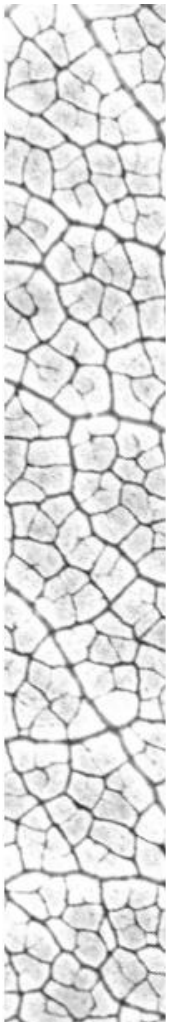

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 7427-7432 (2020)

Study the bacterial inhibitory effect and antioxidant efficacy of Hibiscus sabdariffa extract and punicagranatum peel

Shabeeb Munshid Jasim [1], Abdulridha Ati Jaafar [1*], Assad Shamel Atyea [2]

[1] Department of Animal Production, College of Agriculture, University of Misan, Maysan, IRAQ
[2] Department of Plant Protection, College of Agriculture, University of Misan, Maysan, IRAQ
*Corresponding author: ridha1984iraq@gmail.com

Abstract
Pomegranate peels (punicagranatum) and hibiscus (Hibiscus sabdariffa) were extracted with ethanol at a concentration of 70%, and theirinhibition efficacy was tested against the test organisms (*staphylococcus aureus, Escherichia coli, Pseudomonas, Salmonella, Candida albicans*) with a concentration of 0.1%, 0.2%, 0.3%. It was found that the inhibitory activity increased with the increase in the concentration of the extract, as the concentration of 0.3% gave the highest inhibitory activity againstall the test organisms except for Salmonella bacteria, which was notaffected by the extract of pomegranate peels and for all the concentration.Additionally, both pomegranate peel extract and hibiscus peel show antioxidant efficacy, however, the antioxidant efficacy of pomegranate peel was higher than that of hibiscus extract and for all the concentrations used in the study (100, 200, 300, 400, 500, 600, 700 ppm).

Keywords: *Hibiscus sabdariffa* extract, punicagranatum peel, antioxidant

Jasim ShM, Jaafar AA, Atyea ASh (2020) Study the bacterial inhibitory effect and antioxidant efficacy of Hibiscus sabdariffa extract and punicagranatum peel. Eurasia J Biosci 14: 7427-7432.

INTRODUCTION

In the Malvaceae family, Hibiscus is a genus of flowering plants. In many countries worldwide tea made of hibiscus flowers is known for its many names and is both hot and cold. It is known in Western Africa as bissap, Egyptian and Sudan karkadé, some people call it roselle, the Hibiscus flower's common name (Salib, 2014) where it has been used in many medical and nutritional fields. Studies have shown that hibiscus reduces high blood pressure and contributes to reducing the incidence of cancer as well as preventing the growth of pathological microorganism within the body (Jalalyazdi *et al.* 2019; Guardiola and Mach, 2014). It has previously been observed that hibiscus has an inhibitory efficacy against some pathogenic bacteria (Ruban and Gajalakshmi, 2012). Investigators have demonstrated that Hibiscus contains many important compounds and elements such as sodium, potassium, calcium, vitamin C and anthocyanin (Ali *et al.,* 2005). Hibiscus has been used in Asia and Africa in the manufacture of beverages and dairy products as well as in the manufacture of meat to extend the preservation period and color improvement due to the presence of anthocyanins (Iwalokun and Shittu, 2007; Onibi *et al,* 2007). In the medical field, hibiscus extract has been used in the treatment of some heart diseases, dyspepsia, diuretic, diarrhea, laryngitis, mouth inflammation, and pain reliever (Tolulope, 2007). Many plant extracts, including hibiscus, possess the inhibitory effect on many microorganisms that are found in the different places of the human body causing many diseases such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* and other bacteria that cause infection (Goering *et al.,* 2012).

