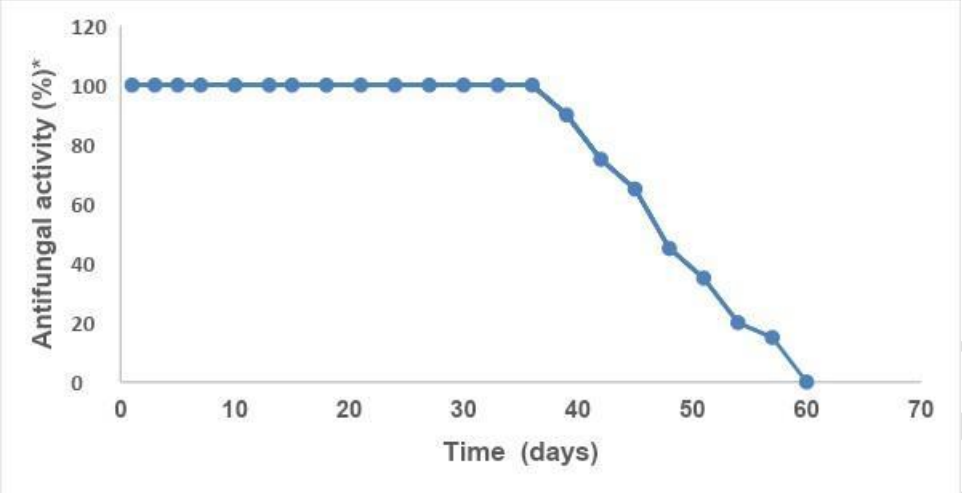


Fig. 4. Half-life of antifungal activity of FCEG to 4°C in 60 days. *C. albicans* (1 x 10^6 yeast/mL). 28°C. 5 days of incubation. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

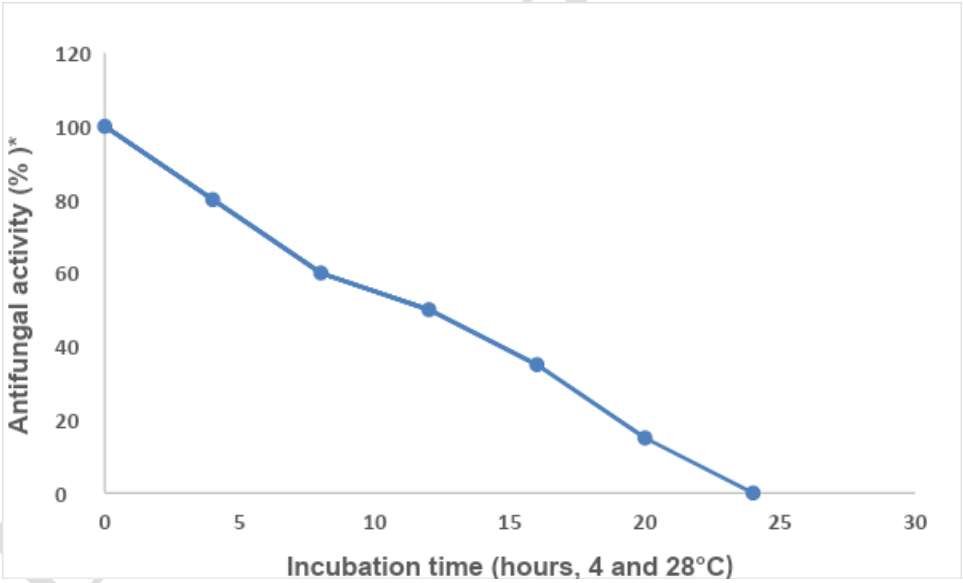


Fig. 5. Effect of tube capping on the antifungal activity of FCEG at 4°C and 28°C. *C. albicans* (1 x 10^6 yeast/mL) 28°C. 5 days of incubation. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

