Ilam University of Medical Sciences

Plant Biotechnology Persa

Online ISSN: 2676-7414

Homepage: https://pbp.medilam.ac.ir

Antimicrobial Effects of Medicinal Plants on *Streptococcus pyogenes*: A Review

Kimiya Karimi[1], Elahe Zarif Fakoor[2], Toktam Ziaee[3], Mahtab Mehboodi[4], Pouria Khodaei Ataloo[5], Masoud Sanati[6], Zeynab Mohammadi[7], Mohammadreza Dorzadeh[7], Kimia Rouzbeh[8]✉, Reyhaneh Heshmati[9]✉

[1] Department of Microbiology, Islamic Azad university, Lahijan, Iran
[2] General Biology, Mashhad, Iran Payam-e noor University of Mashhad, Iran
[3] Department of Parasitology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Department of Medical Microbiology (Bacteriology & Virology), Afzalipour Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[5] Department of Microbiology, School of Medicine, Ardabil University of Medical Science, Ardabil, Iran
[6] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
[7] Department of Microbiology and Virology Faculty of Medicine, Tabriz university of Medical Science, Tabriz, Iran
[8] Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
[9] Department of Biology, Faculty of convergent Science and Technology Science and Research Branch, Islamic Azad university, Tehran, Iran

Article Info

Article type:
Review Article

Article History:
Received: 03 Feburary 2024
Revised: 21 June 2024
Accepted: 28 Auguest 2023
Published Online: 16 Sep 2024

✉ ***Correspondence to:***
Reyhaneh Heshmati
Kimia Rouzbeh

Email:
university.ac55@gmail.com
Kimiarouzbeh222@gmail.com

A B S T R A C T

Streptococcus pyogenes is a pathogen of significant public health concern. It is the most common cause of bacterial pharyngitis and is responsible for serious conditions such as streptococcal toxic shock syndrome, necrotizing fasciitis, impetigo, and scarlet fever. Additionally, *S. pyogenes* is involved in post-streptococcal sequelae, including acute glomerulonephritis, acute rheumatic fever, rheumatic heart disease, and reactive arthritis. The increasing antibiotic resistance of *S. pyogenes* has led to a growing interest in exploring alternative treatments, such as medicinal plants, which may provide effective and safer options for preventing and treating diseases caused by this bacterium. Today, there is an increasing interest in medicinal plants due to less side effects, ease of use, availability, and usually affordability. In conclusion, given the growing antibiotic resistance of *S. pyogenes*, this study investigates the inhibitory effects of medicinal plants against this pathogen. The aim is to assess the potential of these plants in preventing and treating diseases caused by *S. pyogenes*, as well as to elucidate their mechanisms of action on this bacterium.

➢ How to cite this paper
Karimi K, Zarif Fakoor E, Ziaee T, Mehboodi M, Khodaei Ataloo P, Sanati M, Mohammadi Z, Dorzadeh MR, Rouzbeh K, Heshmati R. Antimicrobial Effects of Medicinal Plants on *Streptococcus pyogenes*: A Review. Plant Biotechnology Persa 2024; 6(2): 68-79.

DOI: 10.61186/pbp.6.2.8

Publisher: Ilam University of Medical Sciences

Introduction

Streptococcus pyogenes, (colloquially named "group A streptococcus)" (GAS), is a pathogen of public health significance, infecting 18.1 million people worldwide and resulting in 500,000 deaths each year [1]. This pathogen causes a wide range of manifestations from mild local infections to life-threatening invasive infections. It is usually transmitted through respiratory droplets, contact with skin sores, or through contact with contaminated materials or equipment. Foodborne transmission is also possible, although further research is needed to quantify this route of infection [1-3]. Typically, *Streptococcus pyogenes* infections and associated outcomes are more common in areas of socioeconomic disadvantage. This includes developing countries and disadvantaged or marginalized populations in developed countries [4]. Children, the immunocompromised, and the elderly are at greatest risk for *Streptococcus pyogenes* infections and related sequelae, and transmission rates are highest in schools, daycare centers, hospitals, and residential care homes. This has been attributed to overcrowding and higher levels of social contact in these locations. Prevention and control measures should be aimed at improving living conditions and personal health. Adherence to infection prevention and control practices should be emphasized in high-risk environments. The distribution of resources by governments, especially in underdeveloped countries, should also be considered [5].

Today, there is a growing interest in medicinal plants that are traditionally used to treat human infections. Herbal plants are an excellent source of alternative therapies for many infections, especially bacterial pathogens. However, further clinical studies and the development of standardized herbal formulations are needed to fully harness the therapeutic potential of these natural remedies. In conclusion, Considering the increasing antibiotic resistance to *Streptococcus pyogenes*, this study examines the inhibitory activity of medicinal plants against *Streptococcus pyogenes* and, as a result, their potential in preventing and treating diseases related to this bacterium, as well as the mechanism of action on *Streptococcus pyogenes*.

Streptococcus pyogenes

Streptococcus pyogenes are Gram-positive, catalase-negative and coagulase-negative and beta-hemolytic cocci that grow in pairs or chains. Cells are spherical, non-motile, non-sporulating, 0.5-1.0 μm in diameter and usually appear in chains [6-8]. Interestingly, horizontal gene transfer (HGT) and prophage integration are common among S. pyogenes genomes, giving them genomic flexibility and diversity. These factors collectively create more pathogenic and resistance capabilities and change the regulation of existing genes [6]. GAS strains possess a wide array of virulence factors, including complement inhibitors (M protein, hyaluronic acid capsule, and SIC), leukocidal toxins (SLO and streptolysin S [SLS]), immunoglobulin (Ig) binding proteins (PrtF1/SfbI, FbaA and Sib), and immunoglobulin-degrading enzymes (IdeS, endo-β-N-acetylglucosaminidase of streptococci [EndoS] and SpeB) and superantigens that play an important role in resistance to opsonophagocytosis [2]. M proteins are the major antiphagocytic virulence factors of S. pyogenes. The type specificity of the M protein, of which more than 100 different types are known, is largely determined by an epitope located in the 40 to 50 amino acid residues at the amino terminus. These regions of M proteins have been shown to induce antibodies that have strong bactericidal activity and are unlikely to cross-react with human tissues [9]. The contribution of M proteins to GAS virulence is primarily attributed to their immunomodulatory effects. They can directly bind to and adsorb several host components, including plasmin and fibrinogen, to the surface of streptococci, thereby conferring resistance to innate and adaptive immune responses. M proteins also cause programmed cell death in macrophages by inducing the NLRP3 inflammatory system, which leads to the processing and secretion of pro-inflammatory IL-1β and IL-18 [10]. Therefore, the M protein encoded by the emm gene is an important pathogenic factor and is also an epidemiological marker used worldwide to characterize GAS isolates [9]. The capsule is another virulence factor of this bacterium, which is structurally identical to human hyaluronic acid, a major component of extracellular matrices found in many body tissues, including connective and epithelial tissues. Therefore, the GAS capsule acts to camouflage the pathogen from the host's immune system. GAS capsule mediates adhesion to pharyngeal and skin epithelial cells by directly binding to human cell surface glycoprotein CD44, a primary receptor for human hyaluronic acid. CD44-dependent binding further leads to the activation of cell signaling pathways that disrupt the integrity of the epithelial barrier, thereby allowing GAS to penetrate deeper into the underlying tissues [10]. In addition, Streptolysin O is a cholesterol-dependent hemolysin from S. pyogenes that belongs

