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Research Article

Determination of Antimicrobial Activity of Cream Formulation Developed with *Hibiscus rosa-sinensis* Extract and Probiotic

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ABSTRACT

Medicinal plants are an indispensable source of new and effective pharmaceutical products. In the study, the biological activities of water extract obtained from the *H. rosa-sinensis* leaf was determined and its potential for use in the pharmaceutical and cosmetic industries was investigated. Disc diffusion assay and micro-dilution method against clinical origin test microorganisms were used to assess biological activity. The Extract showed a zone of inhibition on the tested bacteria and yeasts in the 6.85 mm to 10.74 mm range. MIC and MBC or MFC values of the Extract was determined as 6.25-12.50 µg/µL and 12.50-50 µg/µL. Then, the cream formulation containing *H. rosa-sinensis* leaf water extract and/or human milk originated probiotic candidate *Limosilactobacillus fermentum* MA-7 strain have been developed. The Extract and probiotic containing cream formulations showed variable antibacterial and antifungal effects on the tested clinical originated microorganisms. The highest inhibition zone diameters of Cream + Extract + Probiotic formulation group was obtained against *Candida glabrata* RSKK 04019 (9.09 mm) and *Escherichia coli* O157:H7 (9.04 mm). The results indicate that *L. fermentum* MA-7 and *H. rosa-sinensis* water extract, alternative to synthetic antimicrobials, may be used as a natural bioactive ingredient in daily personal care and clinical applications in the near future.

Keywords: *Camellia, Cream formulation, Extract, Natural additive, Probiotic*

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INTRODUCTION

Plants as a source of medicinal compounds have been used for human health since ancient times (Salmerón-Manzano et al., 2020). More than 35% of modern clinical medicines are derived from natural products (Calixto, 2019). *Hibiscus rosa-sinensis* (Malvaceae), is grown as an ornamental plant in tropical climates. The leaf, flower, bark, and root are used as medicine to cure many diseases. The different parts of *H. rosa-sinensis* have anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties that help treat many diseases (Khristi and Patel, 2016). It has been observed that flowers and leaves help treat ulcers and accelerate hair growth (Shandilya and Pathak, 2020). *H. rosa-sinensis* has been used to cure many diseases and is a suitable plant for producing natural medicines (Anil and Ashatha, 2012).
Probiotics provide microbial balance in the intestinal system; they are microorganisms that provide health benefits together with basic nutrition (Kim et al., 2019; Aladeboyeje and Şanlı, 2021; Pandey et al., 2023). The microorganisms most commonly used as probiotics are lactic acid bacteria and are found in the intestinal flora and skin of healthy animals. Probiotics in the skin and gut provide a microbial balance to minimize illness conditions (Nakatsuji et al., 2017). Several studies have shown that the probiotic microorganism *L. fermentum* has antimicrobial, antioxidant, and anti-photoaging properties (Haryani et al., 2023; Palani Kumar et al., 2021). In a study, *L.fermentum* MA-7 and MA-8 strains isolated human milk showed antimicrobial activity against clinical and food-borne bacteria and fungal test microorganisms. These strains also exhibited antioxidant activity (Asan-Ozusaglam and Gunyakti, 2019). In another study, food-borne *Lactobacillus fermentum* MBC2 was shown to delay senescence of the in vivo model organism, the nematode *Caenorhabditis elegans* (Schifano et al., 2019).

The skin covers our body and is our largest organ, but is also exposed to fungal and bacterial threats (Roudsari et al., 2015). The skin functions as a barrier and immune organ that can protect the body against microbial attacks (Harris-Tryon and Grice, 2022). Probiotics have the ability to maintain, optimize and restore the skin's microbiota. Topical applications of probiotics strengthen the natural defense barriers in the skin and have a healing effect on the application area (Woodhams et al., 2020). The creams prepared with herbal extracts can be used in topical applications in pharmacology. Extract-containing creams can protect the skin against damage caused by pathogens (Uçar et al., 2023).
In the study, the potential uses of *H. rosa-sinensis* leaves extract and cream formulations developed with *L. fermentum* MA-7 for the pharmaceutical and cosmetic industries were investigated. Firstly, the biological activity of the water extract from *H. rosa-sinensis* leaf was determined to obtain its use potential as a natural alternative to synthetic antimicrobials. Afterward, the cream formulations containing *H. rosa-sinensis* extract and *L. fermentum* MA-7 were developed, and their antimicrobial activities against clinical test microorganisms were determined.

