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EFFECTS OF AQUEOUS CHILI PEPPER EXTRACTS ON VARIOUS STRAINS OF MICROORGANISMS

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ABSTRACT

Chilli peppers, belonging to the genus Capsicum (family Solanaceae), are widely cultivated for their characteristic pungency, mainly attributed to capsaicin. Capsaicin is soluble in alcohol but poorly soluble in water, with concentrations varying widely from variety to variety. Although numerous studies have documented the antimicrobial, antioxidant, anticarcinogenic, and metabolism-stimulating properties of Capsicum extracts, the impact of aqueous extracts remains less well known. This study evaluated the effects of crude aqueous extracts from eight Capsicum varieties on the growth of four microbial strains: Escherichia coli, Enterobacter cloacae, Staphylococcus aureus and Candida albicans. Contrary to typical antimicrobial expectations, all microorganisms tested showed enhanced growth in the presence of aqueous extracts, with optical densities ranging from 0.4 to 1.8, compared to 0.4-0.6 in the no-extract control media. An optical density of 0.4-0.6 corresponded to a baseline growth rate of 100%, while values above 0.6 to 1.8 reflected an increased microbial growth rate, ranging from above 100% to about 250%, as increasing concentrations of plant extracts were added to the culture medium. Importantly, the aqueous extract from frozen Capsicum annuum var. capia induced the strongest growth stimulation of all strains, even at the lowest concentration. Staphylococcus aureus and Candida albicans consistently showed the highest growth rates on almost all extracts. These results suggest that aqueous extracts of Capsicum may promote microbial proliferation due to the presence of bioavailable nutrients and the limited solubility of capsaicin in water. The findings highlight the critical importance of extraction methods in evaluating the antimicrobial potential of

plant materials and emphasize the need for further research using solvents that efficiently extract bioactive compounds such as capsaicin.
KEY WORDS*: chili peppers, bacteria, yeast, aqueous extracts, capsaicin.*

INTRODUCTION

Capsicum is a genus of plants in the *Solanaceae* family, including sweet and hot peppers, originating from Central and South America (Alonso-Villegas, González-Amaro, Figueroa-Hernández, & Rodríguez-Buenfil, 2023). Widely cultivated for their edible fruits, *Capsicum* is essential in cuisines worldwide, providing diverse flavours and nutritional benefits. The plants have green leaves, white flowers, and colourful fruits in various shapes and sizes (Ibiza, Blanca, Cañizares, & Nuez, 2012). *Capsicum* is the most economically important genus in the *Solanaceae* family, composed of nearly 30 species (Alonso-Villegas et al., 2023). Their fruits, popularly known as peppers or chili, present a bell-shape and a wide range of colours, such as yellow, green, red and orange. They have been largely used by human society as a herb and spice, presenting the first records of usage dating back to 7000 B.C., as part of the Mexican Indian's diet (Pawar et al., 2011). Capsaicin is an active chemical compound in hot peppers, responsible for the burning sensation (Barboza, García, Bianchetti, Romero, & Scaldaferro, 2022; Omolo et al., 2014). This alkaloid stimulates pain receptors, causing the characteristic heat. Capsaicin has multiple uses, including in creams and patches for pain relief due to its analgesic and anti-inflammatory properties, and in self-defence sprays. In moderate doses, capsaicin offers health benefits such as boosting metabolism (Omolo et al., 2014).

When eaten, many hot peppers evoke a sensation of heat and, or pain in the mammalian neurological system, and these adverse effects can be overcome by eating foods containing casein, such as milk, cheese or yogurt. The Maya inhabitants of Mesoamerica have shown that chili peppers were incorporated into several medicinal preparations. These preparations were applied for a variety of ailments including respiratory problems, intestinal ailments, earaches and wounds. They have a wide range of uses, including pharmaceutical, natural dyes and cosmetics, and as an ornamental plant (Omolo et al., 2014).

Peppers (*Capsicum annuum* L.) are a valuable source of bioactive compounds, such as flavonoids, carotenoids and capsaicinoids, with beneficial health effects. These compounds help prevent degenerative diseases including cancer, diabetes, cardiovascular and neurodegenerative diseases such as Alzheimer's and Parkinson's. As they ripen, peppers change colour due to changes in pigment composition (Salehia et al., 2018). Chlorophyll imparts the green hue, carotenoids such as β-carotene and zeaxanthin

cause the yellow and orange colours, and capsanthin and capsorubin are responsible for the red color (Salehia et al., 2018).

