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IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF HIBISCUS FLOWER EXTRACT

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Abstract

Introduction: The key components found in hibiscus flower extract is anthocyanins, which are pigments responsible for the flower's vibrant colors. Anthocyanins are potent antioxidants that help protect the body against oxidative stress, a process linked to various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. This antimicrobial activity makes hibiscus flower extract a potential natural alternative to conventional antibiotics, particularly in light of the growing problem of antibiotic resistance. **Materials and Methods:** The anti-bacterial activity of hibiscus was analyzed by the agar well diffusion method. First, the nutrient agar was uniformly spread on the Petri plates. The two microbial fungal strains Candida and Actinomyces were used to examine the antimicrobial activity. After that, 50, 100, and 150 L of hibiscus were added to the Petri plates. The culture medium was incubated at 37°C for 24 h. Following 24 h incubation, the zone of inhibition was measured. **Results:** In comparison to conventional ascorbic acid, it exhibits DPPH and ABTS radical scavenging activities. It contains many phytocompounds. It has an inhibitory zone against Candida albicans and actinomycetes. **Conclusion:** The findings of this study demonstrate that hibiscus flower extract possesses significant in vitro antioxidant and antibacterial activities. These results support its potential as a natural source of antioxidants and antimicrobial agents. The knowledge gained from this study contributes to the growing body of evidence on the beneficial properties of hibiscus flower extract and paves the way for its potential applications in healthcare and pharmaceutical industries.

Keywords: Hibiscus, Anti-Oxidant, Anti-Bacterial, Actinomycetes, Anthocyanins.

INTRODUCTION

The herb *Hibiscus rosa-sinensis* Linn. (Malvaceae) is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers. In medicine, however, the red flowered variety is preferred. The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers(1).Hibiscus, encompassing a diverse array of species, captivates enthusiasts for both its aesthetic allure and therapeutic potential. Renowned for its flamboyant and intricate flowers, varying in hues from vibrant reds and pinks to soothing whites and yellows, numerous Hibiscus species stand out as ornamental gems in landscaping. Beyond their visual appeal, certain varieties, such as Hibiscus sabdariffa, are treasured for their medicinal properties. The flowers of these plants boast a wealth of bioactive compounds, including polyphenols, flavonoids, and anthocyanins. These compounds contribute to the plant's antioxidant prowess, offering potential health benefits. Hibiscus extracts, often derived from the calyx and sepals, have been studied for their cardioprotective effects, including the regulation of blood pressure and cholesterol levels. Whether adorning gardens with their splendid blooms or serving as a source of natural remedies, Hibiscus exemplifies a multifaceted botanical treasure(2). Antioxidants neutralize free radicals, preventing oxidative stress and cellular damage. Free radicals, produced during metabolic processes or due to

external factors, contribute to inflammation and chronic diseases. Hibiscus, rich in antioxidants like polyphenols and flavonoids, offers potential health benefits by counteracting oxidative stress. Hibiscus flowers, abundant in polyphenols, flavonoids, and anthocyanins, exhibit potent antioxidant properties, safeguarding cells from oxidative damage. These attributes not only contribute to overall health but may also help reduce the risk of specific diseases. Plants produce antioxidant compounds in response to environmental stress, which, when consumed, can enhance your own defenses against oxidative stress, potentially extending lifespan and promoting overall well-being. Leveraging the protective qualities of plant-derived antioxidants aligns with nature's strategy for enhancing human health(3).Extracts from Hibiscus flowers, leaves, and stems demonstrate robust antioxidant potential, suggesting a lowered risk of various diseases. The rise of drug-resistant microorganisms and emerging, previously unknown pathogens poses a significant public health risk, emphasizing the need for effective control measures(4,5).

Hibiscus exhibits antibacterial properties, with certain compounds in its extracts demonstrating the ability to inhibit the growth of bacteria. This natural antibacterial activity suggests potential applications in promoting hygiene and combating bacterial infections. Naturally-occurring phytochemicals found in plants, fruits, and vegetables are bioactive compounds that contribute to the plants' defense mechanisms. Many of these phytochemicals, including polyphenols, flavonoids, and alkaloids, exhibit antioxidant properties, helping to neutralize harmful free radicals in the body and mitigate oxidative stress.