Pomegranate peels have been considered as a medicinal substance and been used since ancient times in the treatment of many diseases because it contains effective compounds, The pomegranate peels contain tannins, which have been used as a treatment ofworm infestations in the digestive tract of humans and animals, as well as for the treatment of diarrhea for its ability to inhibit bacteria and viruses work by stimulating pharyngeal cells and destroying proteins and other structures on the cell wall that bacteria use to stick (Sumner *et al.,* 2005). Existing research recognizes that pomegranate peels contain important alkaloids such as pelletierine, N-Methylpelletierine, isopelletierine, Pseudopelletierine (Sabah Al-Salihi, 2014). The biggest concern for public health is that bacterial strains isolated from various ecosystems are immune to antibiotics used

Received: May 2019
Accepted: March 2020
Printed: December 2020

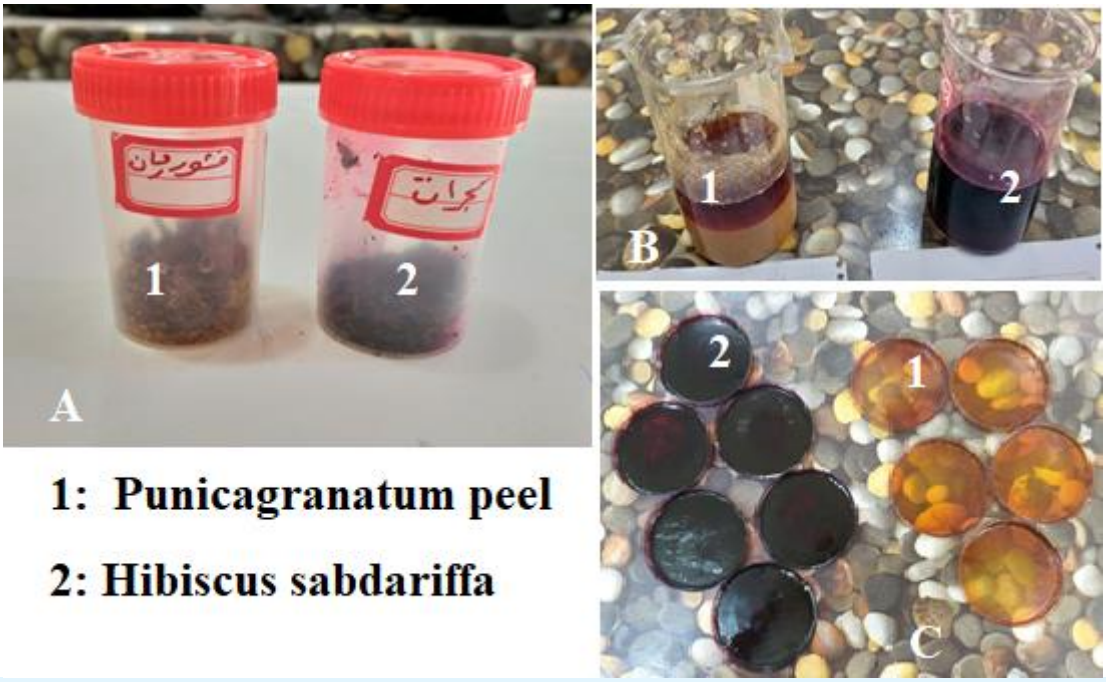

Fig. 1. A: Before Milling, B: powders dissolved in ethanol at a concentration of 70%, and C: after drying

in human medicine, which drastically reduces the treatment options and risks the lives of infected individuals (Banoon *et al.,* 2020), Thus this study aimed to find out the ability of plant extracts (Hibiscus Sabdariffa and Punicagranatum peel) to inhibit pathological microorganisms as well as their use as antioxidants.

