



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 376-380
Received: 03-07-2019
Accepted: 06-08-2019

Yelmate AA
School of Pharmacy, SRTM,
University, Nanded,
Maharashtra, India

Dr. Thonte SS
Channabashweshvar College of
Pharmacy, Latur, Maharashtra,
India

Antibacterial activity of some Indian medicinal plants against methicillin resistant *Staphylococcus aureus* (MRSA)

Yelmate AA and Dr. Thonte SS

Abstract

Staphylococcus aureus (*S. aureus*), a bacterium that causes a variety of skin infection as a result of skin colonization, is becoming increasingly resistant to many commonly used antibiotics. There are a few types of *S. aureus* infections including folliculitis, furuncles, carbuncles, mastitis, impetigo, and cellulitis. Herbs traditionally used for the treatment of inflammation and infection, in extract form have been shown to exhibited anti-bacterial activity against various bacteria. This study aims to examine the anti-bacterial properties of *selected plants* towards *S. aureus*. Plant extraction using ethanol and water was performed. Different concentrations of aqueous and ethanolic extract of all the plants were used in the disc diffusion method for determination of antibacterial activity towards *S. aureus*. It is concluded that all the plant extract has potential to treat skin infections caused by *S. aureus*. Future research is required to confirm the effectiveness of these plants and develop a suitable formulation.

Keywords: Skin infection, anti-bacteria, *Staphylococcus aureus*, Cellulitis

Introduction

Impetigo is a very contagious bacterial infection of the superficial layer of the skin. The bacteria usually infect skin that has been damaged by scratching an insect bite or picking a scab. The lesions may cause soreness and itching, but are typically painless. Fever or any other symptoms of systemic illness are not seen with the cases impetigo.

The condition can be classified as:

- » Primary – there is a direct bacterial invasion of normal skin that has minor breaks;
- » Secondary – the infection is secondary to an underlying skin disease such as eczema or scabies or a result of trauma from burns, bites or lacerations [1]The two main causative organism that cause impetigo are group A, beta-hemolytic streptococci and *Staphylococcus aureus*.

Impetigo is classified as:

Bullous Impetigo

Non-Bullous Impetigo, based on the presence or lack of large blisters, called bullae.

Bullous Impetigo

Bullous impetigo is characterized by bullae, large thin-walled blisters that contain clear or cloudy yellow fluid and measure up to 5 cm in diameter. Bullae are caused by staphylococcal infections, not by streptococci. A certain type of staphylococcal bacteria produces a toxin that causes the large blisters to form. These blisters easily rupture and leave behind a moist area of eroded skin surrounded by a thin ring of the remaining blistered skin. This lesion dries and crusts over, creating a light brown appearance that resembles “varnish”. The lesions are discrete, with little redness or inflammation surrounding them. These large blisters typically occur on the face but may quickly spread to different areas of the skin. A mix of bullous and non-bullous skin lesions may occur.

Non-Bullous Impetigo

This is the more common type of impetigo and is characterized by reddened sores with honey-yellow crusting on them. The sores may initially appear as small blisters that rupture, ooze, and lead to the layer of crusting. The crust typically appears to be “stuck on”. The infection does not disappear easily with topical cleaning. The lesions are painless, often occur around the mouth and nose or on the arms and legs, and resolve without scarring.

These non-bullous lesions are caused by either Streptococcus or Staphylococcus, and in some cases both types of bacteria may be present [11-12].

Correspondence

Yelmate AA
School of Pharmacy, SRTM,
University, Nanded,
Maharashtra, India

Cause

Staphylococcus aureus is the main bacteria that causes non-bullous impetigo; *Streptococcus*

Pyogenes (group a beta-haemolytic streptococcus) causes a smaller number cases, either alone or in combination with *S aureus*. Bullous impetigo is always caused by *S aureus* [1]

Causes: Impetigo is a common cutaneous infection that is especially prevalent in children. Historically, impetigo is caused by either group A β -hemolytic *streptococci* or *Staphylococcus aureus*. Currently, the most frequently isolated pathogen is *S. aureus* [3]

Treatment, Evolution of Bacterial Resistance

S. aureus readily acquires antimicrobial resistance, making its treatment difficult.

For over 60 years, virtually all strains of *S. aureus* are able to produce beta-lactamase (Penicillin's), becoming resistant to beta-lactamase sensitive antibiotics.