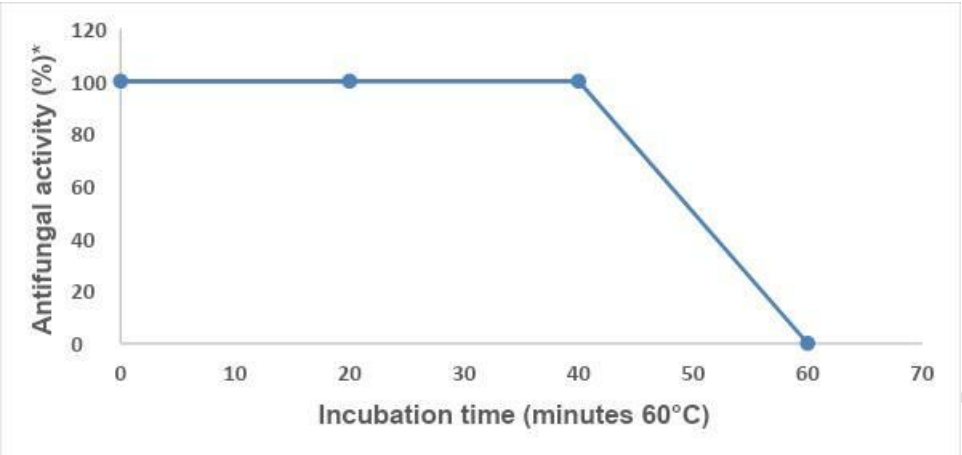


Fig. 6. Effect of tube capping on the antifungal activity of FCEG at 4°C and 28°C. *C. albicans* (1 x 10^6 yeast/mL) 28°C. 5 days of incubation. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

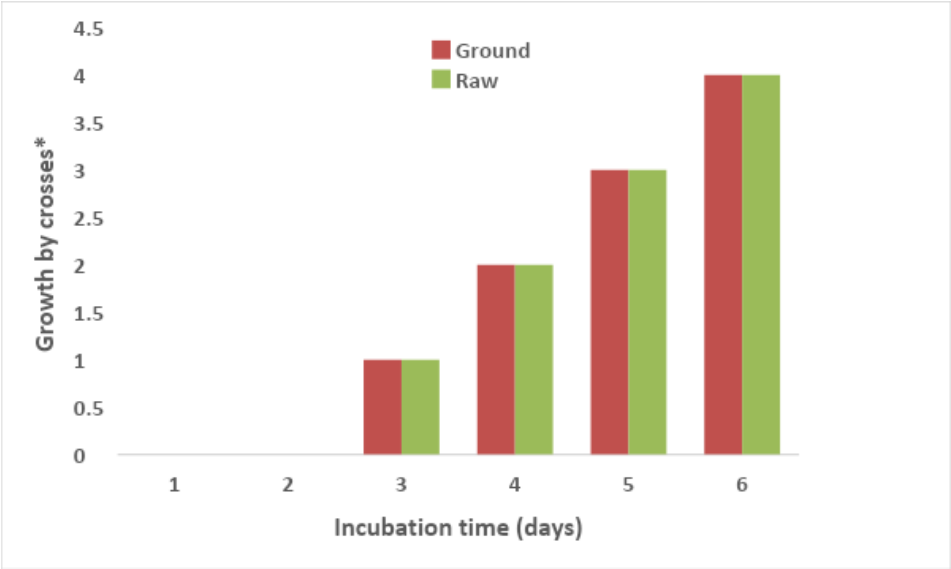
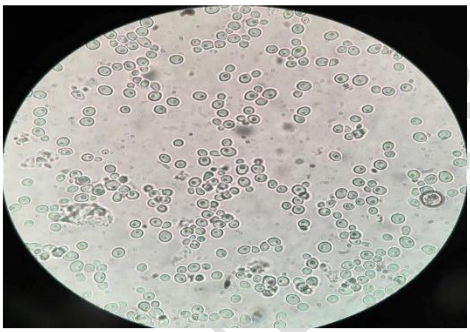


Fig. 7. Effect of adsorption in activated carbon (5 g) on the antifungal activity of FCEG (20 mL). *C. albicans* (1 x 10^6 yeast/mL) 28°C. 5 days of incubation. 100 rpm. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

