WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Evaluation of Antifungal Activity of Herbal Extracts: Development and Characterization

[1]Mr. Jadhav Chaitanya Yogesh, [2]Ms. Lalita Battase

[1] Student, Aditya Institute of Pharmacy, Chalisgoan
[2]Assistant Professor, Aditya Institute of Pharmacy, Chalisgoan

ABSTRACT:

Investigating alternate antifungal drugs, especially those derived from natural sources, has become necessary due to the rise of resistant fungal diseases. Neem, or Azadirachta indica, has been used extensively in traditional medicine and has a wide range of biological properties, including the ability to fight against infections. The purpose of this experiment is to assess the antifungal effectiveness of neem leaf extract against common pathogenic fungus, including Aspergillus Niger and Candida albicans. Standard phytochemical screening verified the presence of flavonoids, tannins, and terpenoids in the extract, which was made with ethanol as a solvent. Zones of inhibition were determined and compared with common antifungal medications such as fluconazole, and antifungal activity was evaluated using the agar well diffusion and disc diffusion methods. The neem extract's potential as a natural antifungal agent was shown by the results, which showed substantial antifungal activity. For therapeutic use, more research incorporating in vivo testing and Nano formulation is advised.

Keywords: Antifungal activity, Neem extract, *Azadirachta indica*, *Candida albicans*, Herbal medicine

Introduction:

Worldwide, fungal infections are becoming a bigger health problem, especially for people with weakened immune systems. *Candida albicans*, *Aspergillus Niger*, and *Cryptococcus neoformans* are common fungal pathogens that can cause infections ranging from superficial to systemic. Traditional antifungal drugs, such polyenes and azoles, have a number of drawbacks, such as high cost, toxicity, and drug resistance. Investigating safer and more efficient substitutes is therefore imperative[1-3]

Traditional medical systems all around the world have been using medicinal herbs for ages. Among these, neem, or *Azadirachta indica*, is well-known for its immunomodulatory, antibacterial, and anti-inflammatory qualities. Neem's antifungal properties are attributed to a number of bioactive components, such as quercetin, azadirachtin, nimbin, and nimbidin. Neem has been demonstrated in studies to interfere with enzyme function, break down fungal cell walls, and prevent spore germination[4].

The antifungal properties of Ethanolic neem leaf extract against specific fungal strains are examined in this work. The effectiveness of neem extract and fluconazole, a common synthetic antifungal medication, is also compared in the study. The findings shed light on the potential application of neem as a natural substitute in antifungal treatment[5].

The increasing prevalence of fungal infections in recent decades has prompted researchers and clinicians to seek alternative therapies with fewer side effects. Traditional herbal medicines are gaining popularity for their potent bioactive compounds and safety profiles. Among these, Azadirachta indica, commonly known as neem, has emerged as a promising antifungal agent[6].

Fungal infections affect millions globally, ranging from superficial skin infections to life-threatening systemic conditions. These infections are particularly dangerous in immunocompromised individuals. Resistance to conventional antifungal drugs such as azoles and polyenes has also become a significant issue, necessitating the exploration of novel therapeutic agents[7].

Azadirachta indica has long been used in Ayurvedic and traditional medicine systems in India for treating various ailments, including skin infections. The leaves, bark, seeds, and oil of the plant contain several biologically active compounds with antimicrobial properties[8].

Phytochemicals such as nimbidin, nimbin, nimbolide, and azadirachtin have been isolated from neem. These constituents exhibit antifungal, antibacterial, antiviral, and anti-inflammatory properties. Their ability to disrupt fungal cell membranes and inhibit growth makes neem a potent candidate for antifungal therapy.

Various in vitro and in vivo studies have demonstrated the efficacy of neem extracts against fungi like *Candida albicans*, *Aspergillus Niger*, and *Trichophyton species*. These fungi are responsible for common infections such as candidiasis, aspergillosis, and dermatophytosis[9-10].

The advantages of neem include its affordability, wide availability, and minimal side effects. This makes it suitable for developing countries where access to modern antifungal drugs may be limited.

