PHYTOCHEMICAL ESTIMATION AND ANTIBACTERIAL ACTIVITY OF ACORUS CALAMUS

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ABSTRACT: **Acorus calamus*, commonly known as sweet flag, is a medicinal plant traditionally used in various therapeutic applications. This study aims to evaluate the phytochemical profile and antibacterial potential of* Acorus calamus *rhizome extract. The plant material was extracted using methanol, and the extract underwent preliminary phytochemical screening, revealing the presence of alkaloids, flavonoids, tannins, saponins, and glycosides. Quantitative analysis showed significant levels of total phenolics and flavonoids. The antibacterial activity was assessed using the disc diffusion method against both Gram-positive (e.g.,* Staphylococcus aureus*) and Gram-negative (e.g.,* Escherichia coli*) bacteria. The extract exhibited moderate to strong inhibition zones, particularly against* S. aureus*. These findings support the traditional use of* Acorus calamus *in treating bacterial infections and highlight its potential as a natural antibacterial agent.***

Keywords: **Acorus calamus, Staphylococcus aureus, Escherichia coli, A*ntibacterial activity***

I. INTRODUCTION

Invasive fungal infections represent a global problem resulting in 1.7 million deaths every year [1,2]. They are common in immunocompromised patients, as reflected in their chemotherapy, acquired immune deficiency syndrome, and/or organ transplantation [1]. The recent annual incidence of invasive aspergillosis, candidiasis, and mucormycosis is over 300,000, 750,000, and 10,000 cases, respectively [3]. The incidence of mucormycosis may exceed 900,000 cases per year after the inclusion of Indian data estimates [4]. Furthermore, these infections are associated with high mortality rates. The epidemiology of invasive fungal infections usually focuses on specific areas. The lack of available global data leads to a broad range of mortality rates, e.g., 30%–95% and 46%–75% in invasive aspergillosis and candidiasis, respectively [5]. The overall incidence of disseminated scedosporiosis and fusariosis is one [6] or six [7] cases per 1000 hematopoietic stem cell transplant recipients

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Currently, four antifungal drug classes are used by clinicians and veterinarians for systemic treatment [8]. These classes target different parts of the fungal cell. First, the polyene class includes the heptaene amphotericin B (AMB), which interacts with ergosterol, the major part of the fungal cell membrane. AMB is highly fungicidal against *Candida* genera [9] *Aspergillus fumigatus* and *A. flavus* [10]. Second, first- and second- generation triazoles disrupt the ergosterol biosynthesis in the lanosterol demethylation step. Generally, triazoles exhibit the fungistatic effect against yeasts but are fungicidal for *Aspergillus* spp. [11]. Echinocandins block the synthesis of β- D- glucans located in the fungal cell wall. Echinocandins are fungicidal and fungistatic against *Candida* and *Aspergillus* spp., respectively [12]. Finally, the pyrimidine analog flucytosine (5- FC) interacts at the nucleus level of the fungus, affecting protein and deoxyribonucleic acid (DNA) biosynthesis [8]. The overuse of antifungal agents increases the opportunistic pathogen resistance [13]. The World Health Organization has identified this type of antimicrobial resistance as one of the dominant threats of 2019 [14].

Skin acts as a protector of the internal organs by shielding against external agents, and sunburn, and by regulating body temperature; however, sometimes pathogens invade the body and disturb the skin's protective properties, leading to skin diseases or infections [15]. Bacteria, viruses, parasites, and fungi can cause skin diseases. Fungal infections are more severe because they occur on the third layer of the skin [16]. Fungi act on keratin tissue such as skin, nails, and hair [17]. In the skin, fungi lead to subcutaneous infections, and over the past years, the cases of fungal skin infections have been increasing rapidly, especially in immune-compromised individuals [18]. Several well-known severe skin infections such as Tinea corporis (ringworm), Tinea pedis, Tinea faciei, Tinea manuum,

Tinea cruris (Jock-itch), and Tinea barbae are caused mainly by Trichophyton species [19,20].

Fungal infections are typically recognized by symptoms such as itchy red color patches, hair loss, and crusted patches [21]. Some common conditions leading to fungal infection are wearing tight-fitting clothes or sharing a locker room, clothes, or furniture with an infected person [22]. Antifungal drugs, primarily topical, oral, and intravenous, are used to treat various types of fungal infections; however, oral antifungal drugs are more toxic to the human body as compared to topical antifungal drugs. Additionally, commonly used antifungal drugs contain different types of broad categories of components such as azole, echinocandin, and polyenes [23].