Flavonoids are secondary plant metabolites, with over 7000 compounds, and play an important role in fruit and plant coloration, along with carotenoids. In *Capsicum*, they accumulate mainly in the peel and are involved in UV protection, growth regulation, antimicrobial defense and pollinator attraction. Flavonoids may be present as aglycones, or O-glycosidic and C-glycosidic derivatives. Among the predominant flavonoids in *Capsicum*, quercetin and luteolin are the most important, accounting for about 41% of the total content in their hydrolyzed form (Antonio, Wiedemann, & Veiga Junior, 2018). Flavonoids, particularly quercetin and luteolin, play a key role in antioxidant protection, reducing oxidative stress and defending plants against environmental factors. The chemical composition of pepper varieties depends on the species, environmental conditions and ripening stage, influencing its use in the food, pharmaceutical and cosmetic industries. Studies have shown that red peppers contain the highest level of bioactive compounds compared to green, yellow and orange varieties. It has a higher content of β-carotene (5.4 µg/g), capsanthin (8.0 µg/g), quercetin (34.0 µg/g) and luteolin (11.0 µg/g), making it one of the most nutritionally valuable vegetables (Antonio et al., 2018).

Carotenoids are pigments classified into two main groups, yellow and red, according to their chemical structure and their ability to absorb visible light. The yellow fraction includes hydrocarbonated (β-carotenoids: β-carotene, α-carotene), hydroxylated (β-cryptoxanthin, zeaxanthin) and epoxide (curubitaxanthin A, violaxanthin) carotenoids, while the red fraction contains ketocarotenoids such as capsanthin and capsorubin, specific to the *Capsicum* genus (Antonio et al., 2018). The carotenoid profile of *Capsicum* fruits varies by species, variety and ripening stage, influencing their colour. During ripening, carotenoids are synthesized and esterified to increase their stability and solubility, with roles in photoprotection, photosynthesis and insect attraction for pollination and seed dispersal.

Capsaicinoids are synthesized in the placenta of chilli fruits by capsaicin-mediated enzymatic condensation of vanillylamine and different fatty acid side chains (Friedman et al., 2019). The capsaicin content of chili peppers typically ranges from 0.1 to 1% of the mass of the pepper (Cedrón, 2013). Involved in defence mechanisms, it causes itching and stinging. This itching, which is common to both animals and humans, is detected by a general pain receptor, on contact with capsaicin, it facilitates the entry of calcium ions into the cells, which are transmitted to the brain in the form of a message (Cedrón, 2013). Biosynthesis of capsaicinoids peaks 30-50 days after flowering, when

the spiciness is most intense throughout the fruit. After this stage, the concentration of capsaicinoids begins to decrease naturally as a result of the plant's metabolism. This process is catalyzed by the enzyme peroxidase, which acts on the vanyl group, the main oxidative region of capsaicinoids. Peroxidase facilitates the oxidative coupling of the phenol from the capsaicinoid, generating derivative compounds, such as 5,5′-dicapsaicin and 4′-O-dicapsaicineter, which are related to lignin metabolites. In mammals, capsaicinoids are metabolized in the liver via cytochrome P450, an enzyme responsible for their conversion by a complex mechanism (Antonio et al., 2018). Cytochrome P450 acts mainly on the vanilloyl group via several biochemical reactions, including aromatic hydroxylation, O-demethylation, benzyl oxidation, ω-hydroxylation and dehydrogenation. *In vivo* and *in vitro* experiments in rat liver cells identified several metabolites of capsaicin, such as vanillylamine, vanillamine, vanillin, vanillyl alcohol, vanillic acid and 8-methylnonanoic acid, indicating the processes by which these substances are broken down and eliminated from the body (Antonio et al., 2018).

The aim of this study was to evaluate the antibacterial activity of six aqueous extracts obtained from different pepper varieties, four hot peppers and two sweet peppers, focusing on the presence of capsaicin and other bioactive compounds. Previous research has demonstrated that chili extracts can inhibit the growth and development of pathogenic microorganisms. This antibacterial effect is primarily attributed to capsaicin, a key bioactive compound known to disrupt bacterial cell membranes and interfere with essential metabolic processes. However, capsaicin is poorly soluble in water, which raises important questions regarding the efficacy of aqueous extracts. Given this limited solubility, it is possible that water-based extraction methods may not efficiently capture the compound's antimicrobial potential. In fact, prior observations have suggested that aqueous extracts of chili peppers may exert only minimal inhibitory effects and, in some cases, may even stimulate microbial growth. This issue becomes particularly relevant when assessing the biological activity of different pepper varieties using aqueous extraction protocols.