to the group of thiol-activated cytolysins. The level of antibodies against streptolysin O (ASO) begins to rise after one week of infection and reaches a maximum level about three to six weeks after infection. The upper limit of normal (ULN) of ASO is 240-320 in the age group of children 6-15 years old. While the ASO response following streptococcal upper respiratory tract infection is usually high, pyoderma caused by *Streptococcus pyogenes* does not elicit a strong ASO response [7]. The findings show that after the initial connection of this bacterium, it forms microcolonies. These macroscopic structures are involved in streptococcal skin infection and acute bacterial tonsillitis. When bacterial cells proliferate, such microcolonies may form complex groups that form organized three-dimensional structures, a sedentary lifestyle commonly referred to as biofilms [3]. It has been found that *Streptococcus pyogenes* forms biofilm both in vitro and in vivo [11]. Therefore, GAS is able to form microcolony and biofilm, and this sedentary lifestyle plays an important role in the pathogenesis of this bacterium [3].

Pathogenicity

Streptococcus pyogenes is a host-adapted bacterial pathogen responsible for a wide variety of diseases. These pathogens colonize the throat or skin and are responsible for a number of purulent infections and non-purulent sequelae. They are the most common cause of bacterial pharyngitis, responsible for streptococcal toxic shock syndrome (STSS), necrotizing fasciitis, impetigo, and scarlet fever. Also, they are involved in post-streptococcal sequelae, including acute glomerulonephritis (APSGN), acute rheumatic fever (ARF), rheumatic heart disease (RHD) and reactive arthritis. APSGN is an immune complex-mediated renal disorder that results in symptoms such as edema, hypertension, urinary sediment abnormalities, and decreased levels of serum complement components. Acute rheumatic fever and rheumatic heart disease are the most serious autoimmune consequences of infection with this bacterium, causing disability and death in children worldwide. *Streptococcus pyogenes* as a "flesh-eating" bacterium that attacks the skin and soft tissues and in severe cases destroys the infected tissues or organs and leads to Tourette's syndrome, tics and movement disorders [12,13].

Prevalence

The frequency of infections caused by S. Pyogenes in different parts of the world is different depending on the clinical manifestations of the infections. GAS has been associated with serious diseases in the past, leading to high morbidity and mortality. In the middle of the 20th century, infections caused by this bacterium decreased. However, during the last two decades, non-suppurative and purulent complications of S. pyogenes infection have increased. This increase in disease burden can be attributed to several factors, including changes in pathogenicity and resistance to antibiotics. GAS infections and complications are different in developed and developed countries. In underdeveloped countries, the prevalence of rheumatic heart disease (RHD) and the incidence of RHD-related mortality are high. On the contrary, in developed countries, the mortality rate due to invasive GAS infection is high [14-16]. The global burden of severe S. pyogenes infection is 18.1 million cases, with 1.78 million new cases per year. The worldwide prevalence of RHD is at least 15.6 million cases, with 282,000 new cases occurring annually. Approximately 233,000 deaths per year are attributed to RHD. Approximately 663,000 new cases of invasive GAS disease are reported each year, with 163,000 deaths per year. 616 million cases of sore throat infections worldwide can be attributed to S. pyogenes. Also, 111 million cases of skin infections in children, developing countries. In the United States, 15% to 30% of pharyngitis cases in children and 5% to 20% of pharyngitis cases per year in adults are due to S. pyogenes. In addition, a resurgence of ARF cases has been observed in children of middle-class US families [17,18]. The findings show that throat infection is more common in temperate climates and its incidence increases in late winter and early spring. In addition, jaundice is more common in children in areas with humid climates. It has also been observed that the severity of invasive skin infections caused by GAS increases from January to April and is associated with host susceptibility to serious infection [18,19].

Treatment

The drug of choice for treating bacterial pharyngitis is oral penicillin for 10 days or IM benzathine penicillin. This treatment is affordable and has a limited spectrum of activity. In non-anaphylactic cases of penicillin allergy, macrolides and first-generation cephalosporins can be used. However, some strains of S. pyogenes have shown resistance to macrolides, and macrolides are used as a third-line treatment for strep throat infections. There are three well-known genotypes showing macrolide resistance: erm (A) for the inducible phenotype of MLSB (macrolide, lincosamide, and streptogramin B), erm (B)

for the constitutive phenotype of MLSB, and mef (A) for the phenotype of M. Severely invasive infections of S. Pyogenes can be treated with vancomycin or clindamycin. In case of soft tissue skin infection by S. pyogenes, intravenous antibiotics and surgery to remove the necrotic tissue are recommended [20-23].