A



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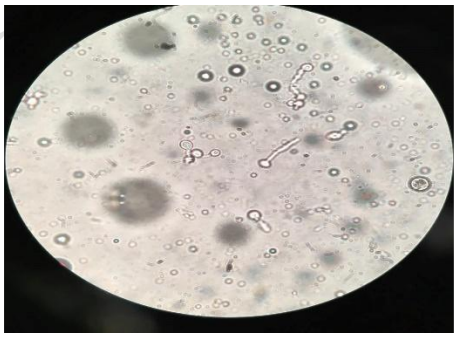

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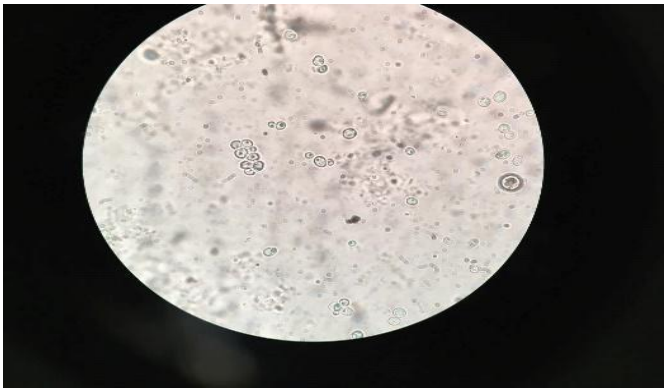

Fig. 8. Effect of FCEG (50 µL) on the filamentation in *C. albicans* in Lee medium.
A.- Control time 0 B.- Positive Control 2½ hours C.- Problem with FCEG (50 µL)

3.2Analisys of the antifungal and antibacterial effect of FCEG on the growth of different fungi.

On the other hand, when we analyze the antifungal effect of FCEG on the growth of different species of fungi and bacteria, it was found that it inhibits completely the growth of all the fungi and bacteria analyzed (fig 9-16), which is like with different reports in the literature, in which the antimicrobial effect of garlic is analyzed under different conditions: such for *Fusarium oxysporum,* it was shown that *A. sativum* was effective at all the extract concentrations tested (25, 50, 75, and 100 g in 100 mL of sterile distilled water), with inhibitions ranging from 41.1 to 76.6% [5], 1% of *A. sativum* extract concentration of 0.0004 mg/mL, inhibit the growth of *T. rubrum* [16], for the ethanolic extract from *A. sativum* bulbs had an antifungal effect on crops of the same dermatophyte at different concentrations (%) [17], the essential oil of garlic for the control of *A. tenuissima*, in which, radial growth in vitro and biomass of this fungus in presence of the garlic essential oil (1000 ppm), the radial growth and biomass production were inhibited 100 and 86.20%, respectively, it compared with control PDA-Tween 80 without oil [18], for lyophilizate garlic, in *C. albicans*, it showed bigger inhibition diameter among 4000 to 5000 µg, a minimum inhibitory concentration of 2500 µg/mL and fungicidal action of 5000 µg [19], and for some dermatophytes [20], also, the inhibitory effect of ajoene on the mycelial phase of *H. capsulatum* [21]. Too, the garlic-chili hydrolysates extracted from maceration and/or Soxhlet extraction with ethanol as solvent, show an effective inhibition on pathogenic fungi like the *Monilia* assuming a similar behavior on it, as well as the hydrolysates of oregano that manage to reach an inhibition percentage of 75.2% at a concentration minimum of 40% for approximately 12 days [22], and the effect against *A. parasiticus* and *A. niger* [23], and Saprolegniasis in rainbow trout (*Oncorhynchus mykiss*) [24] was evaluated, observing that the inhibition is very efficient, and their antimicrobial effect against *E. coli, S. aureus,* and *A. niger* [25,

26,27, and 28], *S. mutans* [29], molds, yeasts, coliforms, and *E. coli* [30] on the Gram positive bacteria *B. subtilis* and *S. epidermidis* and other Gram negative bacteria *E. coli* and *S. dysinteriae* [31].

