JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Assessment of Bioactive Efficacy of *Phyllanthus emblica* Extracts Against Bacterial Strains

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Abstract*:* The antibacterial activity of *Phyllanthus emblica* (Amla) extracts prepared in different solvents; Dimethyl sulfoxide (DMSO), ethanol, and concentrated ethanol against two bacterial cultures: *Escherichia coli* and *Staphylococcus aureus*, is investigated in this study. Agar disk diffusion method was used to measure antimicrobial activity. The findings showed that the selection of the solvent and the recency of the extract had a direct impact on the antimicrobial activity of amla. For *E. coli*, the DMSO prepared extract contained the largest inhibition zone, reflecting higher bioavailability and solubility of active phytochemicals using this solvent. The concentrated ethanol extract contained the highest activity towards *S. aureus*, reflecting a more efficient extraction of effective bioactive compounds against Gram-positive bacteria. Interestingly, three-day stored amla extracts did not show antibacterial action against either of the microorganisms, highlighting the significance of extract freshness in maintaining bio-efficacy.

The results highlight the importance of selecting the solvent and the stability of the extract in determining the most effective antimicrobial potential of the plant-based treatments. Such findings add to our improved knowledge of natural antimicrobial compounds and reveal the pharmaceutical value of amla in the form of natural herbal formulations. Future studies should aim to determine the precise bioactive constituents behind the antibacterial effect, evaluate their stability, and determine if there are synergistic interactions with traditional antibiotics. Such research will provide the foundation for new, environmentally friendly options to replace chemical antimicrobials.

***Index Terms* -** *Phyllanthus emblica*, inhibition zone, *Escherichia coli*, *Staphylococcus aureus*, DMSO

I. INTRODUCTION

With the increasing global menace of antibiotic resistance, especially from antibiotics of soil origin, the necessity to find new, sustainable, and potent medicinal sources is paramount. *Phyllanthus emblica*, or Amla or Indian gooseberry, has been identified as a potential candidate with its numerous health advantages, accessibility, and affordability. This study delves into the potential of amla not just as a natural antimicrobial but also within contemporary nutrition science and its applications towards sustainable agriculture and economic growth (Tsang et al., 2019). As an abundance of bioactive compounds, amla fits within expanding scientific interest in botanically based approaches towards solving world health and environmental issues.

Amla is nutritionally rich and very valuable both as a dietary supplement and medicinally. Some of its major constituents are: Polyphenols: These are chemical compounds, such as gallic acid and ellagic acid, that are strong antioxidants, i.e., they can reduce oxidative stress and can safeguard cells against damage, thus reducing the incidence of chronic illnesses like cancer and neurodegenerative diseases (Karimi Sani et al., 2023). Minerals: Amla has vital minerals like calcium, iron, and phosphorus that are important for bone strength, hemoglobin formation, and cellular processes (Oyeyinka & Afolayan, 2019).

The medicinal properties of amla reside in its broad diversity of bioactive constituents with multiple therapeutic functionalities:

- Antioxidant Effects: The rich composition of ascorbic acid (Vitamin C) and polyphenols endows amla with strong antioxidant properties. These molecules have the ability to scavenge free radicals, retard cell aging, and guard against disorders pertaining to oxidative stress, including cardiovascular disease and diabetes (Arumai Selvan et al., 2018).
- Immunity Boost: Due to its unparalleled vitamin C content much greater than in citrus fruits, amla increases immune function by stimulating the production and activity of white blood cells as well as serving as a natural immunity enhancer (Hu et al., 2018).
- Anti-inflammatory Properties: Amla has exhibited significant anti-inflammatory activity. It is employed in Ayurvedic medicine for the treatment of arthritis and inflammatory bowel disease, to reduce swelling, pain, and stiffness in joints (El-Saber Batiha et al., 2020).
- Cardiovascular Health: Continued use of amla has been associated with reduced total cholesterol, Lower density cholesterol, and triglyceride levels and enhanced high density cholesterol levels, rendering it a natural remedy for heart disease and atherosclerosis (Banerjee et al., 2021).

In conclusion, amla is a powerful and versatile natural product with uses ranging across nutrition, healthcare, and sustainable agriculture. By blending indigenous wisdom with contemporary scientific evidence, *Phyllanthus emblica* has great potential to meet the world's healthcare needs while generating income for rural communities from its production and value addition. Additional research into its bioactive constituents and mode of action will further solidify its position as a valuable alternative to chemical drugs.