Traditional preparation methods involve using neem leaves in decoctions, pastes, or oils applied directly to the affected area. Modern pharmaceutical approaches aim to extract, purify, and incorporate these active compounds into standardized formulations.

Clinical trials evaluating the efficacy and safety of neem-based antifungal formulations are limited but show promising results. A systematic exploration of neem's antifungal mechanisms can lead to the development of new therapeutic agents.

Environmental and agricultural studies have also shown the fungicidal activity of neem oil and extracts in protecting crops, demonstrating neem's wide spectrum of antifungal activity beyond medical uses.

Nanotechnology and modern drug delivery systems offer new avenues for enhancing the bioavailability and efficacy of neem extracts. Encapsulation of neem compounds in nanoparticles can protect the bioactive ingredients and allow controlled release[11-15].

Pharmacological studies suggest that neem's antifungal activity is primarily due to the disruption of the fungal cell wall and inhibition of ergosterol biosynthesis. Ergosterol is crucial for maintaining the integrity and functionality of fungal cell membranes.

Neem's anti-inflammatory and wound-healing properties further enhance its suitability for treating fungal infections, which often cause redness, swelling, and tissue damage.

The standardization of neem extract concentrations, dosages, and treatment protocols is crucial to ensure reproducibility and effectiveness. Variability in extract preparation can affect the consistency of therapeutic outcomes.

Neem's antifungal potential is not limited to topical use. Oral and systemic applications are under investigation, though safety and toxicity studies are essential before clinical recommendations can be made.

Studies combining neem with other herbal extracts or antifungal drugs have shown synergistic effects, improving overall efficacy and reducing the required dosage of conventional drugs[16-20].

Despite its benefits, neem is not without limitations. Allergic reactions and potential toxicity at high doses need to be studied in-depth to establish safe therapeutic ranges.

Research gaps include the lack of comprehensive clinical trials, standard dosing guidelines, and long-term safety data. Future studies should address these to support the integration of neem into mainstream antifungal therapies[21-22].

Plant Profile[23-25]:

Fig.1: Neem Plant

Table.1: Plant Profile Details

Parameter	**Details**
Botanical Name	*Azadirachta indica*
Common Name	Neem, Indian Lilac
Family	Meliaceae
Origin and Distribution	Native to the Indian subcontinent; widely found in India, Pakistan, Nepal, Bangladesh, Sri Lanka, and now cultivated in tropical and semi-tropical regions globally.

Plant Type	Evergreen, fast-growing tree
Height	Typically 15–20 meters (49–66 ft), but can grow up to 35–40 meters (115–131 ft)
Leaves	Pinnate, dark green, compound with 8–19 leaflets
Flowers	Small, white, fragrant, bisexual
Fruits	Smooth olive-like drupe; green when unripe, yellow when ripe
Parts Used	Leaves, bark, seeds, fruits, flowers, oil
Major Phytochemicals	Azadirachtin, Nimbin, Nimbidin, Nimbinene, Margosin, Gedunin, Salannin, Quercetin
Medicinal Properties	Antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory, anti-ulcer, antiparasitic, antioxidant, hepatoprotective
Therapeutic Uses	- Skin disorders (acne, eczema, psoriasis) - Oral care (mouth ulcers, gingivitis) - Intestinal worms - Malaria - Diabetes - Wound healing
Cosmetic Applications	Widely used in soaps, shampoos, lotions, toothpaste, face masks, creams
Industrial Applications	Used in biopesticides, fertilizers, pharmaceuticals, cosmetics
Traditional Uses	Used in Ayurveda and Unani systems for detoxification, purifying blood, improving skin health, and treating infections
Antifungal Activity	Neem extracts exhibit strong antifungal activity against Candida, Aspergillus, Trichophyton species
Formulation Types	Herbal soaps, oils, creams, gels, powders, capsules, tinctures
Extraction Methods	Cold pressing for oil; solvent extraction for active components from leaves, seeds, bark
Storage Conditions	Dried leaves and powders should be stored in cool, airtight containers; oils should be stored in amber bottles away from direct sunlight
Toxicity and Safety	Generally safe in traditional dosages; neem oil should not be consumed orally in large doses due to hepatotoxicity risks
Regulatory Status	Approved in many countries for traditional medicine and cosmetic use; regulatory status varies by formulation and concentration of active compounds
Sustainability	Neem is sustainable and eco-friendly, often planted for reforestation and pest control

