Chelonian Conservation And Biology





Vol. 18 No. 2 (2023) | <u>https://www.acgpublishing.com/</u> | ISSN - 1071-8443

DOI: doi.org/10.18011/2023.11(2).1037.1045

SYNERGISTICS ACTIVITY OF THE OIL EXTRACTS OF HIBISCUS SABDARIFFA

Priyam Bharathidasan

Department of Microbiology, Centre for Infectious Diseases, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University,

Chennai 600077

Dr. Sathish Sankar (*Corresponding author)

Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai-77, Tamil Nadu,India.

Email:sathishsankar.sdc@saveetha.com

Abstract

Introduction

Tropical *Hibiscus sabdariffa*, also referred to as roselle or sorrel, is widely used in both traditional medicine and cooking. This plant, which is native to tropical areas, has drawn notice for possible health advantages. Meanwhile, there are serious health risks related to *Enterococcus faecalis*, a bacterium linked to a number of infections, including endocarditis and urinary tract infections.

Aim

The purpose of this study is to examine the essential oil extracted from *Hibiscus sabdariffa* flowers' synergistic antibacterial properties against *Enterococcus faecalis*.

Materials and methods

Hibiscus sabdariffa essential oil was extracted using the Clevenger apparatus. After thoroughly combining the plant material with distilled water, it was brought to a boil until the oil was visible. To investigate combined antibacterial effects, *Abutilon indicum* crude methanol extract was introduced. A comparison of the methanol extract's and its absence from the antibacterial activity revealed that the latter was more effective against *Enterococcus faecalis*.

Results

The *Hibiscus sabdariffa* essential oil was visible after three hours of boiling. Surprisingly, there were no antibacterial effects seen by the plant oil extract on its own. But when mixed with *Abutilon*



All the articles published by Chelonian Conservation and Biology are licensed under a Creative Commons Attribution-NonCommercial 4.0 International License Based on a work at https://www.acgpublishing.com/

indicum crude methanol extracts, a notable and significant inhibitory activity against *Enterococcus* faecalis was seen.

Conclusion

The outcomes highlight the combination of *Hibiscus sabdariffa* oil extract and *Abutilon indicum* crude methanol extract's strong inhibitory activity against *Enterococcus faecalis*. This cooperative approach raises the possibility of a synergistic antibacterial effect, which may have consequences for the creation of antibiotics that specifically target *Enterococcus faecalis*.

Keywords:- *Hibiscus sabdariffa* oil extract, *Abutilon indicum* crude methanol extract, Clevenger apparatus, Universal heath, Diseases, Well being, Health, International Health policy

Introduction

Hibiscus sabdariffa L., commonly known as roselle and belonging to the Malvaceae family, has a rich history of traditional use. It has been employed as a food item, featured in herbal beverages, both hot and cold, served as a flavor enhancer in the food industry, and utilized as an herbal remedy. (1) The Roselle flower and its calyces are recognized for their antiseptic, diuretic, antioxidant, and antimutagenic attributes. The dried flowers of this plant contain gossipetine and hibiscin, which are anthocyanins. The petals produce the flavanol hibiscritin glucoside, while the calyces are abundant in riboflavin, ascorbic acid, niacin, carotene, calcium, and iron.(2)Typically, the calyces and leaves of the roselle plant are employed in the preparation of jams, jellies, sauces, and pickles. Research indicates that roselle seeds are notably high in protein and oil, with the oil being abundant in unsaponifiable components and phytosterols.(3)

The seeds of the roselle plant, comprising around 20% oil, are acknowledged for their elevated nutritional content and health-enhancing characteristics. (4)This is attributed to the inherent equilibrium of saturated, monounsaturated, and polyunsaturated fatty acids within the seeds. Furthermore, it has been observed that roselle seeds contain substantial quantities of protein, crude fiber, oil, and carbohydrates.(5) The qualities of oils vary primarily due to their compositions, and there is no one-size-fits-all oil from a single source suitable for all purposes. Sorrel seed oil, abundant in both linoleic and oleic fatty acids, is employed in Sudan for producing edible oil. The by-products resulting from this production are utilized as feed for poultry. (6)