MATERIAL AND METHODS

Preparation Leaf Extract

H. rosa-sinensis leaves were collected from Alata Horticulture Research Institute on 18.06.2021 (Mersin/Turkey, 36º38´3.04´N-34º20´58.05´E). They were washed using distilled water and then dried in airy conditions. The sample was then pulverized using a blender. The extraction of the leaf was done using distilled water in a hot water bath for 2 days (6 hours per day). After extraction, the supernatant was filtered using filter paper and evaporated to dryness using a heating plate at 40-50°C. *H. rosa-sinensis* leaf extract was dissolved with dimethyl-sulfoxide (DMSO) to a final concentration of 0.1 mg/mL and sterilized by passing through a syringe filter (0.45 µm). The extract was stored in sterile tubes at +4°C until used.

Pathogenic Microorganisms

Antimicrobial activity of the leaf extract was tested on four pathogenic microorganisms. *Candida glabrata* RSKK 04019 and *C. albicans* ATCC 10231 were cultured in Yeast-Peptone-Dextrose (YPD) media for at 30°C 48 hours. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* O157:H7 were grown in Nutrient-Broth (NB) media for 24 hours at 37°C.

Disc Diffusion Assay

Antimicrobial activity of the extract was obtained using the disc diffusion assay against food-borne or clinical test microorganisms. Test microorganisms were spread on agar medium by dropping 100 µL of cell

suspension (0.5 McFarland). The sterile filter discs (Whatman No:3; Diameter: 6 mm) were impregnated with *H. rosa-sinensis* extract (20 µl, 2 mg/disc) and then placed on the agar medium. The petri dishes were incubated at appropriate conditions. Fluconazole (FCA, 25 µg/disc) and Kanamycin (K, 30 µg/disc) were used as positive controls. The diameter of the zone of inhibition around the discs was measured using Vernier calipers. The experiment was done in three repetitions.

Micro-dilution Method

Minimal inhibitory (MIC) and minimal bactericidal (MBC) or fungicidal (MFC) concentration values of the leaf extract was obtained by micro-dilution method against test bacteria and yeasts. The extract was added to each tube having a growth medium to obtain final concentrations (100-50-25-12.5-6.25-3.12 µg/µl). The culture adjusted to 0.5 McFarland was added to the tubes and incubated under the conditions required for each microorganism. The lowest concentration of the extract that inhibited the growth of the microorganism was determined as MIC value. The sample taken from the tubes was inoculated onto the agar medium to obtain MBC or MFC values. The agar medium was incubated at the appropriate temperature for the test microorganisms. After incubation, the lowest concentration without growth was defined as MBC or MFC values. If the MBC/MIC and MFC/MIC ratio is ≤4, it was determined as bactericidal, and if the MBC/MIC and MFC/MIC rate was >4, it was determined as bacteriostatic effect (Al-Shammari et al., 2022; Baj et al., 2020).

Antimicrobial Activity of Developed New Cream Formulation

Antibacterial and antifungal activities of the new cream formulation containing *H. rosa-sinensis* extract and probiotics were determined by the modified method used in our previous study (Asan-Ozusaglam and Celik, 2023). The developed cream groups included commercial cream, *H. rosa-sinensis* extract and human milk-derived probiotic candidate strain *L. fermentum* MA-7 (Asan-Ozusaglam and Gunyakti, 2019). The antimicrobial activity of commercial cream (C) as control, Cream + Extract (CE) mixture, Cream + Probiotic (CP) mixture and Cream + Extract + Probiotic (CEP) mixture against test microorganisms (*C. glabrata* RSKK 04019, *C. albicans* ATCC 10231, *S. aureus* ATCC 25923 and *E. coli* O157:H7) were determined using the well diffusion assay. The test microorganisms were spread on an agar medium by dropping 100 µL of cell suspension (0.5 McFarland). The cream groups were added to each well (6 mm) in 3 replicates. The petri dishes were incubated at 37°C for bacterial strains and 30°C for yeast. The inhibition zone was measured using Vernier calipers.

Statistical Analysis

Antimicrobial activity data on the test microorganisms were analyzed using the GNU SPSS software. Statistical significance was confirmed by Tukey's post-hoc test and one-way analysis of variance (ANOVA). The difference between the data was considered statistically significant at the $p \leq 0.05$.