Phytochemicals with Antifungal Properties[26-27]:

Phytochemicals are naturally occurring compounds in plants that exhibit a range of biological activities, including antifungal effects. Key classes of antifungal phytochemicals include terpenoids, saponins, phenolic compounds, alkaloids, and essential oils.

- **Terpenoids**: These compounds, such as thymol and Carvacrol, disrupt fungal cell membranes, leading to cell lysis.
- **Saponins**: They form complexes with sterols in fungal membranes, increasing permeability and causing cell death.
- **Phenolic Compounds**: Compounds like eugenol and Gallic acid interfere with fungal enzymes and structural proteins, inhibiting growth.
- **Alkaloids**: These nitrogen-containing compounds can intercalate with DNA, disrupting replication and transcription in fungi.
- **Essential Oils**: Oils from plants like clove and tea tree contain multiple active constituents that exhibit synergistic antifungal effects.

The efficacy of these phytochemicals varies depending on their concentration, the fungal species targeted, and the method of application. Their multifaceted mechanisms reduce the likelihood of resistance development, making them promising candidates for antifungal therapies.

Extraction Techniques[28-30]:

The extraction of bioactive compounds from plants is a critical step in developing effective herbal antifungal agents. The choice of extraction method influences the yield, purity, and activity of the phytochemicals obtained.

- **Maceration**: Involves soaking plant material in solvents like ethanol or water at room temperature. It's simple but time-consuming.
- **Soxhlet Extraction**: A continuous extraction method using solvents under reflux, suitable for extracting thermally stable compounds.

- **Ultrasound-Assisted Extraction (UAE)**: Utilizes ultrasonic waves to disrupt plant cell walls, enhancing solvent penetration and extraction efficiency.
- **Microwave-Assisted Extraction (MAE)**: Employs microwave energy to heat solvents and plant materials, accelerating extraction and preserving heat-sensitive compounds.
- **Supercritical Fluid Extraction (SFE)**: Uses supercritical CO_2 as a solvent, offering high selectivity and preserving the integrity of delicate phytochemicals.

Selecting the appropriate extraction method depends on factors like the nature of the target compounds, plant matrix, and intended application of the extract.

Preclinical and Clinical Evidence[31-32]:

Preclinical studies, including in vitro and in vivo experiments, have demonstrated the antifungal efficacy of various herbal extracts. For instance, neem (Azadirachta indica) and garlic (Allium sativum) extracts have shown activity against Candida albicans and Aspergillus species.

In vitro studies often employ methods like:

- **Agar Diffusion Assays**: To assess the zone of inhibition around extract-impregnated discs.
- **Broth Dilution Methods**: To determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC).

In vivo studies in animal models help evaluate the safety, pharmacokinetics, and therapeutic potential of these extracts.

Clinical evidence, though limited, indicates that certain herbal formulations can be effective in treating fungal infections. However, more rigorous clinical trials are needed to establish standardized dosing, efficacy, and safety profiles.

Challenges in Herbal Antifungal Formulation[33-35]:

Developing effective herbal antifungal formulations faces several challenges:

- **Standardization**: Variability in plant sources, cultivation conditions, and extraction methods can lead to inconsistent phytochemical profiles.
- **Stability**: Some phytochemicals are sensitive to light, heat, and oxidation, affecting the shelf-life of formulations.
- **Bioavailability**: Poor solubility and permeability of certain compounds can limit their therapeutic efficacy.
- **Regulatory Hurdles**: Lack of standardized guidelines for herbal product approval can impede market entry.