MATERIALS AND METHODS

Reagents. The reagents as peptone, sodium chloride, glucose and sodium hydroxide were bought from Carl Roth, Germany. The yeast extract was bought from Difco, USA. Adenine hemisulfate and agar were acquired from Merck, Germany.

Microorganisms strains. The bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and the yeast *Candida albicans* ATCC 10231 were purchased from American Type Culture Collection, Manassas, Virginia, USA.

Enterobacter cloacae DSM 106614 bacterium was acquired from Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany.

Culture media. Bacterial strains were grown in Luria-Bertani (LB) liquid medium comprising 0.5% (w/v) yeast extract, 1% (w/v) NaCl, 1% (w/v) peptone, pH 7.40. For *Candida albicans* culture, Yeast-Peptone-Dextrose (YPD) liquid medium containing 1% (w/v) yeast extract, 2% (w/v) peptone, 0.008% adenine hemisulfate, and 2% (v/v) glucose was used. Both media were prepared with and without 2% (w/v) agar, then autoclaved at 121°C for 30 minutes. The 20% glucose solution was prepared by dissolving 20 g glucose in 100 mL water.

Precultivation conditions of microorganisms. A volume of 5 µL of each bacterial (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Enterobacter cloacae* DSM 106614) and fungal stock strain (*Candida albicans* ATCC 10231) was inoculated on 5 mL of specific medium, LB or YPD, then the cultures were incubated at 30°C, 250 rpm, for 24 hours. Precultures were used for determination of antimicrobial activity of plant extracts.

Plant material. The plant material used was taken from three locations from Romania, Timis County: *Capsicum annuum* var. *anaheim*, *Capsicum annuum capia*, *Capsicum annuum* var. *grassum*, from Liebling village, Liebling Commune, (a lowland agricultural area), *Capsicum annuum* var. *convar*, *Capsicum baccatum* var. *pendulum* from Jabar village, Boldur Commune, and *Capsicum frutescens* var. *cerasiforme* from Cireșu village, Cireșu Commune.

Plant extracts. We weighed 6 g of frozen fruit tissue and 1.6 g of dried fruit tissue from six *Capsicum* varieties. The weighed plant materials were placed in a blender, to which 50 mL of distilled water was added and the mixture was blended for 3 min for the raw fruit extracts and 5 min for the dried fruit extracts. After mixing, the extracts were centrifuged for 10 min at 6000 rpm. They were further filtered by cotton wool and again centrifuged for 10 min at 6000 rpm. The supernatants were sterilized using PVDF filter membranes with a 0.2 µm pore size, and the resulting sterile extracts were used to assess the antimicrobial effect against four microbial strains.

Antimicrobial activity of plant extracts. Volumes of 0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µL of aqueous plant extracts were diluted with 50, 49, 45, 40, 35, 30, 25, 20, 15, 10, 5, 0 µL of sterile distilled water in sterile 96-well Nunc microplates with lids. To these diluted extracts were added 50 µL of culture medium, LB medium for bacterial strains (*Escherichia coli, Staphylococcus aureus*, *Enterobacter cloacae*) and YPD medium for *Candida albicans* strain. Then, 2 µL of each preculture strain were

inoculated into each well. For each strain, the assays were performed in duplicate on different days, each with several duplicates. Optical density (OD) at 600 nm was measured immediately using a Biotek Sinergy H1 microplate reader. Prior to measurement, the plates were linearly shaken for 1 min at a frequency of 731 counts per minute. All plates were incubated overnight at 30°C, 500 rpm in an Edmund Bühler TH 15 incubator hood. After 20 h of incubation, the optical density was measured again under the same conditions.

RESULTS AND DISCUSSIONS

In this study, the antimicrobial effect of several extracts of chili peppers (*Capsicum annuum anaheim*, *Capsicum annuum* var. *convar*, *Capsicum baccatum* var. *pendulum*, and *Capsicum frutescens* var. *cerasiforme*) and sweet peppers (*Capsicum annum capia*, *Capsicum annuum* var. *grassum*) were tested. Fruits were selected based on their availability and accessibility. Aqueous extracts were made for each *Capsicum* fruit.