Non-antibiotic treatment

Treating infectious diseases in humans with antibiotics can lead to adverse side effects and the development of antibiotic-resistant pathogens. Sore throats caused by pharyngitis or tonsillitis are often treated with antibiotics because these conditions frequently result from *Streptococcus pyogenes* infections. To address antibiotic resistance in streptococci, integrating probiotics, such as Lactobacillus, into the treatment regimen could be beneficial [24]. Recent studies have shown that probiotics, specifically Lactobacillus and Bifidobacterium species, may help prevent acute upper respiratory tract infections [25]. A probiotic available on the market, known as BLIS K12 (standing for Bacteriocin-Like Inhibitory Substances), utilizes the S. salivarius strain K12, which was originally isolated from the mouth of a healthy child (26). Streptococcus salivarius is the primary bacterium that colonizes the oral cavity [25]. *Streptococcus salivarius* is known to inhibit the growth of all tested strains of beta-hemolytic (Lancefield group A) S. pyogenes, which is the most common cause of bacterial pharyngitis and tonsillitis and can also be involved in acute otitis media (AOM). This inhibitory effect is attributed to the lantibiotics salivaricin A2 and salivaricin B, both encoded by a 190 kb megaplasmid in strain K12. Derivatives of strain K12 that lack this megaplasmid do not exhibit any inhibitory activity against S. pyogenes [26]. Due to the presence of these two salivaricins, the K12 strain effectively inhibits the growth of S. pyogenes in vitro [27]. In laboratory studies, it's been demonstrated that S. pyogenes is highly susceptible to various BLIS activities originating from S. salivarius, a counterpart found in the human oral cavity [28]. Recent findings suggest that administering the K12 strain orally not only establishes colonization in the oropharynx, releasing two salivaricins that act as antagonists against specific pathogenic strains, but also, via a molecular mechanism that is not fully understood yet, decreases IL-8 plasma concentrations and boosts salivary interferon-γ levels [27]. The strategy involves merging a modified peptide from the C-repeat region of the M protein with an epitope from SpyCEP. This includes M protein peptides J8 and p*17, along with a non-M protein peptide K4S2. Utilizing a conserved Streptococcus A epitope addresses the challenges of strain variability and cross-reactivity seen with vaccines targeting the hypervariable amino-terminal. This method could provide protection against various Streptococcus A strains globally, which is particularly beneficial for developing countries where these strains circulate rapidly. The K4S2 peptide helps produce antibodies that shield host IL-8 from SpyCEP-mediated breakdown, aiding neutrophil recruitment to infection sites [29].

Intravenous immunoglobulin (IVIG) is an investigational supplemental therapy for severe group A streptococcal (GAS) infections, aiming to neutralize toxins and enhance immune responses. Derived from the pooled serum of healthy donors, IVIG contains a variety of antibodies targeting numerous, unspecified bacterial antigens, likely including key toxins and surface-bound virulence factors. These antibodies work by neutralizing toxins and enhancing opsonization, thereby reducing bacterial load and mitigating pro-inflammatory cytokine storms. The variability in virulence factors produced by GAS and the differences in antibody profiles among donors means that the effectiveness of IVIG can vary, necessitating optimization for consistent results [30].

Zinc oxide (ZnO) nanoparticles (NPs) have been researched as potential nanoantibiotics for fighting pathogenic microorganisms and addressing multidrug resistance. These nanoparticles exhibit broad antimicrobial properties against various pathogens, including *Streptococcus pyogenes*. When used alongside antibiotic and anti-inflammatory medications, ZnO nanoparticles can enhance antimicrobial effectiveness against pathogens without promoting antibiotic resistance in both experimental and clinical settings. The Zn2+ ions released from ZnO NPs/MPs trigger antimicrobial responses by disrupting metabolic processes and enzymatic systems in microorganisms. After adsorption, these nanoparticles penetrate the microorganisms, causing cell wall or membrane rupture and inducing oxidative stress through lipid peroxidation, which leads to DNA damage [31].

A novel lysin from S. suis, PlySs2, has shown extensive lytic activity against various Gram-positive pathogens, including S. pyogenes, in vitro. Many streptococcal species are susceptible to PlySs2 lysis in vitro, with PlySs2 exhibiting potent lytic effects on S. pyogenes. This discovery represents a significant

advancement in bacteriophage lysin technology. PlySs2 strikes a balance between specific lysin activity and the broad-spectrum action of antibiotics. Notably, S. pyogenes has not developed resistance to PlySs2 in vitro, suggesting that PlySs2 could become a valuable tool in combating S. pyogenes and other multidrug-resistant Gram-positive bacteria [32].