MATERIALS AND METHODS

Hibiscus and crushed pomegranate peels were purchased from the local markets (Al-Amara Al-Kabeer Market- Al-Attarin). The method mentioned by (Anessing and Perez, 1993; Passos *et al.,* 2019) was followed by weighing 50 grams of each of the hibiscus and crushed pomegranate peels and putting each of them in a 500 ml glass beaker and adding 250 ml of ethyl alcohol to each of them at a concentration of 70%. Then was well mixed and left at room temperature for 24 hours and centrifugation was carried out at a speed of 3000 rpm for a period of 15 minutes.Then a filtration was carried out using the Whatman type filtration tools, the filtrate was evaporated with a rotary evaporator vacuum to obtain a concentrated filtrate under the influence of the vacuum pressure.The concentrated filtrate was poured into glass Petri dishes and was placed in an incubator at 37 ° C for 3 days to obtain a dry powder. The dry powder of hibiscus extract and pomegranate peels was scraped and placed in opaque bottles and kept at 4 ° C until use, as shown in **Fig. 1**. The standard solution was prepared by dissolving 1 g of each of the hibiscus powder and pomegranate peels in 15 ml sterile distilled water. Finally, the following concentrations were prepared (0.1, 0.2, 0.3) mg / ml by adding (1,2,3) ml of the standard solution to (9,8, 7) ml of distilled water to make the final volume of each concentration 10 ml (Weckesser *et al.,* 2007).

Biological effectiveness

Using the culture media called "Muller-Hinton Agar" for determination of the biological effectiveness. Diffusion method was to grow the test organisms, which included (*Staphylococcus aureus, Escherichia coli, Pseudomonas, Salmonella, Candida albicans*). There was obtained from the Misan Health Department / Central Public Health Laboratory.The culture medium used in the inhibitory activity was sterilized with an autoclave at 121° C for 15 minutes, after which the medium was cooled to 50° C. Then poured onto Petri-dishes (plate-count) with a volume of 20 ml for each plate-count and the dishes where left until solidification. The plates were inoculated with the bacterial suspension of each isolate with a 0.1 ml yeast suspension and the suspension was spread using a diffuser lube (glass diffuser). After that, a pit with a diameter of 6 ml was made on the surface of the inoculated culture media using the cork borer (Weckesser *et al*., 2007), The concentrations of plant extracts were transferred (0.3, 0.2, 0.1) to the pits with a volume of 50 µl in each hole, and then the dishes were placed in the incubator at a temperature of 35 ° C for 24 hours. After the incubation period ended, the inhibition diameter was measured by a ruler and the results were recorded.

Antioxidant effectiveness

The ready-made DPPH antioxidant (1,1- Diphenyl-2-Picrylhydrazyl) was used by dissolving 0.004 grams in 100 ml methanol according to the method mentioned by (Shah and Modi, 2015) with some modifications to