Additionally, some phytochemicals possess antibacterial activity, which can inhibit the growth or survival of bacteria, contributing to the plants' natural defense against pathogens. When consumed by humans through a diet rich in fruits and vegetables, these phytochemicals are believed to confer health benefits, potentially reducing the risk of chronic diseases and supporting overall well-being. The dual action of antioxidant and antibacterial activities in these plant-derived compounds underscores their potential role in promoting human health(5). The robust antibacterial activity demonstrated by Picrorhiza kurroa-ZnO extract against Streptococcus mutans, especially at concentrations of 25 and 100 microliters, prompts parallel investigations into hibiscus extracts. If hibiscus exhibits similar antibacterial efficacy against S. mutans, it could signify a natural and effective alternative for reducing the virulence of oral pathogens, offering potential applications in oral health care and contributing to the development of novel antibacterial agents from natural sources(6-7).

Graphene oxide (GO) displays potent antimicrobial properties, effectively combating microorganisms. At lower concentrations, it exhibits minimal or no cytotoxic effects on living cells, making it a promising material for applications requiring antimicrobial benefits without harming cells(7). In this, we show how the chemical compositions, total phenolic and total flavonoid, antioxidants activities (DPPH radical scavenging activity, ABTS radical scavenging activity, reducing power, and FRAP), and anti-inflammatory activity of Hibiscus flower extracts varied depending on the time of harvest. For the purpose of identifying cluster formation and examining the correlations between samples from various harvest times, principal component analysis (PCA) was used. To the best of our knowledge, no studies evaluating the antioxidant and anti-inflammatory properties of Hibiscus extracts have ever been done.

MATERIALS AND METHODS

Preparation of Hibiscus Flower Extract:

In the hibiscus flower extract preparation using ethanol, fresh flowers are harvested, cleaned, and optionally air-dried to prevent mold. After grinding into a coarse powder, the hibiscus is mixed with ethanol (1:5 ratio) and allowed to macerate for 24-48 hours with intermittent agitation. Filtration separates the liquid extract from plant material, and the resulting ethanol-dissolved extract is taken. Ethanol is chosen for its efficient extraction capabilities, and safety precautions are essential during the process of hibiscus extraction.

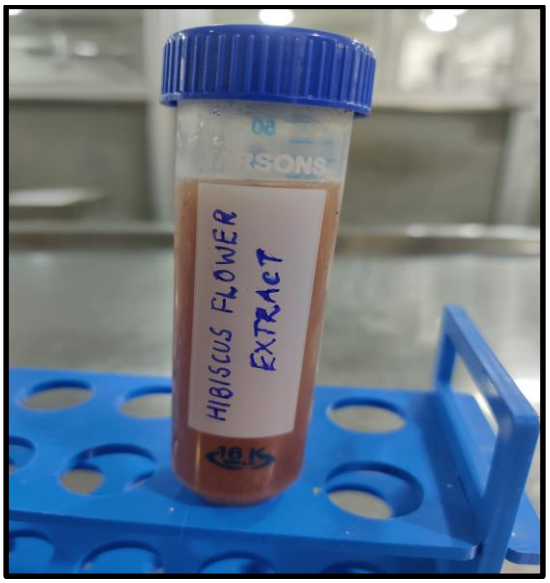


Figure 1: Hibiscus Flower Extract

Antioxidant Assay:

DPPH Assay: Calculate the decrease in DPPH absorbance to gauge the extract's capacity to neutralize free radicals. DPPH radical scavenging activity of each examined sample was performed by the method proposed by (8) A series of the extracts with different dilutions (0.1 mL in 60% ethanol) were mixed with 60% ethanol solution (1.4 mL) and 0.004% DPPH ethanol solution (1 mL). The mixed solution was incubated in the dark for 70 min and measured the absorbance value at 517 nm. Vc was used as the positive control. The DPPH radical scavenging activity was calculated using the equation:

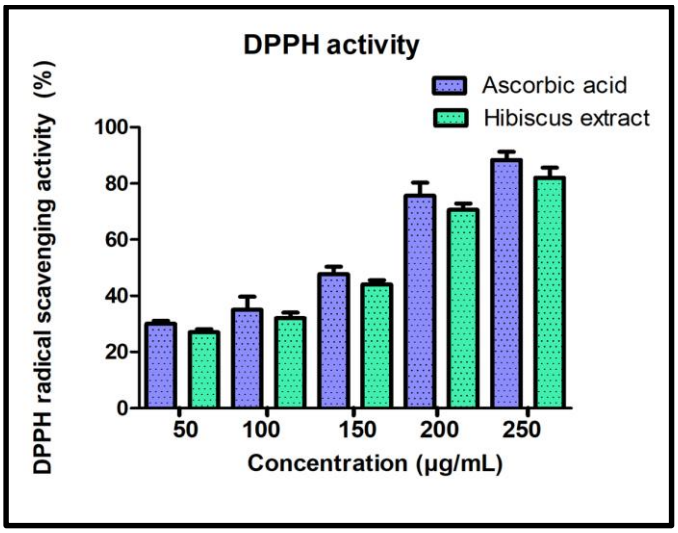
DPPH scavenging activity (%) = (DPPH – Sample/ADPPH) x 100

ABTS Assay: Determine antioxidant capability based on the prevention of the generation of ABTS radical cations.