II. MATERIALS AND METHODS

1. Sample preparation and Extraction: 0.75 g amla **(Fig. 1)** weighed and crushed with ethanol solution (8:2 distilled water: ethanol) in mortar pestle to fine paste **(Fig. 2)**. This amla paste is further used for the preparation of ethanolic and DMSO (Dimethyl sulfoxide) extract.
2. This ethanolic amla extract is divided into 4 tubes as follows: Tube 1: Amla extract with Ethanol, Tube 2: Amla extract with DMSO, Tube 3: Amla extract with Ethanol (kept in 50 degrees water bath for 5 minutes) for concentrated sample, Tube 4: Amla extract with ethanol (incubated at RT for 3 days aka old sample) **(Fig. 3)**.
3. Microbial culture preparation: Sterile Nutrient Agar (HiMedia Laboratories Pvt. Ltd.) was prepared and poured into two sterile test tubes (10ml each) to create agar slants for bacterial culture. Once solidified, the slants were streaked with laboratory isolates of *Escherichia coli* and *Staphylococcus aureus* using sterile nichrome loops. The inoculated slants were then incubated at 37°C for 24 hours to allow bacterial growth. After incubation, bacterial growth from each slant was transferred into 100 mL of sterile saline solution to prepare a bacterial suspension for testing on the prepared extract.
4. Antimicrobial susceptibility testing: St. Mueller Hinton Agar (HiMedia Laboratories Pvt. Ltd.) plates were streaked with *Escherichia coli* and *Staphylococcus aureus* independently. Then 4 sterile discs were dipped in 4 different samples with the help of tweezer and placed in both the plates in respective quadrant aseptically. After which, the plates were kept for pre-diffusion of extract in the refrigerator for 15 minutes (Pongtharangkul & Demirci, 2004), and then plates were incubated for 24 hours in an incubator at 37°C. This incubation period allows for optimal bacterial growth and interaction with the antimicrobial agents present in the amla extracts. (Nyuykonge et al., 2021).
5. Measurement of Inhibition Zones: After 24 hours incubation at 37°C, the diameters of the clear zones surrounding each disc were measured in millimeters (Dewees et al., 1970). These zones indicate the extent of bacterial growth inhibition and were compared across the different amla extracts. (European Committee on Antimicrobial Susceptibility Testing [EUCAST], n.d.)
6. Zones of different amla extract were compared with Norfloxacin (10mcg) from Standard Kirby Bauer chart. (12 mm> is Resistant, 13-16 mm is Intermediate sensitive, 17 mm < is sensitive.) according to Kirby Bauyer Chart.

III. RESULT AND DISCUSSION

The current research sought to assess the antibacterial activity of different solvent-based extracts of *Phyllanthus emblica* (Amla) against *Escherichia coli* and *Staphylococcus aureus* **(Fig. 4)**. The findings reveal that the type of solvent and the freshness of the extract are vital factors affecting the antimicrobial action of amla. In the case of *E. coli*, the maximum zone of inhibition was reported in the amla extract made with DMSO **(Table 2)**. This indicates that DMSO might increase the diffusion and solubility of bioactive compounds like gallic acid, ellagic acid, and flavonoids that are accountable for antibacterial activity. The reduced inhibition zones obtained with ethanol and high ethanol concentration extracts indicate that these solvents might be less effective in extracting or preserving the antibacterial activity of amla constituents compared to DMSO. Significantly, the lack of any inhibitory effect for the 3-day-old ethanol sample is indicative of extensive degradation or loss of active compounds with time, and that freshness of extract is quite essential in guaranteeing optimal antibacterial effectiveness.

Conversely, against *S. aureus*, the concentrated ethanol extract was most active. This reveals that a more concentrated ethanol might be better suited to solubilize phenolic and tannin compounds particularly useful in combating Gram-positive bacteria such as *S. aureus*. The amla extract using normal ethanol had moderate activity, and DMSO was the least active **(Table 1)**. The selective activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) could be due to variations in cell wall structures, permeability, and solvent-dissolved phytochemical interactions.

In both bacterial cultures, the 3-day-old ethanol extract repeatedly did not yield a zone of inhibition, indicating a loss of antimicrobial components potentially by oxidation, microbial contamination, or breakdown of compounds. The observation highlights the need for fresh extracts in order to achieve effective antimicrobial activity. Collectively, these results demonstrate the selective antimicrobial efficacy of amla when combined with appropriate solvents, the more efficacious being DMSO for Gram-negative bacteria and concentrated ethanol for Gram-positive bacteria. This information is useful for the design of targeted herbal antibacterial products and underscores the significance of extraction technique, solvent polarity, and storage time in natural product-derived antimicrobial studies.