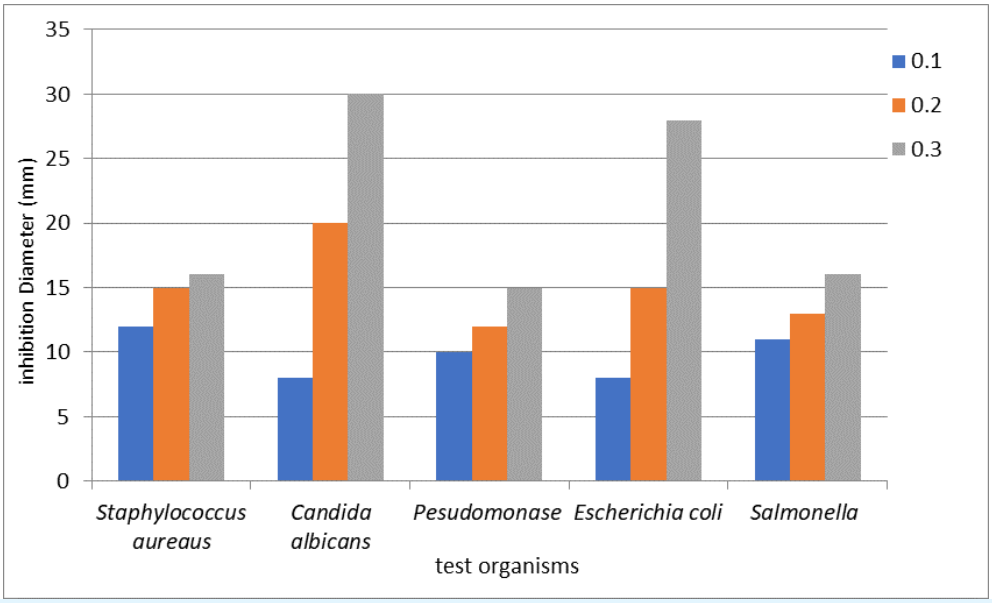

Fig. 2. The inhibitory effect of hibiscus extract

assess the antioxidant activity in hibiscus extract and pomegranate peel. The standard stock solution of hibiscus extract and pomegranate peels was prepared to estimate antioxidant by dissolving 0.04 grams of each extract in 40 ml sterile distilled water and then preparing each of them in seven tubes at concentrations of (100, 200, 300, 400, 500, 600, 700) ppm. G / ml) by adding (2.1, 1.8, 1.5, 1.2, 0.9, 0.6, 0.3) ml of the standard solution into seven test tubes and complete the volume for each tube to 3 ml, withdraw 2 ml of each concentration and add 1 ml of prepared DPPH to it. In addition to the control tube containing 1 ml of DPPH, the tubes were incubated in the dark for 30 minutes, and after the incubation period ended, the absorbance was measured at a wavelength of 517 nm using a Spectrophotometer. The device was reset with methanol and the results were recorded and according equation 1.

Antioxidant efficacy = $\frac{Ao-As}{Ao} * 100$ Eq. 1

Where: Ao = Control, and As= Absorbance Sample

RESULTS AND DISCUSSION

The inhibitory effect of hibiscus extract and pomegranate peel

Fig. 2 shows the inhibitory activity of hibiscus extract, as it can be noted that the highest inhibitory activity is at a concentration of 0.3 against the test revival (*Candida albicans, E. coli, Staphylococcus aureus, Salmonella, and Pseudomonas)*, as it reached (30,28,16,16,15) mm respectively. As for the concentration of 0.1, the inhibitory activity against the test organisms above reached (8,8,12,11,10) mm, respectively.

As for the pomegranate peel extract, it can be seen in **Fig. 3** that the highest inhibitory activity was at a concentration of 0.3 against the tested organisms *(Pseudomonas, Staphylococcus aureus, Candida albicans, E.coli, Salmonella),* which amounted to (35,31,25,19,0) (Mm) respectively. While the least inhibitory activity was at concentration 0.1 against all the above the tested organisms, which amounted to (30,22,16,13,0) mm respectively. It is apparent from this set of results that the inhibitory efficacy of the hibiscus extract and the pomegranate peel increased with the increase in the concentration of the extract due to the increase in the concentration of inhibitors and the active groups in the extract, which led to an increase in the diameter of inhibition. The inhibitory effectiveness of the pomegranate peel extract may be due to the presence of phenols that inhibit the action of enzymes inside the cell and electron transfer and hindering the oxidative phosphorylation process and the clotting of cytoplasmic substances in the bacteria which leads to their killing. This finding is consistent with that of (Gin *et al.,* 2000) who indicated that phenols inhibit the enzymes responsible for the basic metabolic reactions in the bacteria through their non-specialized interference with proteins, which leads to protein metamorphosis and therefore the death of bacteria, This also accords with the earlier observations of Sabah Al-Salihi, (2014) who mentioned that the alcoholic extract of pomegranate peels gave inhibitory activity against pathological bacteria and showed that the higher the concentration of the extract, the greater the diameter of inhibition, Our sets of results are in agreement with Seeram *et al*., (2005) findings which showed that the pomegranate peel extract killed and inhibited the growth of germs isolated from the uterus of sheep infected with uterine infection, also pointed out that the reason for the effect of the pomegranate peel extract on the pathological bacteria is due to its containment of tannins, phenols and flavonoids, which are substances that inhibit bacterial growth. While other studies for other plants like the