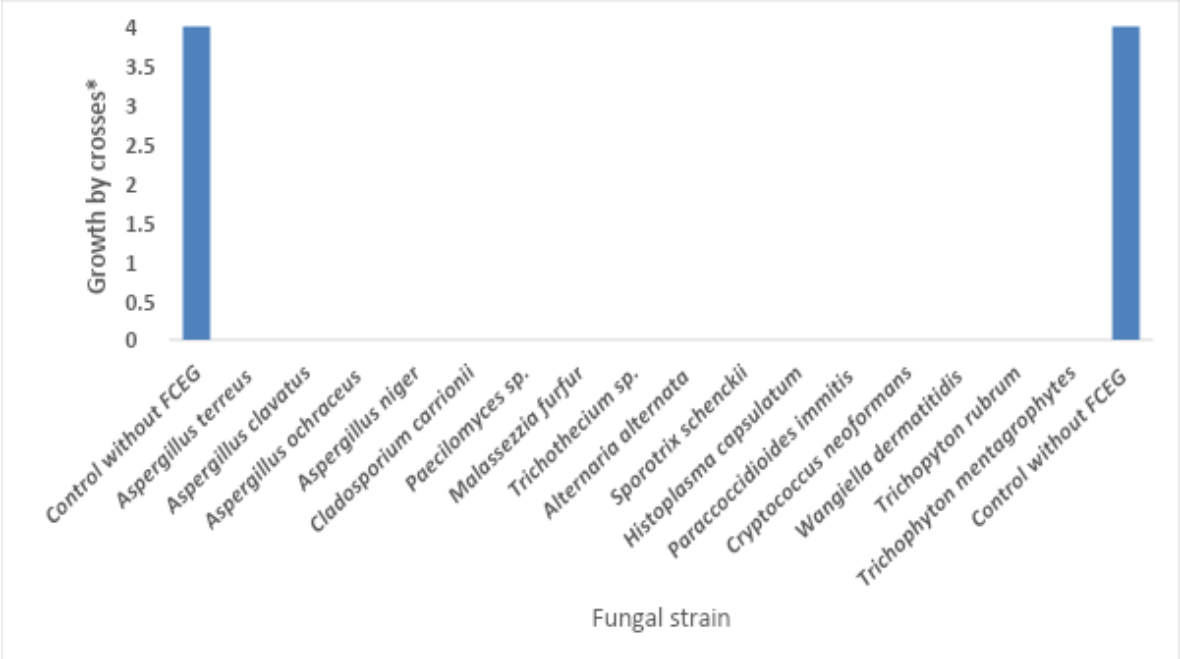

Fig. 9. Effect of FCEG on the growth of different fungal strains. 1 x 10^6 cells/mL. 28°C. 7-15 days of incubation. *+ 2.5 cms, ++ 5.0 cms, +++ 7.5 cms, ++++ 10 cms.