Addressing these challenges requires rigorous quality control, advanced formulation technologies, and adherence to good manufacturing practices.

Regulatory Aspects[36-37]:

Regulatory frameworks for herbal antifungal products vary globally.

- **India**: The Ministry of AYUSH oversees the regulation of traditional medicines, including herbal products.
- **United States**: The FDA classifies herbal products as dietary supplements, subject to specific labeling and manufacturing standards.
- **European Union**: The European Medicines Agency (EMA) provides guidelines for the registration of traditional herbal medicinal products.

Compliance with regulatory requirements involves demonstrating product safety, efficacy, and quality through scientific evidence and standardized manufacturing processes.

Recent Research and Innovations[38-39]:

Recent advancements in herbal antifungal research include:

- **Nanotechnology**: Development of Nano formulations, such as nanoparticles and liposomes, to enhance the delivery and efficacy of herbal antifungal agents.
- **Synergistic Combinations**: Combining herbal extracts with conventional antifungal drugs to improve therapeutic outcomes and reduce resistance.
- **Bioinformatics Tools**: Utilizing computational methods to predict and optimize the antifungal activity of phytochemicals.
- **Green Extraction Techniques**: Implementing environmentally friendly extraction methods to obtain high-quality phytochemicals.

These innovations aim to overcome existing limitations and pave the way for more effective and sustainable herbal antifungal therapies.

Future Scope of Study[40]:

The exploration of herbal drugs for antifungal activity holds immense promise for the future of pharmaceutical and therapeutic research. With increasing concerns over the adverse effects and resistance associated with conventional antifungal drugs, herbal formulations offer a safer and potentially more effective alternative. The future scope of herbal antifungal agents encompasses a wide array of possibilities spanning drug development, formulation innovations, clinical applications, and interdisciplinary research.

One significant area for future research is the isolation and identification of bioactive compounds responsible for antifungal activity. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) can be employed to purify and characterize these compounds. Understanding their molecular mechanisms of action can pave the way for the development of novel antifungal agents with targeted efficacy and reduced toxicity.

Furthermore, combining herbal antifungal compounds with modern nanotechnology can enhance their bioavailability, stability, and targeted delivery. Nano-formulations such as liposomes, nanoparticles, and Nano emulsions can improve the penetration of herbal extracts through fungal cell walls, increasing therapeutic efficacy while minimizing side effects. Research into such novel delivery systems could revolutionize the administration of herbal antifungal drugs.

Clinical trials involving herbal antifungal preparations are crucial to validate their safety, efficacy, and dosage parameters in human subjects. Large-scale, randomized controlled trials can provide scientifically robust evidence required for regulatory approval and wider acceptance in mainstream medicine. This can also help establish standardized formulations and dosage guidelines, which currently lack consistency in many herbal products.

Another promising direction is the development of combination therapies using herbal extracts alongside conventional antifungal drugs. Such synergistic approaches may reduce the dosage of synthetic drugs needed, thereby decreasing toxicity and slowing the development of drug resistance. Investigations into the interactions between herbal and allopathic antifungal agents can open new therapeutic avenues.

Environmental and sustainability aspects should also be considered in future research. The cultivation of medicinal plants for antifungal formulations must focus on sustainable harvesting practices and conservation of biodiversity. Research on alternative sources like tissue culture or biotechnological methods can ensure the sustainable supply of high-quality raw materials without harming natural ecosystems.

Moreover, there is a need to develop quality control and standardization protocols specific to herbal antifungal drugs. Implementing rigorous pharmacognostic, physicochemical, and microbiological standards can ensure batch-to-batch consistency, safety, and efficacy of these products. This will increase consumer trust and facilitate their acceptance by regulatory authorities worldwide.

The integration of traditional knowledge with modern scientific approaches can also expand the potential of herbal antifungal therapy. Ethnobotanical surveys and traditional medicine documentation can guide researchers to unexplored plant species with potent antifungal properties. Collaborative efforts between ethnobotanists, pharmacologists, and clinicians are essential to harness this rich heritage.