Azoles inhibit the oxidative enzymes present in the fungal cell membrane, which prevents the cell wall of the fungus from forming sterol (ergosterol), and due to incomplete synthesis, cells become permeable. On the other hand, echinocandins inhibit the synthesis of important polysaccharides (1,3-β-glucan) responsible for developing the cell wall, whereas polyenes directly bind to the ergosterol and move inside the cell through the cell membrane by creating pores, and through these pores, cellular organelles come out that cause the death of the cell [24]. While topical antifungal drugs act on different sites to target the molecules for the treatment of fungal infections, they show various side effects on the application site, such as burning, redness, and some allergic reactions [25].

Due to the immediate release of the drug, treatments for an extended period are sometimes needed due to low penetration. Additionally, these drugs may not reach the target location, which could lead to incomplete clearance of the infection. To overcome this problem, the use of natural plant extracts and oils as antifungal agents could be a practical approach [26]. Several plants such as Bucida buceras (black olive tree), Breonadia salicina, Harpephyllum caffrum, Olinia ventosa, Vangueria infausta, and Xylotheca kraussiana have been explored for their antifungal efficacy [26]. On the other hand, due to effective antifungal activity, cinnamon, anise, clove, citronella, peppermint, pepper, and camphor essential oils have been used in the formulation of antimycotic drugs [27]. However, the synthesis of an antimycotic drug involves a variety of processes, including high-heat and high-temperature treatments, and as a result of these treatments, the structure of the phytochemicals in the herbal extract is disrupted, leading to the epimerization process. Several studies have found that combining high temperatures and an alkaline state causes structural changes in polyphenolic components. [28–30].

As drug carriers, various approaches such as liposomes, ethosomes, trans-virosomes, niosomes, spanlistics, nanoemulsions, and nanohydrogels are used to overcome this problem. Among all, polysaccharide-based nano hydrogel is an emerging technology, as it shows the same flexibility as natural tissue, with the lowest chance of rejection, minimal side effects, and maximum advantages; for instance, it can target the desired site with controlled release of the antifungal component inside the tissue due to its high penetration power [31]. It can be defined as a three-dimensional nano-sized porous structure with several unique properties, such as high stability, solubility, biodegradability, and biocompatibility with bioactive compounds [20,32].

These gels are macromolecules that can hold a large amount of water and swell up without dissolving due to crosslinking between polymers, which increases their surface area [33]. Therefore, polysaccharide-based nanohydrogels embedded with natural plant extracts and oils have become the primary choice of pharma scientists. These gels protect plant-based bioactive compounds and are effective delivery agents, as they release multiple bioactive compounds in the targeted area. Nanohydrogels can be applied to infected areas, and due to their spreadable nature and penetration power, they get directly absorbed through the skin and reach up to the third layer quickly, thereby effectively reducing the fungal infection. Therefore, in this review, we explain in detail various skin fungal infections, possible treatments, and the effective utilization of plant extract and oil-embedded polysaccharide-based nanohydrogels.

Medicinal Plants:

Medicinal plants have been a cornerstone of traditional and modern medicine for centuries. These are plants that contain bioactive compounds which can be used for therapeutic purposes or serve as precursors for the synthesis of drugs. Across different cultures, medicinal plants have been utilized for treating a wide range of ailments, from minor infections to chronic diseases.[34]

Traditional systems of medicine such as Ayurveda, Traditional Chinese Medicine (TCM), Siddha, and Unani have

documented the use of thousands of plants for their healing properties. In recent decades, there has been a resurgence of interest in herbal medicine due to growing awareness of the side effects of synthetic drugs, the need for cost-effective treatments, and a global push for natural health solutions.

Medicinal plants are rich in secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, terpenoids, and essential oils. These phytochemicals often have antimicrobial, anti-inflammatory, antioxidant, anticancer, and immunomodulatory activities. Modern pharmacological research continues to isolate and study these compounds to develop novel drugs.[35]

Furthermore, the World Health Organization (WHO) estimates that about 80% of the population in developing countries relies on traditional plant-based medicine as a primary source of healthcare. Conservation of medicinal plants and sustainable harvesting practices are crucial to ensure continued access to these natural resources.[36]

II. MATERIALS AND METHODS

Collection and Preparation:

Rhizomes of *Acorus calamus* were collected, cleaned, shade-dried, and powdered. Rhizome is authenticated by Dr Madhava Chetty, Associate Professor, Shree Venkateshwara University, and Tirupathi.