The overall antioxidant capacity was assessed using the ABTS radical scavenging activity. The ABTS working solution (200 lL), which was created by mixing the ABTS stock solution with 2.45 mM potassium persulfate in the dark at room temperature for 12–16 h and diluted with 80% ethanol to reach the absorbance of 0.70 0.05 at 734 nm, was added with various concentrations of Trolox (0.15–1.5 mM) or extract (10 lL). To measure the absorbance at 734 nm in the dark, the mixture was incubated for 2–6 min. mmol/g Trolox equivalent antioxidant capacity (TEAC) was used to express the results.

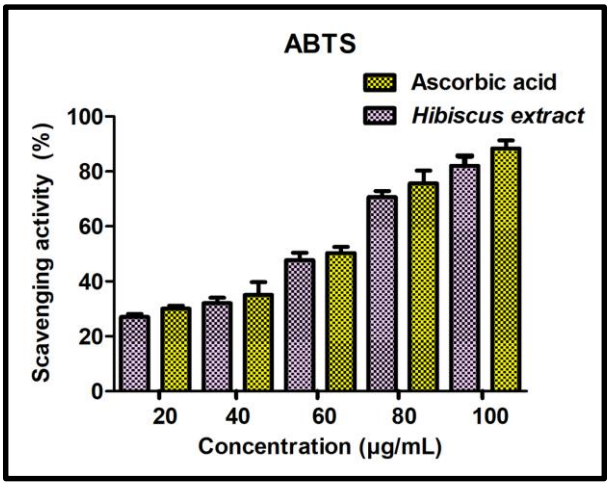
In the antimicrobial assessment of hibiscus flower extract via the agar well diffusion method, nutrient agar is meticulously poured into sterile Petri plates, forming a standardized growth medium. Candida and Actinomyces strains are intentionally selected, ensuring a comprehensive evaluation against both yeast and bacterial microorganisms. After inoculating the agar plates with the microbial strains, hibiscus extract, dissolved in ethanol, is precisely added to wells at varying concentrations (50, 100, and 150 L), facilitating controlled diffusion into the agar. Following a 24-hour incubation at 37°C, zones of inhibition are observed, indicating suppressed microbial growth. The measured diameter of these zones serves as a crucial metric, offering insights into the potency of the hibiscus extract against the tested microorganisms, providing valuable information on its antimicrobial properties.

RESULTS



Graph 1: DPPH Radical Scavenging Activity of Ethanolic Extract of Hibiscus Rosa Sinensis

DPPH is a free radical. DPPH assay is used for the radical scavenging activity of hibiscus. 50 100 150 200 250 concentration of hibiscus was found to be 150/ml.The DPPH radical scavenging activity of Hibiscus rosa sinensis, shows 85% of free radical when compared to standard ascorbic acid.



Graph 2: ABTS Radical Scavenging Activity

ABTS radical scavenging activity is the most common method used to evaluate the antioxidant activity in medicinal plants. 20 40 60 80 100 concentration of hibiscus was evaluated. The inhibition concentration