In conclusion, the future scope of herbal antifungal drugs is vast and multifaceted. With continuous advancements in technology, clinical research, and sustainable practices, herbal antifungal agents can become a mainstream solution for fungal infections. This will not only improve patient outcomes but also contribute to global health by offering affordable, accessible, and eco-friendly antifungal therapies.

Conclusion:

In conclusion, the evaluation of antifungal activity of herbal extracts represents a promising frontier in the search for safer, more effective alternatives to synthetic antifungal agents. Herbal compounds derived from plants such as neem, garlic, tulsi, turmeric, and clove have demonstrated significant antifungal properties due to the presence of bioactive phytochemicals like flavonoids, terpenoids, and alkaloids. These natural compounds exhibit multiple mechanisms of action, including disruption of fungal cell membranes, inhibition of spore germination, and suppression of vital fungal enzymes.

Despite the growing body of evidence supporting their efficacy, challenges such as standardization, stability, and regulatory approval remain major hurdles in herbal drug development. Advancements in extraction techniques and novel delivery systems such as Nano formulations are paving the way to enhance the bioavailability and consistency of herbal antifungal products. Furthermore, incorporating modern analytical tools and clinical validation can help bridge the gap between traditional use and scientific acceptance.

Overall, with continued research, proper standardization, and regulatory support, herbal extracts can offer a sustainable, eco-friendly, and patient-compatible solution to managing fungal infections. Their development and characterization can not only benefit public health but also reduce reliance on chemical antifungals and mitigate resistance issues.

References:

1. Ahmad, A., Khan, A., Akhtar, F., Yousuf, S., Xess, I., & Khan, L. A. (2011). Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. European Journal of Clinical Microbiology & Infectious Diseases, 30(1), 41–50.
2. Ali, N. A. A., Julich, W. D., Kusnick, C., & Lindequist, U. (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology, 74(2), 173–179.

3. Bansod, S., & Rai, M. (2008). Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus* species. Mycoses, 51(4), 316–323.
4. Baswa, M., Rath, C. C., Dash, S. K., & Mishra, R. K. (2001). Antibacterial activity of Karanj (Pongamia pinnata) and Neem (Azadirachta indica) seed oil: A preliminary report. Microbios, 105(408), 183–189.
5. Bhattacharjee, S. K. (2001). *Handbook of Medicinal Plants*. Pointer Publishers.
6. Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods. International Journal of Food Microbiology, 94(3), 223–253.
7. Chandrasekaran, M., & Venkatesalu, V. (2004). Antibacterial and antifungal activity of Syzygium jambolanum seeds. Journal of Ethnopharmacology, 91(1), 105–108.
8. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.
9. Duraipandiyan, V., Ayyanar, M., & Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine, 6(1), 35.
10. Dweck, A. C. (2002). Herbal medicine for the skin: Dermatology and dermatological conditions. Journal of Cosmetic Science, 53(1), 21–38.
11. Edris, A. E. (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytotherapy Research, 21(4), 308–323.
12. Fani, M. M., & Kohanteb, J. (2007). Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. Journal of Oral Science, 49(3), 201–206.
13. Ghosh, A., & Das, B. K. (2008). Antifungal activity of some plant extracts against important fungal pathogens. Journal of Phytopathology, 156(1), 73–77.
14. Gupta, A. K., & Nicol, K. (2004). The use of botanicals in the treatment of fungal skin infections. Clinical Dermatology, 22(2), 160–162.
15. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Springer.
16. Jain, R., Jain, C. L., & Jain, S. (2011). Antifungal activity of different plant parts of Azadirachta indica (Neem). International Journal of Pharmacy and Pharmaceutical Sciences, 3(2), 1–3.
17. Joshi, R. K., & Satyal, P. (2008). Antifungal activities of essential oils: A review. Natural Product Communications, 3(4), 635–639.
18. Kalemba, D., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 10(10), 813–829.
19. Kamatou, G. P., & Viljoen, A. M. (2010). A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. Journal of the American Oil Chemists' Society, 87(1), 1–7.
20. Kim, H. M., Lee, E. H., Cho, S. H., Ahn, C. W., & Shin, M. K. (1998). Effect of Syzygium aromaticum extract on immediate hypersensitivity in rats. Journal of Ethnopharmacology, 60(2), 125–131.
21. Kordali, S., Cakir, A., Mavi, A., Kilic, H., & Yildirim, A. (2005). Screening of chemical composition and antifungal and antioxidant activities of essential oils from three Turkish Artemisia species. Food Chemistry, 91(1), 61–71.
22. Kumar, A., & Shukla, R. (2010). Herbal medicine in the management of fungal infections: Current perspectives and future prospects. International Journal of Pharmaceutical Sciences Review and Research, 3(1), 91–94.
23. Lall, N., & Meyer, J. J. (2000). Antibacterial activity of water and acetone extracts of the roots of Euclea natalensis. Journal of Ethnopharmacology, 72(1–2), 313–316.
24. Lee, S. B., Cha, K. H., Kim, S. M., Park, H. J., & Choi, Y. H. (2007). Antifungal activity of essential oils extracted from medicinal herbs against Trichophyton species. Journal of Microbiology and Biotechnology, 17(10), 1568–1573.
25. Mahesh, B., & Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences, 4(S), 839–843.
26. Maruzzella, J. C., & Sicurella, N. A. (1960). Antibacterial activity of essential oil vapors. Journal of Pharmaceutical Sciences, 49(9), 692–695.
27. Martindale, W. (2005). *The Extra Pharmacopoeia* (35th ed.). Pharmaceutical Press.
28. Mishra, P., & Mishra, P. (2011). Study of antibacterial activity of Ocimum sanctum extract against gram positive and gram negative bacteria. American Journal of Food Technology, 6(4), 336–341.
29. Mothana, R. A., & Lindequist, U. (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of Ethnopharmacology, 96(1–2), 177–181.