Herbal medicines

The use of plants for therapeutic purposes predates the history of mankind and forms the origin of modern medicine. The first recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years [33]. Today, there is an increasing interest in medicinal plants due to less side effects, ease of use, availability, and usually affordability [34]. The World Health Organization estimates that 80% of people use herbal products to improve their health in various ways [35]. Herbs are an excellent source of alternative therapies for many infections. Plants are rich in a wide variety of secondary metabolites (phytochemicals), such as polyphenols, flavonoids, terpenoids, alkaloids, and tannins, which have been shown to have antimicrobial properties [36]. Also, plant essential oils have many properties. which prevents the growth or destruction of microbes. These properties include: 1. The hydrophobic property that causes the essential oil to penetrate into the lipids of the bacterial cell membrane, and with the release of ions and other cell contents, the cell structure is destroyed. 2. The phenolic substances in the essential oil damage the cytoplasmic membrane of the bacterial cell. 3. The carbonic group of essential oil attaches to cell proteins and prevents the role of amino acid and decarboxylase. 4. The reaction of aldehydes with SH groups prevents the growth of microorganisms. 5. The presence of alphatriptin in the essential oil prevents the growth of microbes [37]. In fact, herbal medicine formulas by damaging cell membranes and walls, inhibiting nucleic acid synthesis and increasing intracellular osmotic pressure, activity create antibacterial effects [38]. Essential oils obtained from medicinal plants showed less effectiveness against gram-negative bacteria than gram-positive bacteria, which is caused by the polysaccharide membrane that prevents the penetration of plant essential oils [37]. Also, in some plants the variation in antimicrobial activity was due to the use of different parts of the plant to prepare the extract. For example, the methanolic extract of Peganum harmala (P. harmala) root has the best effect compared to other parts of this plant [34]. In a study, the high antimicrobial activity of L. ethanol extract from Hypericum perforatum Linn. was observed against Lactobacillus plantarum (L. plantarum), Enterococcus faecalis (E. faecalis), Streptococcus mutans (S. mutans) and Streptococcus sobrinus (S. sobrinus) became. While S. sobrinus and L. plantarum were strongly inhibited by their aqueous extracts, a moderate antibacterial effect was observed in S. mutans and E. faecalis [39]. Also, some plants showed different antimicrobial effects in different stages of their growth. Thymus pubescens, Thymus serpyllum and Tanacetum parthenium had a better anti-staphylococcal effect in the flowering stage than in the pre-flowering stage [34]. Rosemary plants are rich in phenolic compounds with high antimicrobial activity against Gram-positive and Gram-negative bacteria [40]. Also, licorice root showed an effect on biofilm, which could be due to the inhibition of cell integrity, which leads to the destruction and leakage of the wall and membrane. Licorice extract showed antimicrobial activity against Gram-positive and Gram-negative bacteria. Most of the antimicrobial effects of licorice root as well as leaves have been reported due to isoflavonoid components, especially glabridin, glycyrrhizin, glabriol, hispaglabridin, B 4′-O-methylglabridin and 3-hydroxyglabrol [36]. In addition, Quercetin showed strong antibacterial activity against bacteria such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis by inhibiting bacterial DNA gyrase and topoisomerase IV [41]. Some Iranian plants evaluated such as Tanacetum parthenium, Thymus vulgaris, Thymus eriocalyx, Thymus persicus, Achillea Millefollum, P. harmala, Hieracium scabrum and Salvia urmiensis with MIC or minimum acceptable inhibitory concentration have antimicrobial activity power, especially against Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus (MRSA)(34). Another study showed that the volatile compounds in Cuminum cyminum and Oliveria decumbens with MIC less than 5 µg/ml are effective against MRSA bacteria [37]. Also, the root extracts of two plants Hypericum scabrum and Hymenocrater calycinus have antimicrobial and anti-biofilm effects [42]. Justicia plant shows antibacterial activities against bacteria such as Staphylococcus aureus, Klebsiella, Pseudomonas which causes secondary lung infection [43]. In addition, Salvia mirzayanii had the strongest activity against Helicobacter pylori with an MIC of 32 µg/mL [44]. It seems that the extracts of Securigera securidaca, Withania sominefra, Rosmarinus officinalis and Aloe vera can

prevent the growth of Streptococcus pneumoniae bacteria [40]. Tincture of Aspidosperma Quebracho Blanco, which is prepared from the bark of the stem. It helps to improve oxygenation which is an effective treatment for asthma. Tincture of 10 to 15 drops three to four times a day stimulates the respiratory center. The strength of the drug in the ethanolic extract is 1.10 [43]. In addition, various anticancer medicinal plants have been identified that show their therapeutic effects by inhibiting cancer-inducing enzymes and hormones, activating DNA repair processes, enhancing the synthesis of protective stimuli, reducing the formation of free radicals, and increasing individual immunity [45]. Ginger, in addition to treating a wide range of unrelated diseases such as arthritis, rheumatism, sore throat, is also used to treat bacterial infections [46]. Also, Ocimum gratissimum and Ocimum sanctum is an aromatic shrub found in tropical regions and used in medicine. It is traditionally used in India to treat various diseases and as an antimicrobial agent [47]. Essential oils of Cinnamomum verum, Cymbopogon citratus, Thymus vulgaris CT thymol, Origanum compactum and Satureja montana showed significant antibacterial activity [48]. The findings show that various cases of gastrointestinal infection (diarrhea, typhoid, cholera, and bacterial enteritis), urinary tract infection, and skin and wound infections due to bacterial pathogens were effectively treated using herbal medicines [49]. Also, many conventional medicines originate from plant sources: a century ago, most effective medicines were plant-based. For example, we can mention aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark) and morphine (from the opium poppy) [33]. Also, penicillin, which ended many deadly epidemics, is derived from plant mold [33]. In general, medicinal plants have been proven to be safe and effective against various human diseases, and their medicinal uses have gradually increased in developed countries [46]. In recent years, bacterial resistance to some antibiotics has increased, and it is very important to choose an alternative method to overcome this antibiotic resistance [40].

Many bioactive plant compounds originating from traditional medicinal plants, such as phenols, terpenoids, alkaloids, flavonoids, isothiocyanates and indoles, inhibit the main drug resistance factors such as efflux pumps, enzyme activity, membrane permeability and other pathogenic mechanisms such as quorum sensing and biofilm development [39]. Both oregano and sage extracts act as a potent anti-biofilm agent with dual action, preventing biofilm formation. A potential explanation for these bactericidal effects of oregano may be the high content of carvacrol and the presence of other phenolic compounds, such as p-cymene, myrcene and γ-terpinene [50]. In addition, the ethanolic extract of the leaves of Strobilanthes cusia Kuntze (S. cusia) showed antibacterial effect on penicillin-resistant Streptococcus pneumoniae (PRSP). Among the chemical compounds identified in the leaves of this plant, triptanthrin probably inhibited PRSP (MIC 25 µg/ml) by inhibiting N-glycan degradation protein in Strep pneumoniae [51]. Furthermore, berberine is an isoquinoline alkaloid isolated from Coptis chinensis Franch and other plants. Berberine is a NorA substrate that accumulates in bacterial cells and leads to DNA damage by binding to DNA. The activity of berberine against Gram-positive bacteria occurs mainly through the mutant temperature-sensitive filament cell division protein Z (FtsZ). Berberine hydrochloride showed moderate to strong activity against Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and multi-drug resistant *E. coli* [38]. Currently, researchers are focusing on developing new antibiotics from medicinal plants. Complex natural products with multiple molecular targets can reduce the incidence of resistance and thus show significant antibacterial activity against Multi Drug Resistant (MDR) bacteria [39]. Therefore, herbal medicines may provide an efficient treatment option for antibiotic-resistant bacteria. However, further studies are needed to investigate potential adverse effects, antibacterial properties and possible synergistic effects with other medicinal plants [37].