To evaluate the antimicrobial potential of the aqueous plant extracts, growth inhibition assays were performed on selected microbial strains, and the results are illustrated in Figures 1 through 6. In these figures, the X-axis represents the dilution factor of the extracts, while the Y-axis indicates the microbial growth rate, expressed as a percentage relative to the untreated control, which was set at 100%. The growth rate was determined based on optical density (OD) measurements at 600 nm. The dilution of the extracts was performed using sterile distilled water and culture media specific to each microorganism. As a negative control, microbial cultures were incubated in either Luria Bertani (LB) medium or Yeast Extract Peptone Dextrose (YPD) medium, both diluted 1:1 with sterile distilled water and without the addition of any plant extract. In contrast, the test conditions included various concentrations of aqueous extracts obtained from raw and dried *Capsicum spp*. fruits. The data show that aqueous pepper extracts did not exhibit inhibitory effects on microbial growth. In fact, a stimulatory effect was observed in all cases, reflected by growth rates above 100%. In addition, with increasing extract concentration, the stimulatory effect was generally enhanced for all microorganisms, although to different degrees. This confirms that water is an inefficient solvent for the extraction of bioactive antimicrobial compounds from *Capsicum spp*. most probably due to the low solubility of capsaicin in water. Consequently, aqueous extracts may lack sufficient concentrations of antimicrobial constituents to produce a measurable inhibitory effect.

The main aim of this research was to determine the nature of the effects exerted by aqueous extracts of peppers on selected microbial strains. *Candida albicans* and *Escherichia coli* demonstrated the highest sensitivity to all *Capsicum* extracts, regardless of whether the source material was fresh or dried.

Figure 1 illustrates the effect of aqueous extracts from *Capsicum annuum* var. *anaheim*, comparing those prepared from raw (A) and dried (B) fruit samples.

The extract obtained from the raw sample showed a weak inhibitory effect on the growth of *Escherichia coli* and *Candida albicans*, comparable to the control samples without any extract added. In fact, all four microorganisms, *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*, showed high growth rates when exposed to dry extracts, in all cases exceeding those of the control. These findings suggest that the drying process may degrade or volatilize key antimicrobial compounds, such as phenolic acids, flavonoids or capsaicinoids, which are known to be sensitive to heat and oxidation. The differences observed between raw and dried samples emphasize the crucial role of preparation methods in preserving the bioactive potential of plant-derived extracts. To elucidate the specific compounds responsible for the antimicrobial activity observed in the crude extracts, further phytochemical analysis is required.

Figure 2 presents the effect of aqueous extracts derived from *Capsicum annuum* var. *convar*, comparing raw and dried fruit preparations.

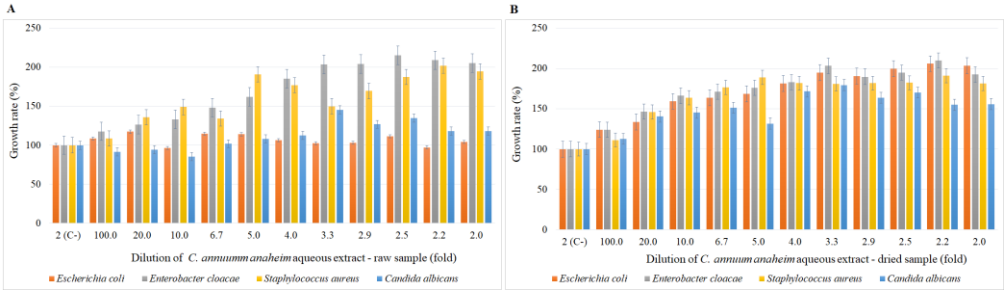

FIG 1. Effect of aqueous extracts of *Capsicum annuum anaheim* from raw (A) and dried (B) samples against different strains of microorganisms *(Escherichia coli, Staphylococcus aureus, Enterobacter cloacae,* and *Candida albicans).* Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Results are expressed as mean growth rate (%) ± standard deviation.

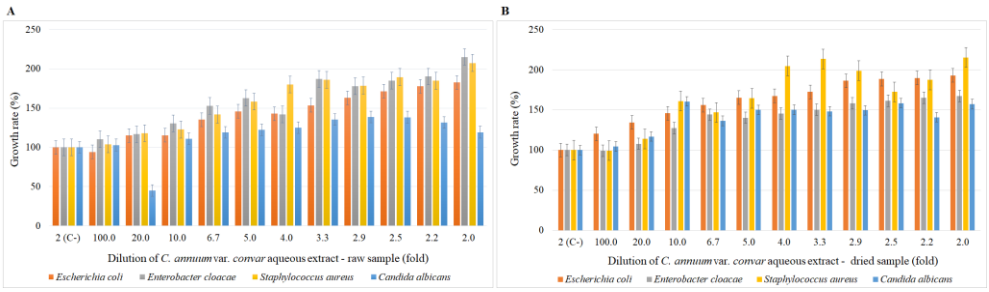

FIG 2. Effect of aqueous extracts of *Capsicum annuum* var. *convar* from raw (A) and dried (B) samples against different strains of microorganisms *(Escherichia coli, Staphylococcus aureus, Enterobacter cloacae,* and *Candida albicans).* Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Results are expressed as mean growth rate (%) ± standard deviation.