RESULTS

The antimicrobial activity of *H. rosa-sinensis* leaf extract was investigated on pathogen test microorganisms using disc-diffusion and micro-dilution methods. The results of the disc-diffusion assay against the tested bacteria and yeast are in Table 1. The inhibition zone diameters of the extract on yeasts were determined as 6.85 mm for *C. albicans* ATCC 10231 and 10.74 mm for *C. glabrata* RSKK 04019 (Figure 1). *H. rosa-sinensis* extract produced a zone diameter of the inhibition as 8.18 mm on *E. coli* O157:H7 and 6.99 mm on *S. aureus* ATCC 25923. The statistically analysis results indicated that the differences between *C. albicans* ATCC 10231 and *S. aureus* ATCC 25923 were insignificant ($p \leq 0.05$), but there was a significant difference against other test microorganisms ($p \leq 0.05$) (Table1). The results obtained showed that *H. rosa-sinensis* leaf extract may be used against bacterial and yeast infections.

Table 1. *Disc-diffusion test results of H. rosa-sinensis leaf extract and trademark antibiotics*

Microorganisms	**Inhibition Zone Diameter (mm±SD)**		
	Extract	**Kanamycin**	**Fluconazole**
***C. glabrata* RSKK 04019**	10.74±0.15^{b}	11.68±1.54^{b}	NA^{b}
***C. albicans* ATCC 10231**	6.85±0.18^{a}	16.34±0.84^{a}	14.48 ±0.57^{a}
***S. aureus* ATCC 25923**	6.99±0.29^{a}	16.37±1.74^{a}	NA^{b}
***E. coli* O157:H7**	8.18±0.17^{c}	17.82±0.42^{a}	NA^{b}
F(sig)	224.843(0.000)	13.558(0.002)	1890.058(0.000)

*****NA: No Activity, *Different letters specify significant differences at $p \leq 0.05$ between samples.

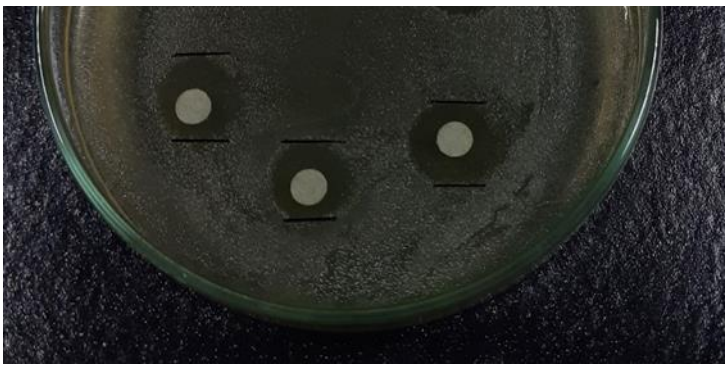

Figure 1. *Inhibition zones of the extract on C. glabrata RSKK 04019*

Udo et al. (2016), the antimicrobial activity of the water extract obtained from *H. rosa-sinensis* leaves was determined using the disc-diffusion experiment. The extract formed an inhibition zone of 12.30 mm on *E. coli* and 11 mm on *S. aureus*. In another study, *H. rosa-sinensis* water leaves extract showed a 20 mm zone of inhibition against *C. albicans* (Pooja et al., 2016). The differences between the current study and the literature may be due to the differences in the extraction method, solvent, and extract concentration used (Al-Zoreky, 2009).

MIC is the minimal concentration of the antimicrobial substance that inhibits visible growth. The MBC or MFC values are the concentration at which microorganism growth is completely inhibited (Kowalska and Dudek, 2021). *H. rosa-sinensis* extract presented the lowest MIC value with 6.25 µg/µL on *S. aureus* ATCC 25923. The extract has a MIC value of 12.5 µg/µL on other test microorganisms. MBC or MFC values of *H. rosa-sinensis* extract varied from 12.50 µg/µL to 50 µg/µL. The extract showed the best antimicrobial activity against *C. glabrata* yeast ATCC 04019 with an MFC value of 12.50 µg/µL. The extract showed cidal effect on *C. glabrata* RSKK 04019, *C. albicans* ATCC 10231 and *E. coli* O157:H7 as MBC/MIC or MFC/MIC values were ≤4. The extract showed static effect as the MBC/MIC ratio on *S. aureus* ATCC 25923 was greater than 4 (Table 2 and Figure 2).