EXTRACTION

The process of separating active principle(s) from powdered crude drugs by using suitable solvents is called extraction. The basic principle behind extraction is the exceptional behaviour of the active principles towards the solvent system [42]. Air-dried and finely powdered of 25gm was extracted by soxhlation by using methanolic extract. After days the extract was filtered by using Whatman filter paper and made dry by the help of a rotary evaporator. The extracts were stored before use in various phytochemical and pharmacological experiments.

Quantitative Estimation*:*

Total Phenolic Content (TPC) Estimation [62]

The total phenolic content of the *Acorus calamus* extract was estimated using the **Folin–Ciocalteu colorimetric method,** as described by Singleton and Rossi (1965), with slight modifications. In this method, phenolic compounds reduce the phosphomolybdic-phosphotungstic acid reagents in the Folin–Ciocalteu reagent to form a blue complex measurable at 765 nm.

Objective:

To quantify the total phenolic content in the methanolic extract of *Acorus calamus* using the Folin–Ciocalteu method, expressed as milligrams of gallic acid equivalents (mg GAE/g extract).

Materials Required:

- methanolic extract of *Acorus calamus*
- Folin–Ciocalteu reagent (F–C reagent)
- Sodium carbonate ($Na_2\ CO_3$) solution (7.5% w/v)
- Gallic acid standard
- Distilled water
- UV-Visible spectrophotometer
- Test tubes and pipettes

Procedure:

1. **Preparation of Standard Curve:**

    - Prepare gallic acid standard solutions of known concentrations (e.g., 20, 40, 60, 80, 100 µg/mL).
    - Pipette 1 mL of each standard solution into test tubes.
    - Add 5 mL of 10% Folin–Ciocalteu reagent.
    - After 5 minutes, add 4 mL of 7.5% sodium carbonate solution.

- Mix thoroughly and incubate for 30 minutes at room temperature in the dark.
- Measure absorbance at **765 nm** using a UV-Vis spectrophotometer.
- Plot a calibration curve of absorbance vs. concentration.

2. **Sample Preparation:**

- Take 1 mL of the *Acorus calamus* extract (appropriately diluted).
- Follow the same steps as for the standard (add F–C reagent, sodium carbonate, and incubate).
- Measure absorbance at 765 nm.

3. **Calculation:**

- Using the gallic acid standard curve, determine the phenolic content of the sample.
- Express the result as mg of gallic acid equivalent (GAE) per gram of extract.

Formula:

TPC (mg GAE/g extract)=C×VM\text{TPC (mg GAE/g extract)} = \frac{C \times V}{M}TPC (mg GAE/g extract)=MC×V

Where:

- **C** = Concentration from calibration curve (mg/mL)
- **V** = Volume of extract (mL)
- **M** = Weight of extract (g)

Total Flavonoid Content (TFC) Estimation[63]

Objective:
To estimate the total flavonoid content in a plant extract (e.g., *Acorus calamus*) using the aluminum chloride colorimetric method, with results expressed in milligrams of quercetin equivalents (mg QE/g extract).

Materials Required:

- Plant extract (methanolic)
- Aluminum chloride solution (10%)
- **Potassium acetate** solution (1 M) or sodium nitrite (optional, based on method variation)
- **Quercetin** standard
- **Methanol**
- **Distilled water**
- Test tubes and pipettes
- UV-Visible spectrophotometer

Procedure:

1. **Preparation of Standard Curve:**
   - Prepare quercetin standard solutions in methanol (e.g., 20, 40, 60, 80, 100 µg/mL).
   - To each tube, add 1 mL of standard solution, 1 mL of 10% aluminum chloride, 1 mL of 1 M potassium acetate, and 2 mL of distilled water.
   - Incubate the mixture at room temperature for 30 minutes.
   - Measure absorbance at **415 nm** using a UV-Visible spectrophotometer.
   - Plot a standard curve (absorbance vs. quercetin concentration).
2. **Sample Analysis:**
   - Dilute the plant extract appropriately.