Abutilon indicum, a member of the Malvaceae family, commonly known as Mallow in English, is utilized for its medicinal properties. Studies have indicated that *Abutilon indicum* exhibits anti-inflammatory, antioxidant, and antimicrobial properties. (7) The plant contains a variety of phytochemicals, including alkaloids, saponins, amino acids, flavonoids, glycosides, and steroids. Essential oil constituents such as α -pinene, mucilage, tannins, caryophyllene, asparagine, caryophyllene oxide, endesmol, farnesol, borenol, geraniol, geranyl acetate, elemene, and α -cineole have been identified in the plant. (8)Additionally, phytoconstituents like β -sitosterol, caffeic acid, fumaric acid, vanillin, p-coumaric acid, p-hydroxybenzoic acid, sesquiterpenes

including lactones (alantolactone and isoalantolactone), hexoses, n-alkane mixtures (C22-34), alkanol, and gallic acid have been reported in various parts of the plant.(9)

Materials and methods

Clevenger apparatus set up:

The Clevenger apparatus is a fundamental instrument in the extraction of essential oils. It is composed of necessary parts that are carefully engineered to ensure effective steam distillation. A graduated receiver, a water condenser, and a round-bottom flask are some of its essential components. The *Hibiscus sabdariffa* plant material is combined with other ingredients in the initial setup of the round-bottom flask, which makes it easier to generate steam and extract essential oils. This flask was selected due to its durability, as it can tolerate high temperatures and facilitate the process of creating steam, which is essential for the isolation of volatile substances.

The water condenser, which is attached to the flask's round-bottom neck, is essential to the closed system that circulates steam. Essential oils are carried by the steam as it rises through the plant material. The efficient cooling and condensing of the steam by the condenser facilitates the extraction of essential oils from water vapor. A quantitative measurement of the extracted oils is obtained by gathering and measuring the condensed essential oils using a graduated receiver situated beneath the condenser. These Clevenger apparatus parts function in unison to maximize the steam distillation process, which makes it a vital and efficient tool for extracting essential oils from plant materials.

Preparation of plant extract:

Thoroughly mixing the mixture of plant material (*Hibiscus sabdariffa*) is an essential step in preparation before starting the boiling process in the Clevenger apparatus. With careful execution, this step ensures that the plant material is distributed uniformly, improving the interaction with steam during the extraction process. The aim is to obtain a homogenous mixture so that every part can be exposed to the steam on a regular basis. This even dispersion maximizes the surface area that comes into contact with the steam and the plant material, which is essential for promoting an effective extraction of essential oils. An improved level of interaction at this point creates the conditions for a deeper extraction, which raises the total yield of volatile compounds—including the highly sought-after essential oils.

Extraction of essential oil:

After the mixture is carefully stirred, heat is applied to it, which starts the boiling process inside the flask with a circular bottom. Steam enriched with volatile components, like essential oils, is released from the plant material when the water in the flask reaches boiling point. These volatile substances vaporize at the high temperature, which enables them to rise with the steam. This procedure successfully extracts the essential oils from the plant material, and the *Hibiscus*

sabdariffa essence is carried by the steam as it passes through the Clevenger apparatus. The condenser then aids in the steam's cooling, which causes the volatile ingredients to condense and the essential oils to gather in the graduated receiver.

Boiling continues in the Clevenger apparatus until the essential oils are successfully extracted from the *Hibiscus sabdariffa* plant material. The length of this stage is variable and depends on various factors, including the desired amount of oil and the unique characteristics of the plant material. The longer heating time facilitates the steam's ongoing interaction with the plant material, which helps the essential oils release and vaporize more gradually. This methodical procedure guarantees the effective extraction of volatile compounds from the plant matrix, including the highly desired essential oils.

The first sign of the oil droplets appears after about three hours of boiling. This is a significant milestone. Essential oils rise to the top and become distinctly different because they are less dense than water. An important marker that marks the beginning of the extraction process's efficacy is the appearance of the first oil droplet. This phenomenon implies that a significant amount of essential oils have gathered in the gathered liquid, and the continuous boiling guarantees a prolonged release and absorption of these valuable aromatic compounds. The visible existence of oil droplets is a concrete indication that the essential oils from the *Hibiscus sabdariffa* plant material were successfully extracted by the Clevenger apparatus.

Results

To make a plant extract, 250 grams of *Hibiscus sabdariffa* and 1 liter of distilled water were carefully combined in the first step of the extraction process. The *Hibiscus sabdariffa* plant material is used to extract essential oils using this aqueous mixture as a base. To ensure that the plant material is distributed uniformly within the solvent and to maximize the contact between water and the components of *Hibiscus sabdariffa*, thorough mixing is essential (Figure 1).