Table 2.*MIC and MBC or MFC values of H. rosa-sinensis extract*

Microorganisms	MIC (µg/µL)	MBC or MFC (µg/µL)	MBC/MIC or MFC/MIC
***C. glabrata* RSKK 04019**	12.50	12.50	1
***C. albicans* ATCC 10231**	12.50	50	4
***S. aureus* ATCC 25923**	6.25	50	8
***E. coli* O157:H7**	12.50	25	2

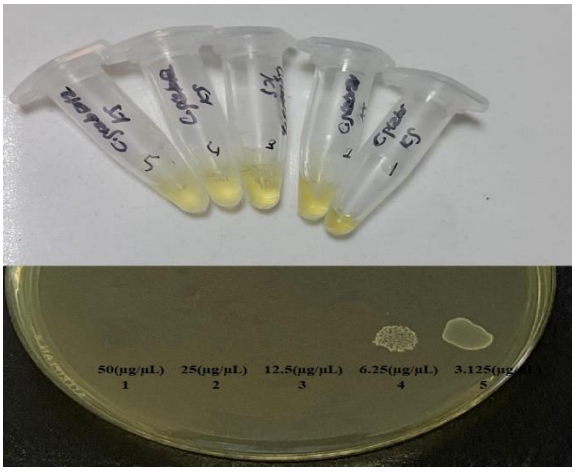

Figure 2. *Micro-dilution test results of the extract on C. glabrata RSKK 04019*

In a study, MIC and MBC values of ethyl acetate extracts from five different cultivars of *H. rosa-sinensis* were determined between 0.625 mg/mL and 20 mg/mL and between 1.25 mg/mL and 20 mg/mL on some test microorganisms (*S. aureus, Streptomyces alboniger, Bacillus subtilis, Staphylococcus epidermidis* and *Micrococcus luteus*) (Patel et al., 2012). *S. aureus* ATCC 25923 had 6.25 µg/µL MIC and 50 µg/µL MBC

values in our study. The difference from the current study may be due to the cultivar of *H. rosa-sinensis*, the solvent and strain of the microorganism used.

Creams are used in cosmetics and clinics to protect and/or improve skin health, *L. fermentum* has good effects on skin health and skin microbiota (Lee et al., 2022). The biological activity results of the developed antimicrobial cream groups prepared with *H. rosa-sinensis* extract and/or probiotic strain *L. fermentum* against the tested bacteria and yeasts are in Table 3. The control (C) group showed no biological activity against other test microorganisms except for *C. albicans* ATCC 10231 (2.08 mm). The antimicrobial activity of the CEP formulation (4.46 mm) containing extracts and probiotics against *C. albicans* ATCC 10231 increased compared to the C group ($p \leq 0.05$). The highest inhibition zones of CE and CEP were determined as 4.10 mm and 9.09 mm against *C. glabrata* RSKK 04019 (Figure 3). The inhibition zone diameter of the CEP group (9.04 mm) against *E. coli* O157:H7 was statistically significant when compared to other test groups ($p \leq 0.05$). CEP has an inhibition zone diameter of 4.18 mm on *S. aureus* ATCC 25923. All CEP groups containing *H. rosa-sinensis* extract and probiotics increased the antimicrobial activity by providing a synergistic effect.

Table 3. *Antimicrobial activity results of cream formulations containing H. rosa-sinensis extract and probiotic*

Microorganisms	**Inhibition Zone Diameter (mm±SD)**				
	C	**CE**	**CP**	**CEP**	**F(Sig)**
***C. glabrata* RSKK 04019**	NA^{a}	4.10±0.08^{b}	2.21±0.12^{c}	9.09±0.12^{d}	4662.291(0.000)
***C. albicans* ATCC 10231**	2.08±0.11^{a}	2.66±0.31^{a}	4.01±0.68^{b}	4.46±0.23^{b}	23.502(0.000)
***E. coli* O157:H7**	NA^{a}	2.33±0.27^{b}	1.25±0.10^{b}	4.18±0.43^{c}	103.648(0.000)
***S. aureus* ATCC 25923**	NA^{a}	2.76±0.28^{b}	NA^{a}	9.04±0.89^{c}	248.375(0.000)

*C:Cream, CE:Cream+Extract, CP:Cream+Probiotic, CEP:Cream+Extract+Probiotic, NA: No Activity

*Different letters specify significant differences at $p \leq 0.05$ between samples.