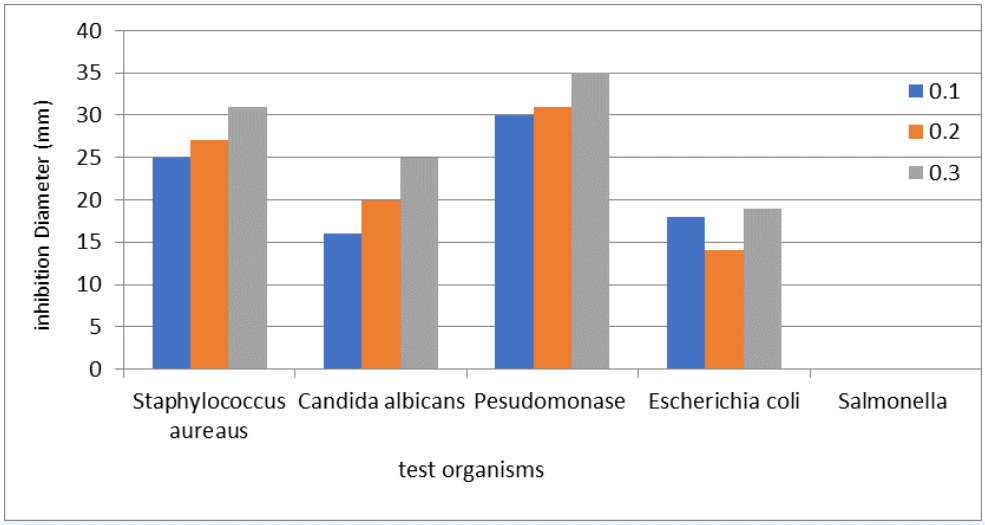

Fig. 3. The inhibitory activity of pomegranate peels

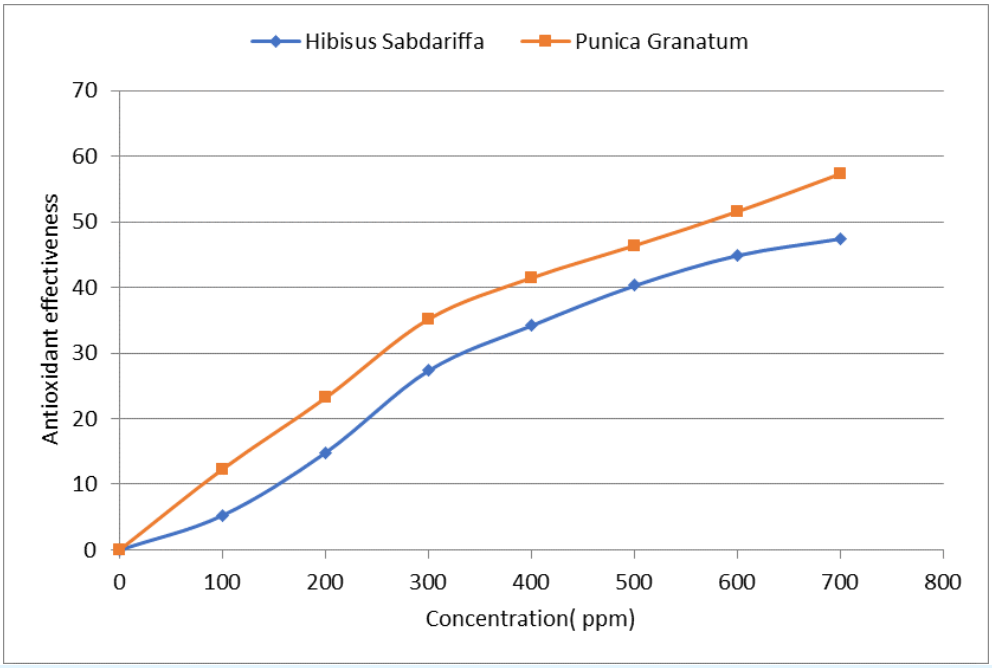

Fig. 4. The antioxidant effectiveness' of hibiscus extract and pomegranate peel

extract of *Uncaria gambir* roxb contain secondary metabolites had the inhibitory activity for cancer cells and able to inhibit the activity of *Escherichia coli* (Unnes *et al.,* 2020).