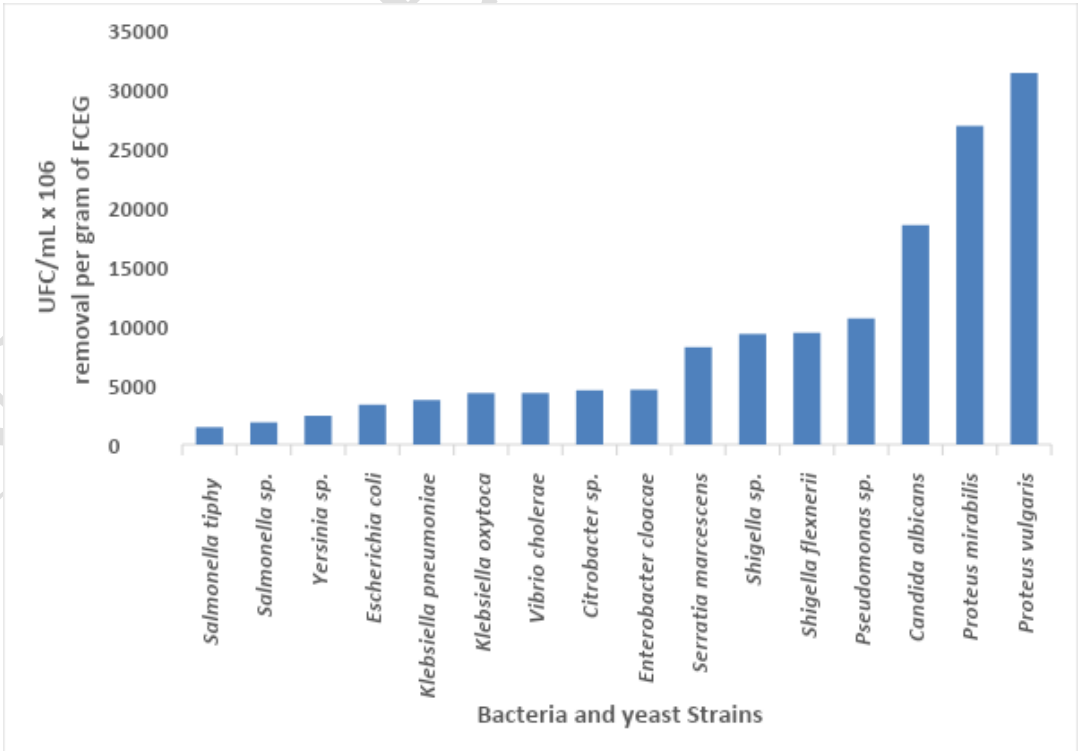

Fig. 10. Effect of FCEG on the growth of different bacteria and yeast strains, by optimum density obtained from McFarland turbidity standard. 1 x 10^6 cells/mL. 35°C. 1-2 days of incubation. (For *C. albicans* 28°C and 5 days of incubation).

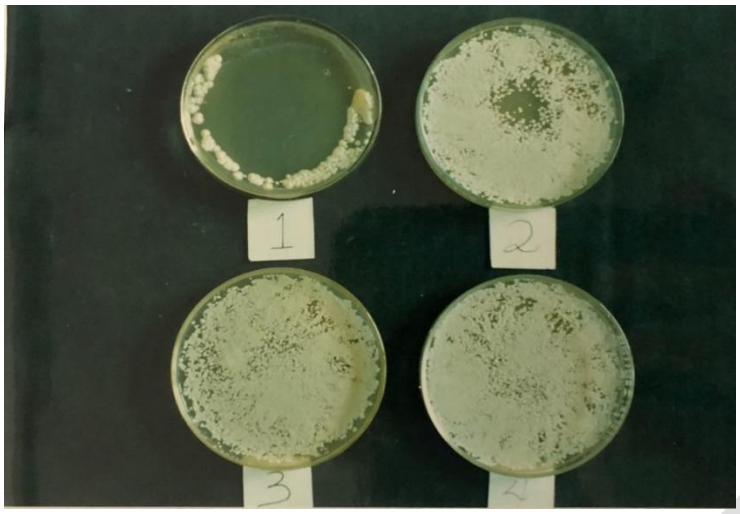

Fig. 11. Effect of different dilutions of FCEG in sterile saline solution on the growth of C*. albicans*.
(1 x10^6 yeast/mL. 28°C. 5 days of incubation).
1.- Control with FCEG
2.- *C. albicans* + FCEG 1.10 (v/v)
3.- *C. albicans* + FCEG 1.100 (v/v)
4.- *C. albicans* + FCEG 1.1000 (v/v)