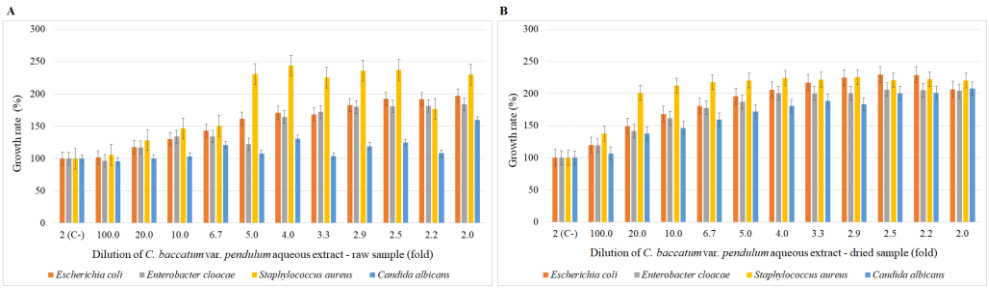

FIG 3. Effect of aqueous extracts of *Capsicum baccatum* var. *pendulum* from raw (A) and dried (B) samples against different strains of microorganisms *(Escherichia coli, Staphylococcus aureus, Enterobacter cloacae,* and *Candida albicans).* Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Results are expressed as mean growth rate (%) ± standard deviation.

Both raw and dried extracts demonstrated no selective inhibitory effect on any of the tested strains. Moreover, as the extract concentration increased, a stimulatory effect on microbial growth was observed in both cases, particularly for *E. coli*, *E. cloacae*, and *S. aureus* with the raw extract, and for all strains with the dried extract, where growth rates exceeded 130%.

The effect of raw and dried *Capsicum baccatum* var. *pendulum* extracts on *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Candida albicans*

is presented in Figure 3. The extract from the raw and dried sample displayed a stimulatory effect on the growth of all tested pathogenic strains.

Figure 4 illustrates the effect of aqueous extracts from raw and dried samples of *Capsicum frutescens* var. *cerasiforme* on four microbial strains. The raw extract exhibited a mild inhibitory effect on *Candida albicans*. The extract from the dried sample exhibited a stimulatory effect on the bacterial strains *Escherichia coli*, *Enterobacter cloacae*, and *Staphylococcus aureus*. The growth of *Candida albicans* was strongly stimulated, reaching a growth rate of over 230%.

Figure 5 presents the comparative antimicrobial effects of aqueous extracts from raw and dried samples of *Capsicum annuum* var. *capia*. The results indicate that both raw and dried fruits extracts exhibited a stimulatory effect on all tested microbial strains, *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans*, as evidenced by increased growth rates relative to the control.

The extract obtained from the raw sample of *Capsicum annuum* var. *grassum* (Figure 6) demonstrated a growth-promoting effect on the bacterial strains *Escherichia coli*, *Enterobacter cloacae*, and *Staphylococcus aureus*, suggesting that the phytochemical composition of the raw extract have included nutrients or bioactive compounds that supported microbial proliferation.

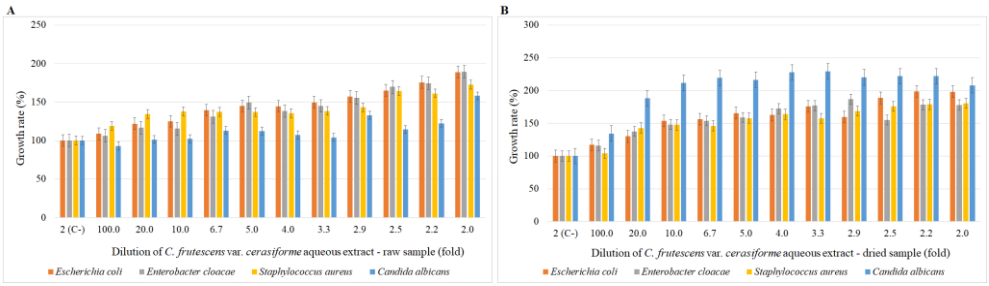

FIG 4. Effect of aqueous extracts of *Capsicum frutescens* var. *cerasiforme* from raw (A) and dried (B) samples against different strains of microorganisms *(Escherichia coli, Staphylococcus aureus, Enterobacter cloacae*, and *Candida albicans).* Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Results are expressed as mean growth rate (%) ± standard deviation.