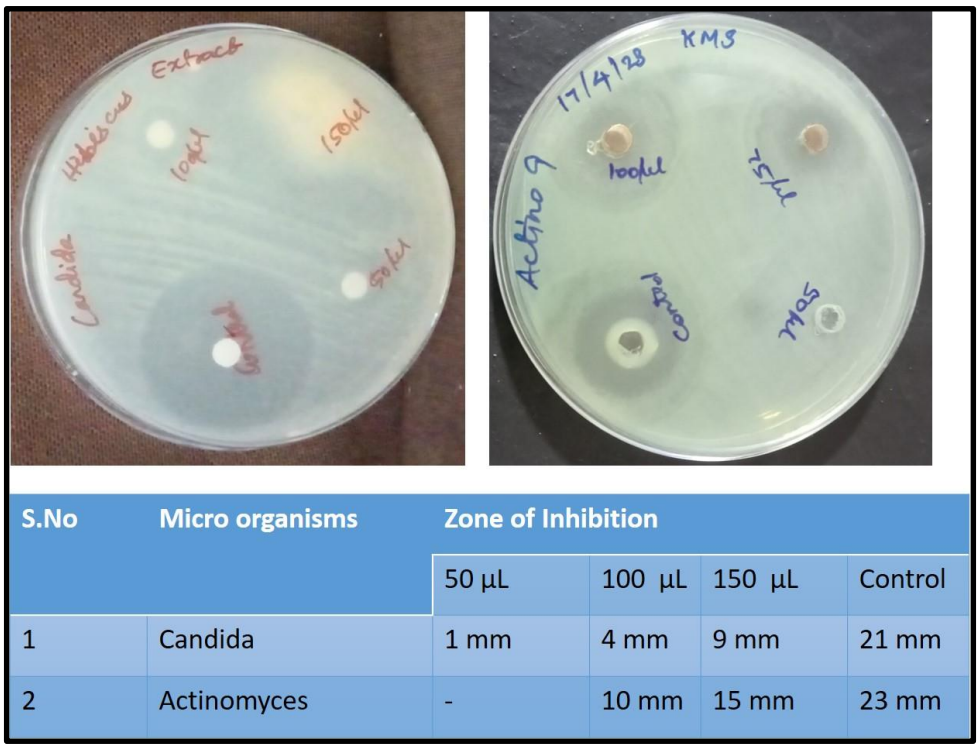
(IC 50) was calculated as 60µg/ml.

Phytochemical Analysis

Table 1: Phytochemical Analysis Of Hibiscus

S.No.	Phytochemical Analysis	Citrus peel extract
1	Tannins	Present
2	Phenolic compounds	Present
3	Saponins	Present
4	Oil and Fat	Absent
5	Protein & Amino acid	Present
6	Flavonoids	Absent
7	Carbohydrates	Present
8	Alkaloids	Present

Phytochemical analysis used to evaluate the phyto compounds present in the medicinal plants. Hibiscus contains tannins, phenolic compounds, saponins, protein and amino acids, and alkaloids carbohydrates.



S.No	Micro organisms	Zone of Inhibition			
		50 µL	100 µL	150 µL	Control
1	Candida	1 mm	4 mm	9 mm	21 mm
2	Actinomyces	-	10 mm	15 mm	23 mm

Figure 2: Activity Of Hibiscus Extract

Antimicrobial resistance (AMR) is one of the major issues all around the world. The microbial species candida and actinomyces were taken for the evaluation minimum inhibition concentration (MIC) is the lowest concentration of plant extract will inhibit the growth of the microorganisms.

The zone of inhibition for candida 1mm, 4mm, 9mm, 21mm, (50,100,150µL control, respectively). The zone of inhibition of actinomyces 10mm,15mm,23mm (100,150µL, control respectively).