Figure 1:- observance of *Hibiscus sabdariffa* oil extract

After the plant extract was prepared, the essential oil extraction was started using the Clevenger apparatus. After stirring the contents of the round-bottom flask, the mixture was brought to a boil, allowing the essential oils to be extracted successfully. This crucial phase of the procedure only took three hours. After this time, the extraction process begins when the first discernible oil droplet appears, signifying that the volatile essential oils have been released from the *Hibiscus sabdariffa* plant material. The Clevenger apparatus makes it easier to extract essential oils from the plant matrix through steam distillation, and the first oil droplet can be seen as a concrete sign of the extraction method's efficacy.

When tested independently, the isolated plant oil extract from *Hibiscus sabdariffa* surprisingly showed no inherent antibacterial activity. But when this oil extract was mixed with the *Abutilon indicum* crude methanol extracts, an interesting change happened. The resulting mixture showed a significant increase in inhibitory activity against the bacterium *Enterococcus faecalis*, also known as *E. faecalis*, which is frequently linked to a number of infections. This finding emphasizes the possibility of synergistic effects resulting from the two extracts interacting. The cooperative behavior of *Abutilon indicum*'s crude methanol and plant oil extracts seems to increase their combined antibacterial potency, indicating a potential synergistic interaction between the bioactive compounds in the two extracts.

To measure the antibacterial efficacy quantitatively, the zone of inhibition—a measurement of the region where bacterial growth is inhibited—was evaluated. The observed inhibition zone measured 3mm when the isolated plant oil extract was used in conjunction with antibiotics. On the other hand, a greater inhibition zone measuring 4mm was observed when the plant oil extract was used in combination with the crude methanol extracts of *Abutilon indicum*. This resulted in a noteworthy increase in inhibitory activity. This substantial variation in the inhibitory zones suggests that the combined extracts may have synergistic antimicrobial effects, especially in inhibiting the growth of *E. faecalis*. The results imply that the combination of the crude methanol extracts of *Abutilon indicum* and the plant oil extract of *Hibiscus sabdariffa* may produce a more effective antimicrobial agent than either extract by itself (Figure 2).



Figure 2:- Antibacterial activity of the plant oil extract

Discussion

In a previous study, *Hibiscus sabdariffa* oil extracts were obtained by the Soxhlet extraction method; the first oil droplets to appear took about 6 hours to appear.(10) But in the present study, oil was extracted using the Clevenger apparatus, and astonishingly, the first evident oil droplet was found in just three hours. This difference in extraction times indicates that, in this specific study, using the Clevenger apparatus turned out to be a more effective method than using the Soxhlet extraction technique.(11)

At every tested concentration, the *Abutilon indicum* extract was shown to be able to inhibit Grampositive bacteria in a previous study.(12) Similar results were seen in the current study when the oil extracts of *Hibiscus sabdariffa* and the crude methanol extracts of *Abutilon indicum* were combined with the Gram-positive bacterium *E. faecalis*. This result demonstrates the noteworthy synergistic antimicrobial activity that was attained by combining the extracts of *Hibiscus*

sabdariffa and Abutilon indicum, which was specifically directed towards a strain of bacteria that is Gram-positive.(13)

The ethanol fruit extracts of *Abutilon indicum* showed notable antimicrobial efficacy against *E. faecalis* in a previous study. (14)This earlier discovery supports the findings of the present investigation, which showed significant inhibition of *E. faecalis* when crude methanol extracts from *Abutilon indicum* and oil extract from *Hibiscus sabdariffa* were combined. The two studies' consistent antimicrobial activity highlights the potential synergistic effects of using extracts from *Abutilon indicum*—alone or in combination with other plant extracts—against *E. faecalis*. (15)

A previous study assessed *Hibiscus sabdariffa*'s antimicrobial activity against a range of oral pathogens, including *E. faecalis*. As per the results, the standard plant extracts exhibited strong antimicrobial activity, particularly against *E. faecalis*. (16) This finding implies that the plant's natural extracts have antibacterial properties by nature. Nonetheless, it is significant that, when focusing only on the *Hibiscus sabdariffa* oil extracts, the current study's results showed minimal antimicrobial activity. Interestingly, a strong antimicrobial effect against *E. faecalis* was seen when combined with the crude methanol extract of *Abutilon indicum*. This demonstrates the complex and possibly beneficial interactions between various plant extracts, demonstrating that the antimicrobial activity of *Hibiscus sabdariffa* can be amplified when combined with extracts from other botanical sources.

Conclusion

The outcomes highlight the strong inhibitory action demonstrated by the combined application of the crude methanol extract from *Abutilon indicum* and the oil extract from *Hibiscus sabdariffa* against *Enterococcus faecalis*. A synergistic antibacterial effect is suggested by this cooperative strategy, which may have implications for the development of antibiotics that specifically target *Enterococcus faecalis*.