IV. CONCLUSION

The current research assessed the antibacterial activity of *Phyllanthus emblica* extracts extracted using varying solvents against *Escherichia coli* and *Staphylococcus aureus*. The findings proved that the solvent used and the extract's age played a considerable role in affecting the antimicrobial activity. DMSO amla extract had the most inhibitory activity against *E. coli*, confirming the better capability of DMSO to extract or stabilize bioactive molecules with activity against Gram-negative bacteria.

On the other hand, ethanol concentrated extract was most inhibited against *S. aureus*, indicating that higher concentrations of ethanol might be more appropriate for the extraction of compounds active against Gram-positive bacteria.
Notably, ethanol extracts aged for three days did not exhibit any inhibition against either bacterial strain, highlighting the importance of stability of the extract and degradation of active constituents with time. These findings indicate that the antimicrobial activity of amla is very sensitive to solvent polarity, concentration, and extract freshness.

V. FUTURE PROSPECTS

Future work could involve isolation and identification of the particular phytochemicals responsible for the noticed antibacterial activity. Stability studies of these compounds may facilitate enhanced shelf-life and formulation procedures. Further testing against a larger panel of microbial strains and investigation of synergistic interactions with traditional antibiotics might establish

Phyllanthus emblica extracts for clinical or pharmaceutical use. Standardized, stable, and solvent-specific amla-based antimicrobial agents may develop as a worthwhile natural recourse to synthetic antibiotics in fighting microbial resistance.

Figures and Tables

Fig. 1. *Phyllanthus emblica* (Amla) sample

Fig. 2. *Phyllanthus emblica* (Amla) ethanolic extract

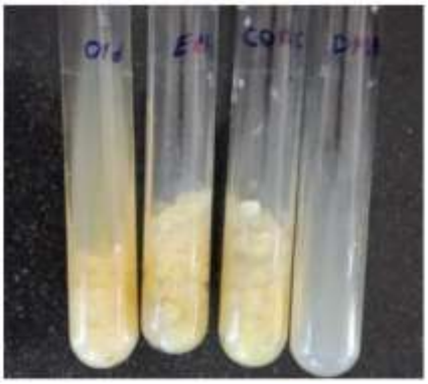

Fig. 3. *Phyllanthus emblica* (Amla) 3 day old, ethanolic, concentrated and DMSO extracts (Left to right)

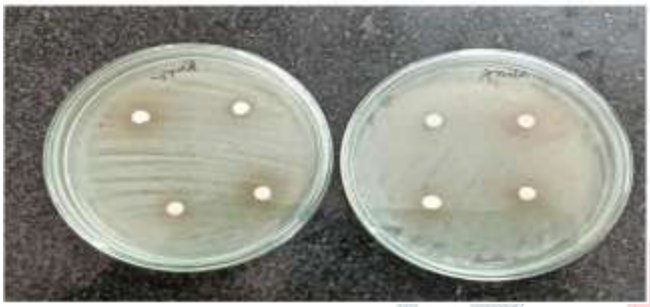

Fig. 4. *Phyllanthus emblica* (Amla) extract discs on *E.coli* and *S. aureus* Mueller Hinton Agar plates

Table 1 Zones of inhibition of different amla extracts against *Staphylococcus aureus*

Sr. No.	Type of amla extract	Zone of inhibition in mm
1	Ethanolic	18
2	Concentrated	20
3	DMSO treated	14
4	03 day old	No zone

Table 2 Zones of inhibition of different amla extracts against *Escherichia coli*

Sr. No.	Type of amla extract	Zone of inhibition in mm
1	Ethanolic	15
2	Concentrated	15
3	DMSO treated	17
4	03 day old	No zone

VI. ACKNOWLEDGMENT

I would like to express my sincere gratitude to my guide, **Ms. Dnyanada Sadanand Ghadi**, for her constant guidance, support, and valuable insights throughout the research work. I extend my heartfelt thanks to our respected **Director, Mrs. Jyoti Gaitonde**, and **I/C Principal, Dr. Mahendra Kanojia**, for providing the necessary facilities and a supportive environment to carry out this project. I am also grateful to **Mrs. Shweta Vijay Khopde**, Department Coordinator, for her encouragement and coordination, and to **Dr. Priyanka Pushkaraj Vartak,** for her valuable suggestions and academic support. Special thanks to **Mr. Vighnesh Borsutkar**, Lab Assistant, and **Mr. Satyadeep Kadam**, Lab Attendant, for their technical assistance and cooperation during the experimental work. This research would not have been possible without their collective support and encouragement.

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