Figure 3. *Inhibition zones of CE and CEP groups against C. glabrata RSKK 04019*

Bhaskar and Nithya (2012), prepared ointments containing ethanol extract obtained from *H. rosa-sinensis* flowers and investigated the wound healing potential. The results indicated that ointments containing the extract had a faster wound-healing ability than the control and standard drug.

CONCLUSION

H. rosa-sinensis leaf extract showed antimicrobial activity against test microorganisms. It has been determined that the extract may have potential use in the pharmaceutical industry. In the cream formulation developed (Cream+Extract+Probiotic), antimicrobial activity increased with the synergistic effect of the extract and the probiotic candidate strain. Thus, it was determined that it may be appropriate to use the developed cream groups with high biological activity as a preservative in cosmetic products.

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Conflict of interest

The authors declared no conflict of interest.

Author contribution

All authors contributed equally.

Ethical approval

During the writing process of the study titled "***Determination of Antimicrobial Activity of Cream Formulation Developed with Hibiscus rosa-sinensis Extract and Probiotic***", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. Ethics committee approval is not required.

REFERENCES

Aladeboyeje, O., and Şanlı, N. Ö. (2021). Fermented traditional probiotic beverages of Turkish origin: a concise review. *International Journal of Life Sciences and Biotechnology*, *4*(3), 546-564. https://doi.org/10.38001/ijlsb.936982

Al-Shammari, K. I., Batkowska, J., Gryzińska, M., Wlazło, Ł., Ossowski, M., & Nowakowicz-Dębek, B. (2022). The use of selected herbal preparations for the disinfection of Japanese quail hatching eggs, *Poultry Science*, *101*(10), 102066. https://doi.org/10.1016/j.psj.2022.102066

Al-Zoreky, N. S. (2009). Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International Journal of Food Microbiology, *134*(3), 244-248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

Anil, K., & Ashatha, S. (2012). Review on *Hibiscus rosa sinensis*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *3*(2), 534-538. http://www.ijrpbsonline.com/files/13-3213.pdf

Asan-Ozusaglam, M., & Celik, I. (2023). White pitahaya as a natural additive: potential usage in cosmetic industry. *Foods and Raw materials*, *11*(1), 57-63. https://dx.doi.org/10.21603/2308-4057-2023-1-552

Asan-Ozusaglam, M., & Gunyakti, A. (2019). *Lactobacillus fermentum* strains from human breast milk with probiotic properties and cholesterol-lowering effects. *Food Science and Biotechnology*, *28*, 501-509. https://doi.org/10.1007/s10068-018-0494-y

Baj, T., Biernasiuk, A., Wróbel, R., & Malm, A. (2020). Chemical composition and in vitro activity of *Origanum vulgare* L., *Satureja hortensis* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. essential oils towards oral isolates of *Candida albicans* and *Candida glabrata*. *Open Chemistry*, *18*(1), 108-118. https://doi.org/10.1515/chem-2020-0011

Bhaskar, A., & Nithya, V. (2012). Evaluation of the wound-healing activity of *Hibiscus rosa sinensis* L (*Malvaceae*) in Wistar albino rats. *Indian Journal of Pharmacology*, *44*(6), 694. https://doi.org/10.4103%2F0253-7613.103252

Calixto, J. B. (2019). The role of natural products in modern drug discovery. *Anais da Academia Brasileira de Ciências*, *91*. https://doi.org/10.1590/0001-3765201920190105

Harris-Tryon, T .A., & Grice, E. A. (2022). Microbiota and maintenance of skin barrier function. *Science*, *376*(6596), 940-945. https://doi.org/10.1126/science.abo0693

Haryani, Y., Halid, N. A., Guat, G. S., Nor-Khaizura, M. A. R., Hatta, A., Sabri, S., ... & Hasan, H. (2023). Characterization, molecular identification, and antimicrobial activity of lactic acid bacteria isolated from selected fermented foods and beverages in Malaysia. *FEMS Microbiology Letters*, *370*, fnad023. https://doi.org/10.1093/femsle/fnad023

Khristi, V., & Patel, V. H. (2016). Therapeutic potential of *Hibiscus rosa sinensis*: A review. *International Journal of Nutrition and Dietetics*, *4*(2), 105-123. http://dx.doi.org/10.17654/ND004020105