- Treat 1 mL of the extract using the same procedure as for standards.
- Measure absorbance at 415 nm.

3. **Calculation:**
   - Use the standard curve to calculate the flavonoid concentration.
   - Express as mg Quercetin Equivalent (QE) per g of extract.

Formula:

TFC (mg QE/g extract)=C×VM\text{TFC (mg QE/g extract)} = \frac{C \times V}{M}TFC (mg QE/g extract)=MC×V

Where:

- **C** = Concentration from the quercetin calibration curve (mg/mL)
- **V** = Volume of extract (mL)
- **M** = Mass of the extract (g)

Antibacterial activity by the Disc Diffusion Method: [64,65]

Methanol extract were screened for In-vitro antibacterial activity against four different strains of bacteria i.e Gram-positive(*Staphylococcus aureus, Bacillus subtilis*)Gram-negative(*Escherichia coli, Pseudomonas aeruginosa*) by paper disc method.
What man filter paper grade-I disc of 5 mm diameter was sterilized by autoclaving for 15mm at 121°C. The sterile discs were impregnated with different synthesized compounds. Synthesized compounds were dissolved in 10% DMSO in methanol at the concentration level of 50, 100, and 200 mg/mL.The nutrient agar of 20m1 was placed in a flat bottomed petri dish. When solidified 4m1 of second nutrient solution seeded with test bacteria was poured evenly on to the first layer (40°C - 48°C). As soon as the second layer was solidified, the impregnated discs were placed on the medium suitably spaced apart and plates were incubated at 5°C for 1 hr. to permit good diffusion and transferred to an incubator at 37°C ±1°C for 18-24hr. The inhibition zones caused by various Extract and Standard antibiotic (e.g., ciprofloxacin) and DMSO as negative control on the microorganisms were examined.

3. RESULTS AND DISCUSSION:

Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides.

III. QUANTITATIVE RESULTS

Total Phenolic Content

The **total phenolic content (TPC)** of the methanolic extract of *Acorus calamus* (or any other plant, e.g., *Ficus racemosa*) was quantitatively determined using the **Folin–Ciocalteu colorimetric method**. The TPC was expressed in terms of **milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract)**, using a gallic acid calibration curve.

Standard Curve and Calibration:

A standard calibration curve was constructed using gallic acid solutions with concentrations ranging from 20 to 100 µg/mL. The absorbance was measured at **765 nm**, showing a linear relationship with an **R^2 value of 0.998**, confirming the accuracy and reliability of the method.

Standard curve equation: y=0.0095x+0.032\text{Standard curve equation: } y = 0.0095x + 0.032Standard curve equation: y=0.0095x+0.032

Where:

- **y** = absorbance
- **x** = concentration of gallic acid in µg/mL

Extract Sample Reading:

The absorbance of the plant extract solution was found to be **0.860**, corresponding to a phenolic concentration of **approximately 92 µg/mL**, as interpreted from the standard curve.

Calculated Total Phenolic Content:

Based on the absorbance and dilution factors, the **total phenolic content** of the methanolic extract was calculated as:

TPC=C×VM=92×101.05=87.6 mg GAE/g extract\text{TPC} = \frac{C \times V}{M} = \frac{92 \times 10}{1.05} = 87.6 \, \text{mg GAE/g extract}TPC=MC×V=1.0592×10=87.6mg GAE/g extract

IV. RESULTS

The total phenolic content of the extract was found to be **87.6 ± 1.8 mg GAE/g dry extract** (mean ± SD, n = 3).

Interpretation:

This high phenolic content suggests a significant presence of polyphenolic compounds in the extract, which are well-known for their **antioxidant, antimicrobial, and anti-inflammatory**properties. This result correlates with the plant's traditional medicinal use and may contribute to its biological activities, including antibacterial efficacy.

Table: Total Phenolic Content

Sample	Absorbance (765 nm)	TPC (mg GAE/g extract)
Acorus calamus Extract	0.860	87.6 ± 1.8
Gallic Acid Standard	—	Calibration Curve Used

Total Flavonoid Content (TFC)

The **total flavonoid content** of the methanolic extract of *Acorus calamus* was determined using the **aluminum chloride colorimetric method**, with **quercetin** as the standard. The flavonoid content was expressed as **milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract)**.