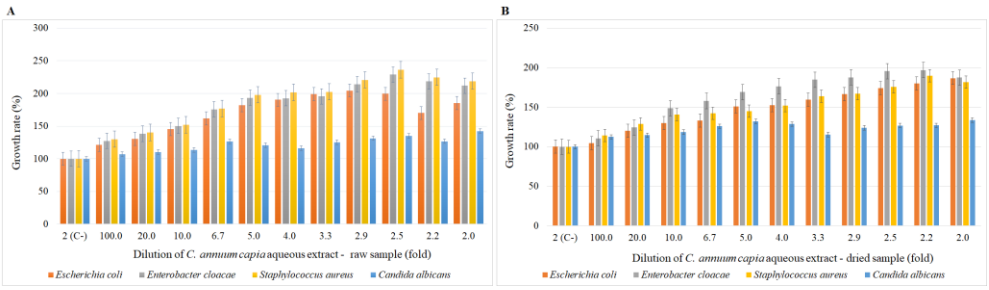

FIG 5. Effect of aqueous extracts of *Capsicum annuum* var. *capia* from raw (A) and dried (B) samples against different strains of microorganisms *(Escherichia coli, Staphylococcus aureus, Enterobacter cloacae,* and *Candida albicans).* Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Results are expressed as mean growth rate (%) ± standard deviation.

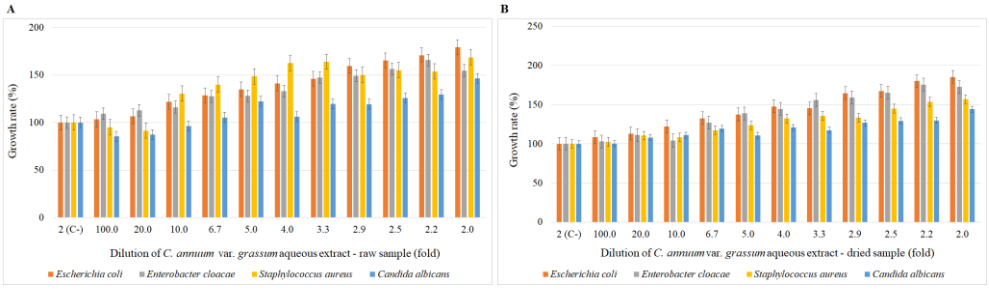

FIG 6. Effect of aqueous extracts of *Capsicum annuum* var. *grassum* from raw (A) and dried (B) samples against different strains of microorganisms *(Escherichia coli, Staphylococcus aureus, Enterobacter cloacae,* and *Candida albicans).* Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Results are expressed as mean growth rate (%) ± standard deviation.

Both aqueous extracts obtained from raw and dried fruits showed a stimulatory effect on the growth of all microbial species tested. This consistent growth stimulation of all strains suggests that water as a solvent does not efficiently extract antimicrobial compounds from *Capsicum* fruits and instead provide nutrients or conditions favorable for microbial proliferation as sugars and vitamins. These findings highlight the importance of selecting appropriate extraction methods to accurately assess the antimicrobial potential of plant materials.

Further studies using alternative solvents or extraction techniques are needed to isolate and evaluate the bioactive compounds responsible for the antimicrobial activity.

CONCLUSIONS

Chili pepper extract is known for its antibacterial properties, mainly attributed to capsaicin and other bioactive compounds. Capsaicin exerts its antimicrobial effect by disrupting cell membrane integrity and interfering with vital bacterial metabolic processes, thereby inhibiting the growth of pathogenic microorganisms such as *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. However, in this study, aqueous extracts of chili peppers exhibited a predominantly stimulatory rather than inhibitory effect on microbial growth in all six varieties tested. This result is due to the low solubility of capsaicin in water, which limits the extraction of its antimicrobial constituents and may instead provide nutrients or favorable conditions that promote microbial proliferation. These findings emphasize the critical importance of extraction methods in assessing the antimicrobial potential of plant-derived compounds. Future research should focus on optimizing extraction techniques using solvents that better solubilize capsaicin and related bioactive to accurately assess their antimicrobial efficacy and potential therapeutic applications.

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