Different effects of Medicinal plants on *Streptococcus pyogenes*

Streptococcus pyogenes, a major human pathogen, causes a wide variety of invasive systemic infections such as acute pharyngitis, skin and soft tissue infections, especially necrotizing fasciitis. The emergence of antibiotic resistance in this bacterium and treatment failure has become a global concern [52]. For this reason, interest in the use of plant extracts for the treatment of streptococcal pharyngitis has increased due to several therapeutic challenges associated with conventional antibiotics1. Medicinal plants may affect S. pyogenes in several ways: (1) inhibition of protein synthesis. Flavonoids and terpenoids can inhibit bacterial protein synthesis by binding to bacterial ribosomes. (2) Bacterial membrane damage: Phenols

and terpenoids can damage bacterial cell membranes, which leads to leakage of cell contents and bacterial death. (3) cell wall damage. (4) Inhibition of metabolism: flavonoids and terpenoids can inhibit the metabolism of bacteria by inhibiting important enzymes in the metabolism of bacteria. (5) inhibition of biofilm formation(36, 53). For example: methanolic extract of Red Galangal Rhizome (Alpinia purpurata (Vieill) K. Schum) is effective against *Streptococcus pyogenes* [53]. Another study showed that Boesenbergia pandurata, Eleutherine americana and Rhodomyrtus tomentosa have high antibacterial potential against this bacterium. An isolated compound named Rhodomyrtone from the leaves of Rhodomyrtus tomentosa showed strong antibacterial activity against many pathogenic Gram-positive bacteria including S. pyogenes [54]. In addition, thyme, oregano, sage, barberry, purple coneflower, and licorice are common medicinal plants used in traditional or folk remedies in Canada to treat streptococcal pharyngitis. Both licorice root extract and sage leaf extract were the most effective antibacterial agents, killing 99.99% of the initial bacterial load within 3 hours at 2x MIC [36]. Also, essential oils (Eos) of oregano and sage prevent the growth and formation of S. pyogenes biofilm [50]. Al-Mezori and his colleagues, by examining basil oil, eucalyptus oil and dill oil, showed that basil and eucalyptus oil have limited inhibition against Strep pyogenesis, while dill oil showed strong inhibitory effects against Strep pyogenesis (16 mm) [55]. Another study showed that thyme, cinnamon, lemongrass, tea tree, lavender, oregano, clove, palmarosa or cajeput EOs are active against S. pyogenes. Also, Cinnamomum verum EO containing cinnamaldehyde showed the highest activity. Other oils containing phenolic derivatives (eugenol and basil containing estragole) were less active. The presence of free phenol increases the antibacterial activity against Strep pyogenes. Also, the components of EOs can interfere with the electron transport chain of bacterial mitochondria and change energy production [48]. EOs contain a large number of phytochemicals, therefore, their mode of action involves multiple targets in S. pyogenes cells, rather than a single mechanism. Therefore, further studies are needed to identify multiple mechanisms of action, effective and safe dosage of EOs for clinical trials and active pharmaceutical compounds for further development as plant-derived drugs [50]. Recently, a diverse range of phytochemical antibacterial agents have been reported to suppress the growth of *Streptococcus pyogenes*, including polyphenols (ie, flavonoids) and terpenes. A recent review describes the mechanisms of inhibition of different Streptococcus species, including S. pyogenes, by phytochemicals through preventing pharyngeal bacterial adhesion, glycolytic enzyme inhibition and pH drop, biofilm reduction, and changing cell surface hydrophobicity [56]. In addition, it seems that the compounds with lower molecular mass (such as Carvacrol, Thymol, Caffeic acid, γ-terpinene, β-pinene and α-pinene) enter plants through the peptidoglycan layer and act on the cytoplasmic membrane of this bacterium. Also, exposure to these compounds causes morphological changes, such as changes in the structure of the cell surface, which may lead to the leakage of cytosolic fluids outside the cells [48]. The findings show that bacteria exposed to carvacrol derived from oregano and thyme EOs have shown morphological changes on the bacterial cell surface. Oregano EOs, which are rich in carvacrol, may lead to morphological changes in the length and diameter of S. pyogenes cocci, which may be due to the lipophilic properties of the EO and its compounds that target bacterial membranes. Although previous studies have attempted to explain the mode of action of EOs, the antibacterial mechanisms are still unclear [50]. It has been mentioned that carvacrol in combination with ciprofloxacin shows a synergistic effect. This alternative strategy can lead to a reduction in the dose of antibiotics and thus reduce the adverse effects of treatment [48]. Another study stated that Baicalein showed synergy with ceftazidime against *Streptococcus pyogenes*. Also, it inhibits penicillinase activity to enhance the antibacterial effects of penicillins in a dose-dependent manner. In addition, it can treat bacterial infection by inhibiting biofilm formation and protein synthesis, affecting bacterial membrane permeability, and inhibiting the activity of succinate dehydrogenase, malate dehydrogenase, and DNA topoisomerase I and II [38].

The results of a study showed that the alcoholic and aqueous extracts of Satureja bachtiarica have the greatest effect on gram-positive bacteria S. pyogenes, which may be due to the presence of thymol, carvacrol and their precursors. Also, Origanum vulgare and Salvia officinalis essential oils show antibacterial and anti-biofilm activities against *Streptococcus pyogenes*. Methanolic and ethyl acetate extracts of Plectranthus amboinicus showed a dose-dependent inhibition against the biofilm of this bacterium isolated from pharyngitis patients [57]. Another study by Minami and colleagues investigated the effectiveness of three parts (fruit, stem and leaf) of Lonicera caerulea var.emphyllocalyx extract (LCEEs) against S. pyogenes infection in mice. They found that oral administration of LCEE

increased mortality in a mouse model and its stem and leaf extracts were significantly more effective than fruit extracts. Also, mouse lymph node cells and mesenteric lymph node cells treated with LCEE suppressed the excessive production of inflammatory cytokines such as TNF-α compared to untreated cells [58]. Verbascum thapsus methanolic leaf extract showed significant inhibitory activity against S. pyogenes (inhibition zone 22.9 ± 0.64 mm), while the inhibition zone of Ciproflaxin/Flucanozole (21.6 ± 0.58) was less. This plant is a promising new way to discover antibacterial drugs, especially in the field of the development of drug-resistant microbial strains, as it provides encouraging clues for future research [59]. Therefore, plants may produce biologically active compounds that may be valuable in the treatment of diseases caused by S. pyogenes. However, their active components should be isolated and their toxicity and therapeutic activity evaluated in vivo [54].