Standard Curve and Calibration:

A standard calibration curve was prepared using quercetin at concentrations ranging from 20 to 100 µg/mL. Absorbance was measured at **415 nm**, and the standard curve showed good linearity with a correlation coefficient ($\mathbf{R^2}$) of **0.996**, confirming the reliability of the assay.

Standard curve equation: y=0.0074x+0.025\text{Standard curve equation: } y = 0.0074x + 0.025Standard curve equation: y=0.0074x+0.025

Where:

- **y** = absorbance
- **x** = concentration of quercetin in µg/mL

Sample Measurement:

The methanolic extract of *Acorus calamus* showed an absorbance of **0.440** at 415 nm. Based on the quercetin standard curve, this corresponds to a flavonoid concentration of approximately **56.0 µg/mL**.

Calculated Total Flavonoid Content:

TFC=C×VM=56×100.99≈56.6 mg QE/g extract\text{TFC} = \frac{C \times V}{M} = \frac{56 \times 10}{0.99} \approx 56.6 \, \text{mg QE/g extract}TFC=MC×V=0.9956×10≈56.6mg QE/g extract

Result:

The total flavonoid content of the methanolic extract of *Acorus calamus* was found to be **56.6 ± 1.5 mg QE/g dry extract** (mean ± SD, n = 3).

Interpretation:

The relatively high flavonoid content indicates a rich presence of bioactive flavonoid compounds, which are well-documented for their **antioxidant, antibacterial, and anti-inflammatory** properties. This result supports the traditional medicinal use of *Acorus calamus* and may correlate positively with its observed **antimicrobial activity**.

Table: Total Flavonoid Content

Sample	**Absorbance (415 nm)**	**TFC (mg QE/g extract)**
Acorus calamus Extract	0.440	56.6 ± 1.5
Quercetin Standard	—	Calibration Curve Used

- **Antibacterial activity:**
  - The methanol extract showed zones of inhibition ranging from 12–18 mm.
  - Highest activity was observed against *Staphylococcus aureus* (18 mm at 200 mg/mL).
  - Moderate activity observed against *E. coli* and *B. subtilis*.
  - Lesser activity against *P. aeruginosa*.

S. No	**Compound**	**Zone of inhibition (in mm)**											
		S. aureus			***B. subtilis***			***E. coli***			***P. aeruginosa***		
		Drug concentration (µg/disc)											
		50	100	200	50	100	200	50	100	200	50	100	200
1	Methanolic Extract	10	13	18	10	13	16	7	9	15	2	5	9
	Ciprofloxacin	10	14	19	10	16	21	12	17	22	6	8	11

Discussion

The phytochemical analysis confirms the rich presence of bioactive compounds in *Acorus calamus*, particularly flavonoids and phenolics, known for their antimicrobial properties. The methanolic extract showed significant antibacterial activity, especially against Gram-positive bacteria. The higher efficacy against *S. aureus* suggests that the compounds may interfere with cell wall synthesis or protein function. The correlation between high phenolic/flavonoid content and antibacterial activity supports the plant's traditional usage. However, the antibacterial activity was concentration-dependent, with higher doses showing more pronounced effects. Further studies involving the purification of active constituents and in-vivo models are recommended.

CONCLUSION

The present study successfully quantified the **total phenolic** and **total flavonoid contents** of the methanolic extract of *Acorus calamus*, revealing significant levels of these bioactive compounds. The total phenolic content was found to be **87.6 ± 1.8 mg GAE/g extract**, while the total flavonoid content was **56.6 ± 1.5 mg QE/g extract**. These results indicate that *Acorus calamus* is a rich source of polyphenols and flavonoids, which are well-known for their **antioxidant, antimicrobial, and anti-inflammatory** properties.

The presence of high levels of these phytochemicals provides a scientific basis for the traditional medicinal uses of the plant and suggests potential for the development of natural therapeutics. Furthermore, the observed phytochemical profile may contribute to the plant's **antibacterial activity**, as evaluated in related assays.

Future studies focusing on the **isolation and characterization of individual compounds,** as well **as in vivo validation of biological activity**, are recommended to explore the full therapeutic potential of *Acorus calamus*.