DISCUSSION

The results of the study indicate promising antioxidant and antimicrobial properties in Hibiscus rosa sinensis. The DPPH assay revealed that Hibiscus extract, at concentrations ranging from 50 to 250 µg/ml, exhibited optimal radical scavenging activity at 150 µg/ml, with an impressive 85% efficacy compared to the standard ascorbic acid. Furthermore, the ABTS assay demonstrated a strong antioxidant potential, with an IC50 of 60 µg/ml, reinforcing Hibiscus as a potent source of antioxidants. Phytochemical analysis identified the presence of various beneficial compounds, including tannins, phenolic compounds, saponins, proteins and amino acids, alkaloids, and carbohydrates in Hibiscus. The antimicrobial assessment against Candida and Actinomyces showcased notable inhibitory effects, as evidenced by the zone of inhibition ranging from 1 mm to 21 mm for Candida and 10 mm to 23 mm for Actinomyces at different concentrations. These findings underscore the multifaceted therapeutic potential of Hibiscus, highlighting its significance in combating oxidative stress and microbial challenges, thus contributing to the ongoing efforts to address global issues such as antimicrobial resistance. The research highlights the impact of extraction methods on the antibacterial properties of flower material, with ethanol extraction showing significant effectiveness against Salmonella sp. (20.40 ± 1.54) mm, indicating its potential as an alternative source for developing targeted antibacterial agents through further exploration of bioactive compounds(9). The ethanol extract from dried leaves of *Hibiscus tiliaceus* L. demonstrated notable antioxidant activity with an IC50 of 86.5µg/ml, compared to ascorbic acid (15.00µg/ml). Additionally, the extract exhibited antimicrobial effects against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella paratyphi*, endorsing its potential use in traditional medicine and warranting further exploration(10).This comprehensive study on 25 Mexican Hibiscus sabdariffa varieties highlights their diverse phytochemical composition, antioxidant potential, and antibacterial activity. Variability in phenolic, flavonoid, and anthocyanin contents, along with distinct antioxidant capacities, underscores the significance of calyx color intensities. The antibacterial efficacy against both Gram-negative and Gram-positive strains, particularly the potency against Gram-positive bacteria, adds therapeutic value. Multivariate analysis based on anthocyanin content aids in variety classification, emphasizing the importance of precise material descriptions for accurate research outcomes(11).Study was to evaluate the antibacterial activity of selenium nanoparticles synthesized using Capparis decidua. Antibacterial activity was studied by inhibition zone against *E.coli* and *Lactobacillus* using Agar well diffusion method which was characterized by a clear zone(12). The collective findings from diverse studies on different Hibiscus species reveal a rich tapestry of therapeutic potential. Hibiscus rosa sinensis stands out for its robust antioxidant properties demonstrated through DPPH and ABTS assays, coupled with a varied phytochemical profile encompassing tannins, phenolic compounds, saponins, proteins, amino acids, alkaloids, and carbohydrates. Its significant antimicrobial activity against Candida and Actinomyces suggests broader applications in addressing microbial challenges, including antimicrobial resistance. Similarly, *Hibiscus tiliaceus L*. showcases noteworthy antioxidant activity and promising antimicrobial effects against pathogenic strains like *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella paratyphi*, supporting its traditional medicinal potential. The extensive phytochemical diversity observed among 25 Mexican *Hibiscus sabdariffa* varieties, with varying antioxidant capacities and antibacterial effects against Gram-negative and Gram-positive strains, further underscores the importance of calyx color intensities.

This comprehensive understanding emphasizes the need for tailored approaches in harnessing the therapeutic benefits of different Hibiscus species, ranging from antioxidant-rich applications to combating microbial challenges in traditional medicine.

FUTURE SCOPE

The compelling results obtained from this study on *Hibiscus rosa sinensis* lay a robust foundation for future research and applications. Firstly, the identified antioxidant properties, particularly the high radical scavenging activity observed in the DPPH assay, and the low IC50 in the ABTS assay, suggest the potential utilization of Hibiscus as a natural antioxidant supplement. Further investigations could delve into isolating and characterizing specific compounds responsible for these activities, enhancing our understanding of its therapeutic mechanisms.

The rich phytochemical profile, encompassing tannins, phenolic compounds, saponins, proteins, amino acids, alkaloids, and carbohydrates, opens avenues for exploring Hibiscus in various industries, including pharmaceuticals, nutraceuticals, and cosmeceuticals. Isolating and studying individual phytochemicals could lead to the development of novel therapeutic agents or functional ingredients.

Additionally, the significant antimicrobial activity against *Candida* and *Actinomyces* highlights Hibiscus as a potential candidate for addressing microbial challenges. Further research could focus on elucidating the specific mechanisms of action and conducting in vivo studies to validate its efficacy in real-world scenarios.

Considering the global concern of antimicrobial resistance, Hibiscus may offer a sustainable and natural alternative to conventional antimicrobial agents. Exploring the potential synergistic effects of its various compounds and their interactions with existing antibiotics could contribute to the development of combination therapies.

In conclusion, the multifaceted therapeutic potential of Hibiscus, as evidenced by its antioxidant and antimicrobial properties, warrants in-depth exploration. Future research avenues should focus on isolating active compounds, understanding their mechanisms of action, and conducting clinical trials to validate the translational potential of Hibiscus-based interventions, ultimately contributing to the broader strategies for combating oxidative stress and microbial challenges, including the pressing issue of antimicrobial resistance.

CONCLUSION

The present study concludes ethanolic extract of hibiscus which has DPPH, ABTS radical scavenging activity. IC_{50} concentration of DPPH 150µg/ml and ABTS 60µg/ml. We have concluded many phytochemicals present in our hibiscus show good antimicrobial resistance against candida and actinomyces. So, the hibiscus which can be applied to develop new antimicrobial and healthy ingredients.

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Conflict of Interest

None to declare.

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