Table 1. Medicinal Plants on *Streptococcus pyogenes*

plant	family	using part	Extraction	Inhibition zone	MIC (µg/ml)	MBC	MBIC	Reference
sage	Lamiaceae	leaf	ethanol extracts		62.5	125	125 (2 × MIC)	[36]
purple coneflower	Asteraceae	flower	ethanol extracts		62.5	125	250 (4 × MIC)	[36]
Licorice	Fabaceae	root	ethanol extracts		62.5	125	250 (4 × MIC)	[36]
oregano	Lamiaceae	Flowering shoots	essential oils		0.5	0.5	0.5	[50]
sage	Lamiaceae	leaves	essential oils		0.5	0.5	0.5	[50]
Boesenbergia pandurata	Zingiberaceae	rhizome	Rhizome extract	7mm	3.91-31.25	7.81-62.50		[54]
Eleutherine americana	Iridaceae	bulb	Bulb extract	23mm	250	250-500		[54]
Rhodomyrtus tomentosa	Myrtaceae	flower	Flower extract	17 mm	3.91-31.25	3.91-62.50		[54]
Cyclea peltata	Menispermaceae	tuber	Methanolic extract	12mm	0.5 and 0.75)			[60]
Euphorbia hirta	Euphorbiaceae	Entire plant	Methanolic extract	13	0.75 شک) (دارم			[60]
Quercus infectoria	Fagaceae	nut gal	ethanol extract	26	500	1000		[61]
Piper betle	Piperaceae	leaves	ethanol extract	23	500	500		[61]
Rhodomyrtus tomentosa	Myrtaceae	Leaf/stem	ethanol extract		7.8/1000	62.5/>1000		[61]
Verbascum thapsus	Scrophulariaceae	leaf	Methanol Ethyl acetate	22.9 ± 0.64 18.6 ± 0.12	500 125	62.50 31.25		[59]
Lantana camara	Verbenaceae	Leaf,flower	methanol ethyl acetate		2 ≤ 0.125			[62]
Cistus parviflorus	Cistaceae	Aerial Parts	methanolic	22.4 ± 0.58(mean ± S.D)	0.98			[63]
thyme	Lamiaceae	different types	essential oils	31mm	1:4 dl/mL	>1:64 dl/mL		[64]

Conclusion

The use of medicinal plants in treating bacterial infections has become increasingly crucial due to the growing problem of antimicrobial resistance. As bacteria develop resistance to conventional antibiotics, the need for alternative treatment options has become more pressing.

Medicinal plants have been used for centuries to treat various ailments, including bacterial infections. These plants contain a wide range of bioactive compounds, such as alkaloids, terpenoids, and phenolic compounds, which possess antimicrobial properties. The use of herbal medicine has gained popularity as a complementary or alternative treatment option for bacterial infections, particularly in regions where access to conventional antibiotics is limited.

In the present study, a collection of effective medicinal plants against *Streptococcus pyogenes* was collected and the effect of each was mentioned.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by none mentioned.

Funding

Not applicable.

References

1. 1. Avire NJ, Whiley H, Ross K. A Review of Streptococcus pyogenes: Public Health Risk Factors, Prevention and Control. Pathogens. 2021;10(2):248.
2. 2. Walker MJ, Barnett TC, McArthur JD, Cole JN, Gillen CM, Henningham A, et al. Disease manifestations and pathogenic mechanisms of group A Streptococcus. Clinical microbiology reviews. 2014;27(2):264-301.
3. 3. Brouwer S, Barnett TC, Rivera-Hernandez T, Rohde M, Walker MJ. Streptococcus pyogenes adhesion and colonization. FEBS letters. 2016;590(21):3739-57.
4. 4. Efstratiou A, Lamagni T. Epidemiology of Streptococcus pyogenes. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet] 2nd edition. 2022.
5. 5. Ralph AP, Carapetis JR. Group a streptococcal diseases and their global burden. Host-pathogen interactions in streptococcal diseases. 2012:1-27.
6. 6. Ibrahim J, Eisen JA, Jospin G, Coil DA, Khazen G, Tokajian S. Genome analysis of Streptococcus pyogenes associated with pharyngitis and skin infections. PLoS One. 2016;11(12):e0168177.
7. 7. Spellerberg B, Brandt C. Laboratory diagnosis of Streptococcus pyogenes (group A streptococci). 2022.
8. 8. Langlois DM, Andreae M. Group A streptococcal infections. Pediatrics in Review. 2011;32(10):423-30.
9. 9. Luca-Harari B, Darenberg J, Neal S, Siljander T, Strakova L, Tanna A, et al. Clinical and microbiological characteristics of severe Streptococcus pyogenes disease in Europe. Journal of clinical microbiology. 2009;47(4):1155-65.
10. 10. LaRock DL, Johnson AF, Wilde S, Sands JS, Monteiro MP, LaRock CN. Group A Streptococcus induces GSDMA-dependent pyroptosis in keratinocytes. Nature. 2022;605(7910):527-31.
11. 11. Ferretti JJ, Stevens DL, Fischetti VA. Streptococcus pyogenes: basic biology to clinical manifestations [Internet]. 2016.
12. 12. You Y, Davies MR, Protani M, McIntyre L, Walker MJ, Zhang J. Scarlet fever epidemic in China caused by Streptococcus pyogenes serotype M12: epidemiologic and molecular analysis. EBioMedicine. 2018;28:128-35.
13. 13. Lynskey NN, Jauneikaite E, Li HK, Zhi X, Turner CE, Mosavie M, et al. Emergence of dominant toxigenic M1T1 Streptococcus pyogenes clone during increased scarlet fever activity in England: a population-based molecular epidemiological study. The Lancet Infectious Diseases. 2019;19(11):1209-18.
14. 14. Stevens DL. Invasive group A streptococcus infections. Clinical Infectious Diseases. 1992;14(1):2-13.
15. 15. Martin JM, Green M, Barbadora KA, Wald ER. Erythromycin-resistant group A streptococci in