References

- 1. Hibiscus sabdariffa L. A phytochemical and pharmacological review. Food Chem. 2014 Dec 15;165:424–43.
- 2. Higginbotham KL. Aqueous Extracts of Hibiscus Sabdariffa as Antimicrobials in Foods. 2013.
- 3. Mariod AA, Tahir HE, Mahunu GK. Roselle (Hibiscus sabdariffa): Chemistry, Production, Products, and Utilization. Academic Press; 2021. 272 p.
- 4. Google Docs [Internet]. [cited 2023 Nov 29]. 7 scopus.pdf. Available from: https://drive.google.com/file/d/1sibudXkw_aBvPq6AAJ3q7u5Mhko_p5eF/view?usp=sharing&usp=embed facebook

- 5. Naeem MA, Zahran HA, Hassanein MMM. Evaluation of green extraction methods on the chemical and nutritional aspects of roselle seed (Hibiscus sabdariffa L.) oil. OCL. 2019;26:33.
- 6. Chau CW. Screening and Optimization of Oil Seed Extraction Using Soxhlet Method. 2013. 54 p.
- 7. Raja RR, Kailasam KV. Abutilon indicum L (Malvaceae)-Medicinal Potential Review. Pharmacognosy Journal [Internet]. 2015 Sep 20 [cited 2023 Nov 15];7(6). Available from: http://www.phcogj.com/sites/default/files/10.5530pj.2015.6.2.pdf
- 8. Website [Internet]. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85146302612&doi=10.52711%2f0974-360X.2022.00924&partnerID=40&md5=93acffe823158e659108341e18b0825a
- 9. Devarkar V. View of A Review on Phytochemical Constituents of Abutilon indicum (Link) Sweet An Important Medicinal Plant in Ayurveda [Internet]. [cited 2023 Nov 15]. Available from: http://www.plantaescientia.com/ojs/index.php/ps/article/view/54/37
- 10. Hagr T, Adam I. Phytochemical Analysis, Antibacterial and antioxidant Activities of Essential Oil from Hibiscus sabdariffa (L) Seeds, (Sudanese Karkadi). Progress in Chemical and Biochemical Research. 2020 Jul 1;3(3):194–201.
- 11. Google Docs [Internet]. [cited 2023 Nov 29]. 2.pdf. Available from: https://drive.google.com/file/d/1sMsX9xi00bqxsfp-Lgbvn_g1iEVnhvMa/view?usp=sharing&usp=embed_facebook
- 12. Kaushik D, Kaushik P, Sharma C. EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ABUTILON INDICUM. 2010 [cited 2023 Nov 29]; Available from: https://www.semanticscholar.org/paper/EVALUATION-OF-ANTIOXIDANT-AND-ANTIMICROBIAL-OF-Kaushik-Kaushik/2a72265160322546f53ff690b6f36b027402f166
- 13. Website [Internet]. Available from: https://www.scopus.com/record/display.uri?eid=2-s2.0-85101096035&origin=resultslist&sort=plf-f&src=s&nlo=&nlr=&sid=63b046f1ad6f1a81cacad22b000fae45&sot=aff&sdt=cl&cluster=scoprefnameauid%2c%22Lakshmi%2c+T.%2357221436939%22%2ct%2c%22Than gavelu%2c+L.%2357221436939%22%2ct%2bscosubjabbr%2c%22DENT%22%2ct%2bscoexactsrctitle%2c%22International+Journal+Of+Dentistry+And+Oral+Science%22%2ct%2 c%22Oral+Diseases%22%2ct&sl=55&s=AF-ID%28%22Saveetha+Dental+College+And+Hospitals%22+60109334%29&relpos=14&citeCnt=0&searchTerm=

- 14. Identification of biological activities of Abutilon indicum fruit by in silico and in vitro approach. Karbala International Journal of Modern Science. 2018 Sep 1;4(3):287–96.
- 15. Website [Internet]. Available from: https://www.scopus.com/record/display.uri?eid=2-s2.0-85092912484&origin=inward&txGid=5a0481005cb86983ccc2fe9d54b6d590
- 16. Abass AA, Al-Magsoosi MJN, Kadhim WA, Mustafa R, Ibrahim SA, Aljdaimi AI, et al. Antimicrobial effect of Red Roselle (Hibiscus Sabdariffa) against different types of oral bacteria. J Med Life. 2022 Jan;15(1):89.