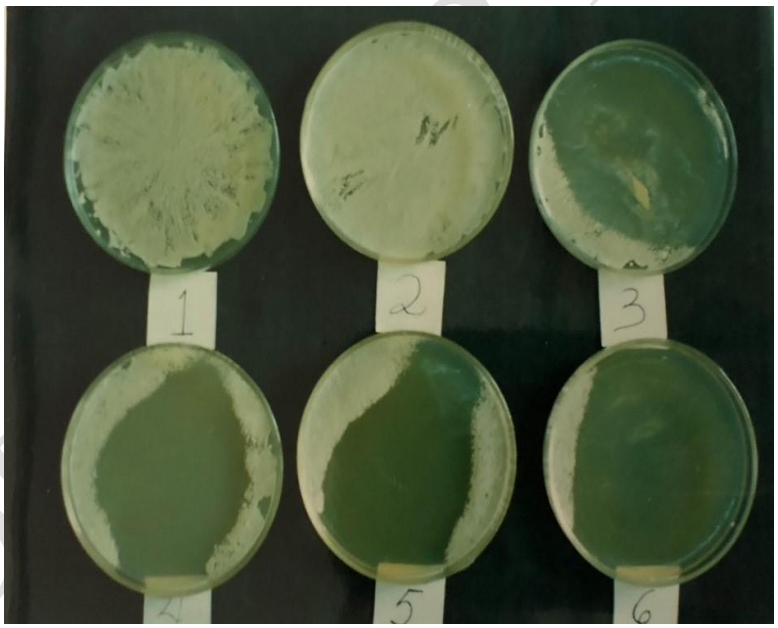

Fig. 12. Effect of FCEG in sterile saline solution on the growth of different concentrations of C*. albicans*.
(50 µL. 28°C. 5 days of incubation).
1.- *C. albicans* 1 x10^6 yeast/mL without FCEG
2.- *C. albicans* 10 x10^6 yeast/mL without FCEG
3.- *C. albicans* 5 x10^6 yeast/mL with FCEG
4.- *C. albicans* 6 x10^6 yeast/mL with FCEG
5.- *C. albicans* 7 x10^6 yeast/mL with FCEG
6.- *C. albicans* 8 x10^6 yeast/mL with FCEG

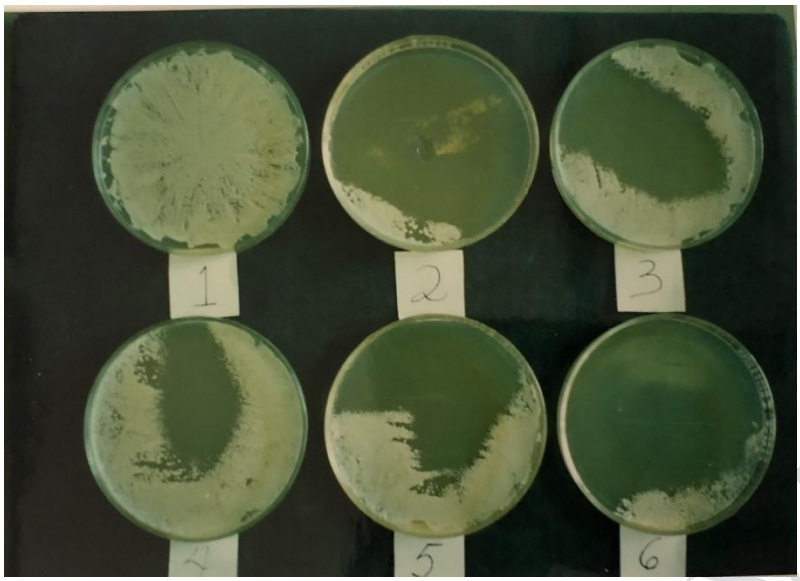

Fig. 13. Effect of different concentrations of FCEG on the growth of C*. albicans*.
(1 x10^6 yeast/mL. 28°C. 5 days of incubation).
1.- *C. albicans* 1 x10^6 yeast/mL without FCEG
2.- *C. albicans* 10 x10^6 yeast/mL with 50 µL of FCEG
3.- *C. albicans* 1 x10^6 yeast/mL with 10 µL of FCEG
4.- *C. albicans* 1 x10^6 yeast/mL with 20 µL of FCEG
5.- *C. albicans* 1 x10^6 yeast/mL with 30 µL of FCEG
6.- *C. albicans* 1 x10^6 yeast/mL with 40 µL of FCEG