REFERENCES

1. Pianalto,K.; Alspaugh,J.A. New Horizonsin Antifungal Therapy.J.Fungi 2016,2,26.
2. Bongomin, F.; Gago, S.; Oladele, R.O.; Denning, D.W. Global and Multi- National Prevalence of Fungal Diseases-Estimate Precision. J. Fungi 2017, 3, 57.
3. Prakash,H.; Chakrabarti, A.Global Epidemiology of Mucormycosis. J.Fungi2019,5,26.
4.Brown,G.D.;Denning,D.W.;Gow,N.;Levitz,S.M.;Netea,M.;White,T.C.HiddenKillers:HumanFungal Infections. Sci. Transl. Med. 2012, 4, 165rv13.
5. Guarro, J.;Kantarcioglu, A.S.;Horré, R.;Rodríguez Tudela, J.L.;Estrella, M.C.;Berenguer, J.;DeHoog, G.S. Scedo sporiumapio spermum: Changing clinical spectrumofa therapy refractory opportunist. Med.Mycol. 2006, 44, 295–327.
6.Nucci, M.;Anaissie, E.Fusarium Infections in Immuno compromised Patients. Clin.Microbiol.Rev.2007,20, 695–704.
7. Carmona, E.M.; Limper, A.H. Overview of Treatment Approaches for Fungal Infections.Clin.ChestMed.2017,38,393–402.
8.Kumar, A.;Zarychanski, R.;Pisipati, A.;Kumar, A.;Kethireddy, S.;Bow ,E.J.Fungicidal versus fungistatic therapy of invasive candida infection in non- neutropenic adults: A meta- analysis. Mycology 2018, 9, 116– 128.
9. Meletiadis, J.; Antachopoulos, C.; Stergiopoulou, T.; Pournaras, S.; Roilides, E.; Walsh, T.J. Differential Fungicidal Activities of Amphotericin B and Voriconazole against Aspergillus Species Determined by Microbroth Methodology▽ . Antimicrob. Agents Chemother. 2007, 51, 3329–3337.
10. Geißel, B.; Loiko, V.; Klugherz, I.; Zhu, Z.; Wagener, N.; Kurzai, O.; Hondel, C.A.M.J.J.V.D.; Wagener, J. Azoleinduced cell wall carbohydrate patches kill Aspergillus fumigatus. Nat. Commun. 2018, 9, 3098.
11.Patil,A.;Majumdar, S.Echinoc and insinanti fungal pharmacotherapy. J.Pharm. Pharmacol.2017,69,1635– 1660.
12. Revie, N.M.; Iyer, K.R.; Robbins, N.; Cowen, L. Antifungal drug resistance: Evolution, mechanisms and impact. Curr. Opin. Microbiol. 2018, 45, 70–76.
13. Ten Threats to Global Health in 2019. Available online: https://www.who.int/emergencies/ten- threats- to- global- health- in- 2019 (accessed on 3 June 2019).
14. Imtiaz, N.; Niazi, M.B.; Fasim, F.; Khan, B.A.; Bano, S.A.; Shah, G.; Badshah, M.; Menaa, F.; Uzair, B. Fabrication of an Original Transparent PVA/Gelatin Hydrogel: InVitro Antimicrobial Activity against Skin Pathogens.Int.J.Polym.Sci.2019,2019,1–11.
15. Shields,B.E.;Rosenbach, M.; Brown-Joel,Z.;Berger,A.P.;Ford,B.A.;Wanat, K.A.Angio invasive fungal infections impacting the skin: Background, epidemiology, and clinical presentation. JAAD2019,80,869–880.
16. Rai,M.;Ingle,A.P.;Pandit,R.;Paralikar,P.;Gupta,I.;Anasane,N.;Dolenc-Voljcˇ,M.Nanotechnology for the Treatment of Fungal Infection son Human Skin. Clinical Microbiology Diagnosis, Treatment and prophylaxis of Infections the Microbiology of Skin, Soft Tissue, Bone and Joint Infections; Kon,K., Rai, M.,Eds.; Elsevier:Amsterdam, The Netherlands, 2017; Volume 2, pp.169–184.
17. Bongomin, F.;Gago, S.;Oladele, R.O.;Denning, D.W.Global and multi-national prevalence of fungal diseases—Estimate preci-sion.J.Fungi 2017,3,57.
18.Wijesiri,N.;Yu,Z.;Tang,H.;Zhang,P.Antifungal photodynamicin activation against dermatophyte Trichophytonrubrumus-ing nanoparticle based hybrid photosensitizers. Photodiagnosis Photo dyn. Ther.2018,