Kim, S. K., Guevarra, R. B., Kim, Y. T., Kwon, J., Kim, H., Cho, J. H., Kim, J. B., & Lee, J. H. (2019). Role of probiotics in human gut microbiome-associated diseases. *Microbiol. Biotechnol*, *29*(9), 1335-1340. https://doi.org/10.4014/jmb.1906.06064

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*, *10*(2), 165. https://doi.org/10.3390/pathogens10020165

Lee J.Y., Kim Y., Kim J.I., Lee H.Y., Moon G.S. and Kang C.H. (2022). Improvements in Human Keratinocytes and Antimicrobial Effect Mediated by Cell-Free Supernatants Derived from Probiotics. *Fermentation*, *8*(7), 332-339. https://doi.org/10.3390/fermentation8070332

Nakatsuji, T., Chen, T. H., Narala, S., Chun, K. A., Two, A. M., Yun, T., ... & Gallo, R. L. (2017). Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Science Translational Medicine*, *9*(378), eaah4680. https://doi.org/10.1126/scitranslmed.aah4680

Palani Kumar, M. K., Halami, P. M., & Serva Peddha, M. (2021). Effect of *Lactobacillus fermentum* MCC2760-based probiotic curd on hypercholesterolemic C57BL6 mice. *ACS Omega*, *6*(11): 7701-7710. https://doi.org/10.1021/acsomega.1c00045

Pandey, A., Mazumder, A., & and Das, S. (2023). A critical evaluation of beneficial gut flora: Probiotics, prebiotics, postbiotics and synbiotics to treat constipation and haemorrhoids. *Allelopathy Journal, 59(*2), 187-196.

Patel, R., Patel, A., Vaghasiya, D., & Nagee, A. (2012). Antimicrobial evaluation of *Hibiscus rosa-sinensis* plant extracts against some pathogenic bacteria. *Bulletin of Environmental and Scientific Research*, *1*(3-4), 14-17. http://www.besr.org.in/

Pooja, K. A., Dudeja, S., Chauhan, R., Hemalata, S., Beniwal, V., Chhokar, V., & Kumar, A. (2016). Antimicrobial activity of ethno-medicinal plants against cariogenic pathogens. *Journal of Medicinal Plants Studies*, *4*(3), 283-290. https://www.plantsjournal.com/archives/2016/vol4issue3/PartD/4-4-37-333.pdf

Roudsari, M. R., Karimi, R., Sohrabvandi, S., & Mortazavian, A. M. (2015). Health effects of probiotics on the skin. *Critical Reviews in Food Science and Nutrition*, *55*(9), 1219-1240. https://doi.org/10.1080/10408398.2012.680078

Shandilya, S., & Pathak, V. (2020). Chemical constituents & pharmacological effects of *Hibiscus rosa-sinensis* (china rose)–a review. *World Journal of Pharmaceutical Research*, *10*(1), 858-869. https://doi.org/10.20959/wjpr20211-19535

Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Public Health. 2020 May 12;17(10):3376. https://doi.org/10.3390/ijerph17103376

Schifano, E., Zinno, P., Guantario, B., Roselli, M., Marcoccia, S., Devirgiliis, C., & Uccelletti, D. (2019). The foodborne strain Lactobacillus fermentum MBC2 triggers pept-1-dependent pro-longevity effects in *Caenorhabditis elegans*. *Microorganisms*, *7*(2), 45.

Uçar, M. A., Derun, E. M., & Pişkin, M. B. (2023). Determination of usage potential of *Hypericum perforatum, Hypericum capitatum, Centaurea cyanus* extracts and creams in the cosmetic industry. *Sigma*, *41*(3):443-450. 10.14744/sigma.2023.00051

Udo, I. J., Ben, M. G., Etuk, C. U., & Tiomthy, A. I. (2016). Phytochemical, proximate and antibacterial properties of *Hibiscus rosa-sinensis* L. Leaf. *Journal of Medicinal Plants Studies*, *4*(5), 193-195.

Woodhams, D. C., Rollins-Smith, L. A., Reinert, L. K., Lam, B. A., Harris, R. N., Briggs, C. J., & Bigler, L. (2020). Probiotics modulate a novel amphibian skin defense peptide that is antifungal and facilitates growth of antifungal bacteria. *Microbial Ecology*, *79*, 192-202. https://doi.org/10.1007/s00248-019-01385-9