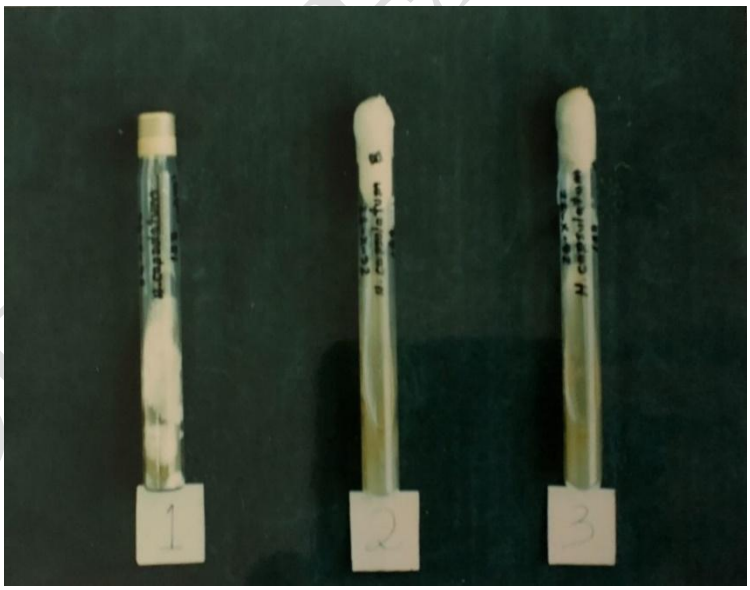

Fig. 14. Effect of FCEG on the growth of *H. capsulatum*.
(28°C. 15 days of incubation).
1.- *H. capsulatum* without FCEG
2.- *H. capsulatum* with 50 µL of FCEG
3.- *H. capsulatum* with 50 µL of FCEG

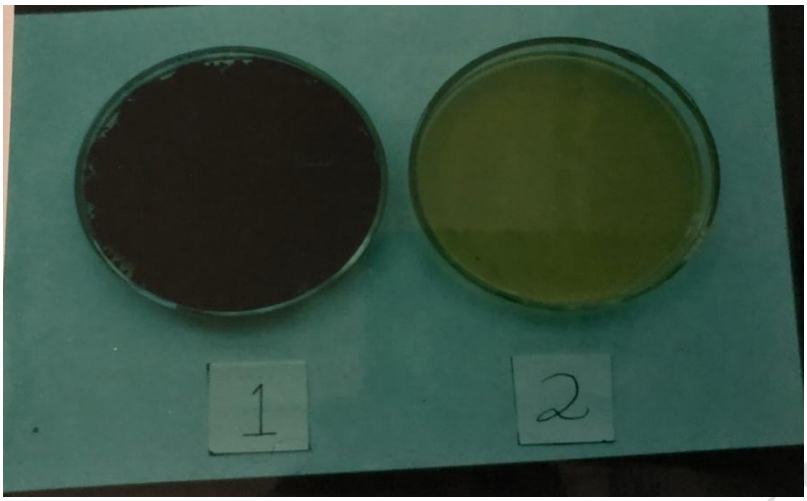

Fig. 15. Effect of FCEG on the growth of *Wangiella dermatitidis* (28°C. 5 days of incubation).
1.- *Wangiella dermatitidis* without FCEG
2.- *Wangiella dermatitidis* with 50 µL of FCEG