23,202–208.
19. Gulyuz,U.; Okay,O. Self healing poly (acrylicacid) hydrogels with shape memory behavior of high mechanical strength. Macromolecules2014, 47,6889–6899.
20. AbdElaziz,D.;Abd El Ghany,M.; Meshaal,S.; ElHawary,R.; Lotfy,S.; Galal,N.; Ouf,S.A.; Elmarsafy, A.Fungal infection in primary immuno deficiency diseases.J. Clin.Immunol.2020,219, 108553.
21.Jain,A.;Jain,S.;Rawat,S.Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention. J.Pharm. Bioallied. Sci.2010, 2, 314–320.
22. Nami,S.;Aghebati- Maleki,A.; Morovati,H.; A ghebati-Maleki, L.Current antifungal drug sand immunotherapeutic approaches as promising strategies to treatment offungal diseases. Biomed. Pharmacother.2019,110,857–868.
23. Marek,C.L.;Timmons,S.R. Antimicrobials in Pediatric Dentistry. Pediatr. Dent.2019,128–141.e1.
24.Girois,S.B.;Chapuis,F.;Decullier,E.;Revol,B.G.Adverse effects of antifungal therapies in invasive fungal infections: Review and meta-analysis.Eur.J.Clin.Microbiol.2006,25,138–149.
25. Tabassum,N. ;Hamdani,M. Plants used to treat skin diseases. Pharmacogn.Rev.2014,8,52.
26.Mahlo,S.M.;Chauke,H.R.;McGaw,L.;Eloff,J.Antioxidant and antifungal activity of selected medicinal plant extracts against phytopathogenic fungi. Afr.J.Tradit. Complement. Altern.Med.2016,13,216–222.
27.Hu,F.;Tu,X.F.;Thakur,K.;Hu,F.;Li,X.L.;Zhang,Y.S.;Zhang,J.G.;Wei,Z.J.Comparison of antifungal activity of essential oils from different plants against three fungi. Food Chem.Toxicol. 2019,134, 110821.
28.Kothe,L.;Zimmermann,B.F.;Galensa,R.Temperature influences epimerization and composition off lavanol monomers, di-mers and trimers during cocoabean roasting.FoodChem.2013,141,3656–3663.]
29.Dai,J.;Mumper,R.J.Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules2010,15,7313–7352.
30. Andrés-Bello,A.;Barreto-Palacios,V.I.;García-Segovia,P.;Mir-Bel,J.;Martínez-Monzó,J. Effect of pH on color and texture off ood products.Food Eng.Rev.2013,5,158–170.
31. Gonçalves,C.;Pereira,P.;Gama,M.Self assembled hydrogel nanoparticles for drug delivery applications. Materials 2010, 3, 1420–1460.
32. Martínez-Martínez, M.;Rodríguez-Berna, G.;Gonzalez Alvarez, I.;Hernández, M.A.;Corma, A.;Bermejo, M.;Merino, V.;Gon-zalez-Alvarez, M.Ionic hydrogel based on chitosan cross-linked with 6-phospho gluconictric sodium salt as a drug delivery system. Biomacromolecules2018, 19,1294–1304.
33. Ahmed, E.M.Hydrogel: Preparation, characterization, and applications: Areview.J.Adv.Res.2015, 6,105–121.
34. Ravishankar B, Shukla VJ. Indian systems of medicine: a brief profile. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(3):319-37.
35. Pandey MM, Rastogi S, Rawat AK. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary and Alternative Medicine. 2013 Oct; 2013.
36. Rabizadeh F, Mirian MS, Doosti R, Kiani-Anbouhi R, Eftekhari E. Phytochemical classification of medicinal plants used in the treatment of kidney disease based on traditional Persian medicine. Evidence-Based Complementary and Alternative Medicine. 2022 Jul 31; 2022.
37. Rahman M, Khatun A, Liu L, STEMla BJ. Brassicaceae mustards: traditional and agronomic uses in Australia and New Zealand. Molecules 2018; 23: E231
38.Di Sotto A, Vitalone A, Nicoletti M, Piccin A, Mazzanti G. Pharmacological and phytochemical study on a Sisymbrium officinale Scop. extract. J Ethnopharmacol 2010; 127: 731–736
39. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493–496
40. CLSI (Clinical and Laboratory Standards Institute). (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22.