schoolchildren in Pittsburgh. New England Journal of Medicine. 2002;346(16):1200-6.
16. 16. Quinn A, Ward K, Fischetti VA, Hemric M, Cunningham MW. Immunological relationship between the class I epitope of streptococcal M protein and myosin. Infection and immunity. 1998;66(9):4418-24.
17. 17. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. The Lancet infectious diseases. 2005;5(11):685-94.
18. 18. Veasy LG, Wiedmeier SE, Orsmond GS, Ruttenberg HD, Boucek MM, Roth SJ, et al. Resurgence of acute rheumatic fever in the intermountain area of the United States. New England journal of medicine. 1987;316(8):421-7.
19. 19. Olafsdottir L, Erlendsdóttir H, Melo-Cristino J, Weinberger D, Ramirez M, Kristinsson K, et al. Invasive infections due to Streptococcus pyogenes: seasonal variation of severity and clinical characteristics, Iceland, 1975 to 2012. Eurosurveillance. 2014;19(17):20784.
20. 20. Choby BA. Diagnosis and treatment of streptococcal pharyngitis. American family physician. 2009;79(5):383-90.
21. 21. Kim S. Optimal diagnosis and treatment of group A streptococcal pharyngitis. Infection & Chemotherapy. 2015;47(3):202.
22. 22. Al-Hamad AM. Streptococcal throat: Therapeutic options and macrolide resistance. Saudi Medical Journal. 2015;36(9):1128.
23. 23. Ikebe T, Hirasawa K, Suzuki R, Isobe J, Tanaka D, Katsukawa C, et al. Antimicrobial susceptibility survey of Streptococcus pyogenes isolated in Japan from patients with severe invasive group A streptococcal infections. Antimicrobial agents and chemotherapy. 2005;49(2):788-90.
24. 24. Rahmawati N, Syukri M, Darmawi D, Zachreini I, Zulfiani U, Yusuf M, et al. Haematological Features of White Rats (Rattus norvegicus) Infected with S. pyogenes and Administered with Probiotics (Yogurt). The Scientific World Journal. 2022;2022(1):2899462.
25. 25. Wilcox C, Stuart B, Leaver H, Lown M, Willcox M, Moore M, et al. Effectiveness of the probiotic Streptococcus salivarius K12 for the treatment and/or prevention of sore throat: a systematic review. Clinical Microbiology and Infection. 2019;25(6):673-80.
26. 26. Di Pierro F, Adami T, Rapacioli G, Giardini N, Streitberger C. Clinical evaluation of the oral probiotic Streptococcus salivarius K12 in the prevention of recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes in adults. Expert opinion on biological therapy. 2013;13(3):339-43.
27. 27. Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS. Use of Streptococcus salivarius K12 in the prevention of streptococcal and viral pharyngotonsillitis in children. Drug, healthcare and patient safety. 2014:15-20.
28. 28. Tagg JR, Harold LK, Jain R, Hale JD. Beneficial modulation of human health in the oral cavity and beyond using bacteriocin-like inhibitory substance-producing streptococcal probiotics. Frontiers in Microbiology. 2023;14:1161155.
29. 29. Reynolds S, Pandey M, Dooley J, Calcutt A, Batzloff M, Ozberk V, et al. Preclinical safety and immunogenicity of Streptococcus pyogenes (Strep A) peptide vaccines. Scientific Reports. 2021;11(1):127.
30. 30. Johnson AF, LaRock CN. Antibiotic treatment, mechanisms for failure, and adjunctive therapies for infections by group A Streptococcus. Frontiers in microbiology. 2021;12:760255.
31. 31. Jin S-E, Jin H-E. Antimicrobial activity of zinc oxide nano/microparticles and their combinations against pathogenic microorganisms for biomedical applications: From physicochemical characteristics to pharmacological aspects. nanomaterials. 2021;11(2):263.
32. 32. Gilmer DB, Schmitz JE, Euler CW, Fischetti VA. Novel bacteriophage lysin with broad lytic activity protects against mixed infection by Streptococcus pyogenes and methicillin-resistant Staphylococcus aureus. Antimicrobial agents and chemotherapy. 2013;57(6):2743-50.
33. 33. Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian pacific journal of cancer prevention. 2003;4(4):281-8.
34. 34. Baradaran M, Jalali A. A Review on Antibacterial Effects of Iranian Herbal Medicine on Methicillin-Resistant Staphylococcus aureus. Jundishapur J Chronic Dis Care. 2019;8(4):e96058.
35. 35. Abedi R, Moradkhani S, Afsharmanesh G, Rangchian M. General physicians' attitude and decision-making toward herbal medicines prescription: a scenario-based cross-sectional study in Hamadan, Iran. Journal of Herbal Medicine. 2024.
36. 36. Wijesundara NM, Rupasinghe HPV. Bactericidal and Anti-Biofilm Activity of Ethanol Extracts Derived from Selected Medicinal Plants against Streptococcus pyogenes. Molecules. 2019;24.
37. 37. Khahan-Yazdi I, Shabani M, Tajvidi-Monfared M, Vahidi Emami H, Mojab F, Shams S, et al. Effectiveness of medicinal plant essential oils on drug-resistant bacteria in Iran: A systematic review. Journal of Medicinal Plants. 2023.
38. 38. Liang J, Huang X, Ma G. Antimicrobial activities and mechanisms of extract and components of herbs in East Asia. RSC Advances. 2022;12:29197 - 213.
39. 39. Zhao S, Geng Y, Shi J, Qian J, Yang Y, Dai D, et al. Chinese herbal compound for multidrug-resistant or extensively drug-resistant bacterial pneumonia: a meta-