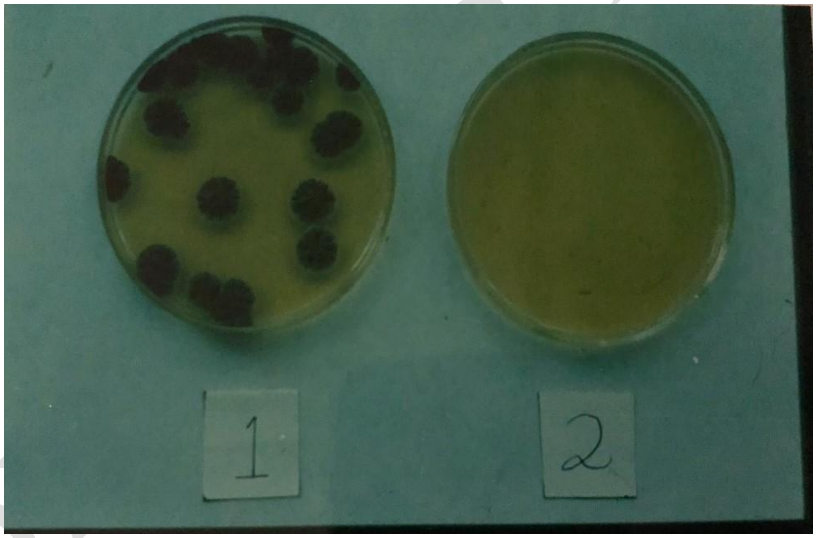

Fig. 16. Effect of FCEG on the growth of *Cladosporium carrionii* (28°C. 5 days of incubation).
1.- *Cladosporium carrionii* without FCEG
2.- *Cladosporium carrionii* with 50 µL of FCEG

4. Conclusion

Actually, the pharmaceutical industry has largely focused on high-throughput biochemical screening programmed for the discovery and development of new drugs, the use of natural products for medicinal and antimicrobial purposes is an ancient practice [15, 45], and the use of garlic, to which it is common to attribute a wide variety of properties, among which are: antifungal and antileishmanial activity [4, 7], but, until 1944 when Cavallito and Bailey [46] isolated and described the

properties of allicin, the compound responsible for garlic's characteristic pungent odor, that researchers gained a clearer insight into the chemical wonder carefully packaged by nature in the composite bulbs of this edible *Allium*, and thus began decades of extensive research on allicin, “the heart of garlic” [47], and the ajoene [(E, Z 4,5,9-tritiadodeca-1,6,11-triene-9-oxide] is an organosulfur compound derived from garlic, from which systematically

demonstrated its potential inhibitory activity *in vitro* and *in vivo* on most of the fungi that cause human mycoses this compound acts selectively on the plasma membrane by inhibiting the synthesis of phosphatidylcholine, with accumulation of phosphatidylethanolamine and, consequently, causing cell death [15, 48, and 49], and the effects of garlic oil were studied with *Penicillium funiculosum* as a model strain. Results showed that the minimum fungicidal concentrations (MFCs, v/v) were 0.125 and 0.0313 % in agar medium and broth medium, respectively, suggesting that the garlic oil had a strong antifungal activity. The main ingredients of garlic oil were identified as sulfides, mainly including disulfides (36 %), trisulfides (32 %) and monosulfides (29 %) by gas chromatograph-mass spectrometer (GC/MS), which were estimated as the dominant antifungal factors [50]. Too, was studied their powder has a good adsorption capacity for mercury [51], and in this work we analyze some characteristics of FCEG, and their effect on the growth of different bacteria and fungi, with the next conclusions:

1.- The dilution of the FCEG with sterile saline solution 0.85% (w/v) inhibits its antifungal properties.
2.- The different concentrations of FCEG analyzed have different inhibitory. The minimum inhibitory concentration of FCEG was between 40 and 50 µL (0.8 and 1 mg/mL of protein.
3.- As the concentration of *C. albicans* yeasts increases, the effectiveness of the FCEG partially decreases. No growth was observed at 1 x 10^6, 2 x 10^6, and 3 x 10^6 yeasts/mL), and only little growth was observed at the other concentrations.
4.- FCEG has a half-life between 45-60 days at 4°C, while in uncovered containers it loses its activity at 24 hours, both at 4°C and 28°C.
5.- FCEG loses its antifungal activity at 60 minutes to 60°C.
6.- Activated charcoal treatment also inhibits antifungal activity of FCEG.
7.- FCEG significantly inhibits germ tube induction in *C. albicans.*
8.- The different species of fungi and bacteria analyzed were inhibited in their growth by the FCEG.

Competing interests

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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