30. Murthy, M. K., & Raghavendra, M. P. (2011). Evaluation of antimicrobial activity and phytochemical analysis of selected medicinal plants against human pathogenic bacteria. Journal of Pharmacy Research, 4(2), 498–500.

31. Ncube, N. S., Afolayan, A. J., & Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African Journal of Biotechnology, 7(12), 1797–1806.

32. Nostro, A., Germano, M. P., D'Angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology, 30(5), 379–384.

33. Palombo, E. A., & Semple, S. J. (2001). Antibacterial activity of traditional Australian medicinal plants. Journal of Ethnopharmacology, 77(2–3), 151–157.

34. Pandey, A. K., & Tripathi, N. N. (2010). Antifungal potential of some herbal essential oils against Fusarium species. Archives of Phytopathology and Plant Protection, 43(16), 1600–1605.

35. Pereira, A. C., & Ghosh, S. (2012). Herbal therapeutics for fungal infections: A review. International Journal of Research in Pharmacy and Chemistry, 2(2), 232–238.

36. Prasad, D., & Kumar, M. S. (2012). Antifungal activity of plant extracts against dermatophytes isolated from clinical samples. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3(3), 1205–1210.

37. Raj, M. R., & Dhanasekaran, S. (2008). Evaluation of antifungal activity of herbal formulations. Indian Journal of Pharmaceutical Sciences, 70(5), 616–618.

38. Rai, M., Pandey, A. K., Gade, A., & Rathod, D. (2016). Current trends in phytotherapy and nanotechnology for the treatment of fungal infections. Critical Reviews in Microbiology, 42(4), 485–494.

39. Rana, I. S., Rana, A. S., & Rajak, R. C. (2011). Evaluation of antimicrobial activity in methanolic extracts of some medicinal plants. Biological Forum – An International Journal, 3(2), 91–93.

40. Rath, C. C., Dash, S. K., & Mishra, R. K. (2009). Evaluation of antimicrobial activity and phytochemical screening of selected medicinal plants. Journal of Natural Products, 2, 23–28.