analysis and trial sequential analysis with association rule mining to identify core herb combinations. Frontiers in Pharmacology. 2023;14:1282538.
40. 40. Keikhaie KR, Fazeli-Nasab B, Jahantigh HR, Hassanshahian M. Antibacterial activity of ethyl acetate and methanol extracts of Securigera securidaca, Withania sominefra, Rosmarinus officinalis and Aloe vera plants against important human pathogens. Journal of Medical Bacteriology. 2018;7(1-2):13-21.
41. 41. Siriwong S, Thumanu K, Hengpratom T, Eumkeb G. Synergy and mode of action of ceftazidime plus quercetin or luteolin on Streptococcus pyogenes. Evidence-Based Complementary and Alternative Medicine. 2015;2015(1):759459.
42. 42. Hamidi M, Toosi AM, Javadi B, Asili J, Soheili V, Shakeri A. In vitro antimicrobial and antibiofilm screening of eighteen Iranian medicinal plants. BMC Complementary Medicine and Therapies. 2024;24(1):135.
43. 43. Chaughule RS, Barve RS. Role of herbal medicines in the treatment of infectious diseases. Vegetos. 2024;37(1):41-51.
44. 44. Atapour M, Zahedi MJ, Mehrabani M, Safavi M, Keyvanfard V, Foroughi A, et al. In vitro susceptibility of the Gram-negative bacterium Helicobacter pylori to extracts of Iranian medicinal plants. Pharmaceutical Biology. 2009;47(1):77-80.
45. 45. Sharma AN, Dewangan HK, Upadhyay PK. Comprehensive Review on Herbal Medicine: Emphasis on Current Therapy and Role of Phytoconstituents for Cancer Treatment. Chemistry & Biodiversity. 2024:e202301468.
46. 46. Rukundo A, Omara D, Majalija S, Odur S, Alafi S, Okech SG. Antibacterial activity of ethanolic and aqueous extracts of Zingiber officinale on Streptococcus pneumoniae and Pseudomonas aeruginosa. bioRxiv. 2023:2023.01. 03.522596.
47. 47. Varghese RM, Kumar SA, Rajeshkumar S. Antibacterial activity of herbal formulation against common oral pathogens. Bioinformation. 2023;19(5):663.
48. 48. Sfeir J, Lefrançois C, Baudoux D, Derbré S, Licznar P. In vitro antibacterial activity of essential oils against Streptococcus pyogenes. Evidence-Based Complementary and Alternative Medicine. 2013;2013(1):269161.
49. 49. Heidari-Sureshjani M, Tabatabaei-Yazdi F, Alizadeh-Behbahani B, Mortazavi A. Antimicrobial effect of aqueous, ethanol, methanol and glycerin extracts of Satureja bachtiarica on Streptococcus pyogenes, Pseudomonas aeruginosa and Staphylococcus epidermidis. Zahedan journal of research in medical sciences. 2015;17(7).
50. 50. Wijesundara NM, Rupasinghe HV. Essential oils from Origanum vulgare and Salvia officinalis exhibit antibacterial and anti-biofilm activities against Streptococcus pyogenes. Microbial pathogenesis. 2018;117:118-27.
51. 51. Han X, Jin L, Zhao Z, Xu X, Liu S, Huang Y, et al. Combining the In Silico and In Vitro Assays to Identify Strobilanthes cusia Kuntze Bioactives against Penicillin-Resistant Streptococcus pneumoniae. Pharmaceuticals. 2023;16(1):105.
52. 52. Adil M, Khan R, Rupasinghe HV. Application of medicinal plants as a source for therapeutic agents against Streptococcus pyogenes infections. Current Drug Metabolism. 2018;19(8):695-703.
53. 53. Putri DPU, Dewi LM, Bestari RS. Antibacterial Effectiveness Test of Methanol Extract of Red Galangal Rhizome (Alpinia purpurata (Vieill) K. Schum) Against Streptococcus pyogenes and Klebsiella pneumonia Bacteria. Eureka Herba Indonesia. 2024;5(2):436-40.
54. 54. Limsuwan S, Voravuthikunchai SP. Anti-Streptococcus pyogenes activity of selected medicinal plant extracts used in Thai Traditional Medicine. Tropical Journal of Pharmaceutical Research. 2013;12(4):535-40.
55. 55. Al-Mezori HOQ, Yasin MR, Ali WN, Khedir AM. Comparative Study Of Synthetic Antibiotic Amoxicillin With Volatile Oil Extracted From Some Traditional Medicinal Plants On Bacteria Human Pathogen (Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli). Journal of Pharmaceutical Negative Results. 2023:859-67.
56. 56. Macé S, Hansen LT, Rupasinghe HV. Anti-bacterial activity of phenolic compounds against Streptococcus pyogenes. Medicines. 2017;4(2):25.
57. 57. Manimekalai K, Srinivasan P, Dineshbabu J, Guna G, Teepica Priya Darsini D. Anti-biofilm efficacy of Plectranthus amboinicus against Streptococcus pyogenes isolated from pharyngitis patients. Asian J Pharm Clin Res. 2016;9(4):348-54.
58. 58. Minami M, Nakamura M, Makino T. Effect of Lonicera caerulea var. emphyllocalyx extracts on murine Streptococcus pyogenes infection by modulating immune system. BioMed Research International. 2019;2019(1):1797930.
59. 59. Lone AS, Ravindran K, Jeandet P. Evaluation of Antimicrobial activity and Bioactive compound analysis of Verbascum thapsus L. A folklore medicinal plant. Phytomedicine Plus. 2024:100560.
60. 60. Delfani S, Mohammadrezaei-Khorramabadi R, Abbaszadeh S, Naghdi N, Shahsavari S. Phytotherapy for Streptococcus pyogenes. Journal of Pharmaceutical Sciences and Research. 2017;9(5):513.
61. 61. Limsuwan S, Subhadhirasakul S, Voravuthikunchai SP. Medicinal plants with significant activity against important pathogenic bacteria. Pharmaceutical biology. 2009;47(8):683-9.

62. 62. Agbo IA, Hlangothi B, Didloff J, Hattingh A, Venables L, Govender S, et al. Analysis of the correlation between phytochemical content and wound-healing potential of Lantana camara ethyl acetate and methanol extracts. Journal of Medicinal Herbs. 2023;14(2).
63. 63. El-Shibani FA, Sulaiman GM, Abouzied AS, Al Ali A, Abdulkarim AK, Alamami AD, et al. Polyphenol fingerprint, biological activities, and in silico studies of the medicinal plant Cistus parviflorus L. extract. ACS omega. 2023;8(50):48269-79.
64. 64. Maqbul MS, Bokhari YA, Basalib SG, Alhelali SN, Omar BMM, Khan AA, et al. A Comparative Study of Different Types of Thyme Essential Oils Against Streptococcus Pyogenes to Determine their Biochemical and Antimicrobial Properties. Oriental Journal of Chemistry. 2020;36(2):220.