The red tea extract (hibiscus) contains some effective compounds such as (resin, saponins, phenols and tannins) as well as organic acids such as ascorbic acid, Hibiscus acid, tartaric acid, malic acid, as well as the presence of Anthocyanin pigment and other phenolic compounds such as Hibicus protocatechuic acid, which is found in the aqueous extract of the flower of the tea plant (hibiscus). Due to its evident effectiveness against bacteria, it has been used as a treatment in traditional medicine for diarrhea, intestinal infections, and skin infections. The ability of the hibiscus plant to inhibit various types of test organisms is also attributed to the fact that it contains many secondary metabolism products, as the plant flowers contain the compounds of Anthocyanins, Flavonoids, Phenolic acid. These compounds have proven effective antibacterial, antioxidative and anticarcinogenic. It also contains many organic acids such as citric acid, malic acid, tartaric acid and a high percentage of ascorbic acid (Izquierdo-Vega et al., 2020), The hibiscus tea plant has the ability to inhibit bacterial growth better than some antibiotics.

Antioxidant activity at hibiscus and pomegranates peel extracts

Fig. 4 illustrate the antioxidant efficacy of hibiscus's and pomegranate peel extract as the highest antioxidant activity reached 57.36% and 47.47%, respectively, to

inhibit peroxides at a concentration of 700 ppm. While the lower efficacy for them was 12.33% and 5.27%, respectively at a concentration of100 ppm. The sample absorbent was on 517 nm. We conclude from this that of the antioxidant activity at pomegranate peels was the highest inhibition of peroxides compared with the antioxidant activity at hibiscus and in the all concentrations prepared form samples the study.

Increasing the antioxidant efficacious the hibiscus plant with an increase in the molecular weight, because was found the phenolic compounds and carotenoids in the hibiscus plant act as hydrogen donor compounds that have the ability to convert free radicals into more stable compounds, and thus the increased at antioxidant effectiveness of hibiscus by increasing these compounds. Piovesana *et al.* (2019) results were consistent with Devatkal and Naveena, (2010) which found that pomegranate peel extract gave antioxidant efficient when added to goat meat andstored for 6 days.

CONCLUSION

Through the study that was conducted on Hibiscus sabdariffa and pomegranate peel powder, we conclude that Hibiscus sabdariffa extract and pomegranate peel possesses a high ability to inhibit pathological micro-organisms as well as their antioxidant efficacy and therefore these plant extracts can be used and taken in the form of therapeutic preparations that help in treating pathogens caused by pathological bacteria.

REFERENCES

Ali, B. H., Wabel, N. A., & Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(5), 369-375. https://doi.org/10.1002/ptr.1628

Anesini, C., & Perez, C. (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. *Journal of ethnopharmacology*, *39*(2), 119-128. https://doi.org/10.1016/03788741(93)90027-3

Banoon, S., Ali, Z., and Salih, T. (2020). Antibiotic resistance profile of local thermophilic Bacillus licheniformis isolated from Maysan province soil. Comunicata Scientiae, 11, e3291. https://doi.org/10.14295/cs.v11i0.3291

Devatkal, S. K., & Naveena, B. M. (2010). Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat science*, *85*(2), 306-311. https://doi.org/10.1016/j.meatsci.2010.01.019

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food chemistry*, *48*(10), 4581-4589. https://doi.org/10.1021/jf000404a

Goering, R., Dockrell, H., Zuckerman, M., Roitt, I., & Chiodini, P. L. (2012). *Mims' medical microbiology*. Elsevier Health Sciences.

Guardiola, S., & Mach, N. (2014). Therapeutic potential of Hibiscus sabdariffa: a review of the scientific evidence. *Endocrinología y Nutrición (English Edition)*, *61*(5), 274-295. DOI: 10.1016/j.endoen.2014.04.003

Iwalokun, B. A., & Shittu, M. O. (2007). Effect of Hibiscus sabdariffa (calyce) extract on biochemical and organoleptic properties of yogurt. *Pakistan Journal of Nutrition*, *6*(2), 172-182.

Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sánchez-Gutiérrez, M., Morales-González, J. A., Vargas-Mendoza, N., Gómez-Aldapa, C. A., Castro-Rosas, J., Delgado-Olivares, L., Madrigal-Bujaidar, E., andv Madrigal-Santillán, E. (2020). Organic Acids from Roselle (Hibiscus sabdariffa L.)—A Brief Review of Its Pharmacological Effects. *Biomedicines*, *8*(5), 100. DOI: 10.3390/biomedicines8050100

Jalalyazdi, M., Ramezani, J., Izadi-Moud, A., Madani-Sani, F., Shahlaei, S., & Ghiasi, S. S. (2019). Effect of hibiscus sabdariffa on blood pressure in patients with stage 1 hypertension. *Journal of Advanced Pharmaceutical Technology & Research*, *10*(3), 107. doi: 10.4103/japtr.JAPTR_402_18

Onibi, G. E., & Osho, I. B. (2007). Oxidative stability and bacteriological assessment of meat from broiler chickens fed diets containing Hibiscus sabdariffa calyces. *African Journal of Biotechnology*, *6*(23). DOI: 10.5897/AJB2007.000-2435

Passos, M. M., Fernandes, E. P., Gualberto, S. A., Silva, S. L. C., and Da Silva, D. C. (2019) Total Phenolics and Flavonoids Determination and Antioxidant Capacity Evaluation of Poincianella Bracteosa (Fabaceae) Extracts. *Periódico Tchê Química*. 16(32):87-95.

Piovesana, A., Rodrigues, E., & Noreña, C. P. Z. (2019). Composition analysis of carotenoids and phenolic compounds and antioxidant activity from hibiscus calyces (Hibiscus sabdariffa L.) by HPLC-DAD-MS/MS. *Phytochemical Analysis*, *30*(2), 208-217. https://doi.org/10.1002/pca.2806

Ruban, P., & Gajalakshmi, K. (2012). In vitro antibacterial activity of Hibiscus rosa–sinensis flower extract against human pathogens. *Asian pacific journal of tropical biomedicine*, *2*(5), 399-403. doi: 10.1016/S2221-1691(12)60064-1

Sabah Al-Salihi, S. (2014). Inhibitory Effect of Aqueous Extract of Pomegranate Peel on Escherichia. *Kirkuk University Journal-Scientific Studies*, *9*(1), 11-18. DOI: 10.32894/kujss.2014.89138

Salib, J. Y. (2014). Polyphenolic Compounds from Flowers of Hibiscus: Characterization and Bioactivity. In *Polyphenols in Plants* (pp. 231-239). Academic Press. https://doi.org/10.1016/B978-0-12-397934-6.00012-7

Seeram, N. P., Adams, L. S., Henning, S. M., Niu, Y., Zhang, Y., Nair, M. G., & Heber, D. (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *The Journal of nutritional biochemistry*, *16*(6), 360-367. https://doi.org/10.1016/j.jnutbio.2005.01.006

Shah, P., & Modi, H. A. (2015). Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. *Int J Res Appl Sci Eng Technol*, *3*(6), 636-41.

Sumner, M. D., Elliott-Eller, M., Weidner, G., Daubenmier, J. J., Chew, M. H., Marlin, R., Raisin, C.J., and Ornish, D. (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. *The American journal of cardiology*, *96*(6), 810-814. https://doi.org/10.1016/j.amjcard.2005.05.026

Tolulope, M. (2007). Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. *Journal of Medicinal Plants Research*, *1*(1), 009-013. https://doi.org/10.5897/JMPR.9000430

Unnes, S., Sumarni, W., Diliarosta, S., and Ramadanty, I. (2020). The reconstruction of scientific knowledge about bajakah kalalawit (uncaria gambir roxb) bioactivity as ethnomedicine. *Periódico Tchê Química*. 17(35):524-535.

Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K., & Schempp, C. Á. (2007). Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine*, *14*(7-8), 508-516. https://doi.org/10.1016/j.phymed.2006.12.013