These enzymes hydrolyze the beta lactam ring, and they are, so far, the main mechanism of resistance to beta lactam antibiotics. 13Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected in 1961. Cases of infections caused by MRSA in the community were reported in the 80's, but the importance of this group has increased significantly in recent years. MRSA infections are no longer confined to hospital settings, but rates of Community-associated MRSA (CA-MRSA) vary widely among studies.

The presence of MRSA as impetigo's causative agent in non-hospitalized patients is considered

Unusual and with heterogeneous distribution. Staphylococcal impetigo is usually caused by *S. aureus* strains that possess the Exfoliative toxin gene. On the other hand, community MRSA clones (CA-MRSA) do not have the Exfoliative toxin gene, but the Panton-Valentine-Leucodin (PVL) gene. Staphylococci that possess PVL gene cause suppurative cutaneous infections such as abscesses and furuncles. Therefore, concern about MRSA in community-acquired infections, should be greater in the presence of furuncles and abscesses and smaller in impetigo [4]

Cellulitis

Cellulitis is a diffuse, deep, acute inflammation of the skin including the dermis and subcutaneous tissue. It often follows an acute or chronic trauma, and is an important cause of hospital admissions. About 10% of infections-related hospital admissions in the US annually are due to cellulitis. Cellulitis occurs when an infectious organisms invades the dermis of the skin (usually through a break). This disruption of the skin can result from several causes including fungal infections (like onychomycosis, and tinea pedis), venous leg ulcers, pressure ulcers, and web spaces. The natural presence of low temperature, low pH and skin flora play an important role in reducing pathogenic colonization on the skin surface.

Under the microscope, cellulitis is characterized by lymphatic dilation, dermal edema, along with diffuse, heavy neutrophil infiltration around blood vessels. Histiocytes, lymphocytes, and granulation tissue may be observed in late cases.

Causes

In adults with intact immune system, the most common cause of cellulitis is group A streptococci (*Streptococcus pyogenes*). Another important but less common organism is *Staphylococcus aureus*.

However, the exact incidence and prevalence of each causative organisms is still an area of debate, due to the

challenging nature of accurate diagnosis, and the fact that most cellulitis cases are treated without determining the causative agent. Therefore, most cases of cellulitis are empirically treated [5]

Streptococci and *S. aureus* are the most common pathogens identified in patients with cellulitis.

Cellulitis is an inflammatory condition of sub-cutaneous connective tissue under the skin. Bacteria, most commonly Streptococci and Staphylococci, when they get beneath the skin tissue through possible cuts or bruises, play a big role in the pathogenesis of this condition [6]

Pathophysiology of Cellulite

Cellulite occurrence seems to be attributable to structural, inflammatory, morphologic, and biochemical alterations in the subcutaneous tissue. There is evidence that hormones influence the formation of cellulite. Estrogen stimulates lipogenesis and inhibits lipolysis, resulting in adipocyte hypertrophy. This mechanism may partially explain the greater prevalence of this condition in women. Other evidence of the hormonal influence on cellulite is its presence in most women, its usual onset at puberty, and its exacerbation during pregnancy, nursing, menstruation and its connection with oral contraceptive use. There is evidence that deficiencies in lymphatic drainage and microvascular circulation are associated with cellulite development. In areas in which circulation and lymphatic drainage are decreased, such as the buttocks and thighs, they are more predisposed to the increase of micro edema within the subcutaneous fat layers, leading to accentuation of skin irregularities. Post inflammatory alterations, genetics, weight gain, and lifestyle may also be contributing factors to the development of cellulite [7]

Medicinal plants have a very promising future because there are about millions of plants are around the world, and most of them having important medical activities and have not investigate yet, and their medical activities could play a valuable role in the treatment of diseases in future studies [8]. There is no life without plants. Plants are the essential source for foundation of medicine. Numbers of important drugs that are still in use today are derived from traditional medicinal herbs [9]. Human beings totally depends on the plants for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, Flavours, fertilizers. Plants, especially used in Ayurveda can provide biologically active chemical constituents which plays an important role in the the treatment of diseases with enhanced activity and /or reduced toxicity [9]

Vitex negundo Linn.

Vitex negundo Linn. (Verbenaceae) is a woody, aromatic shrub growing to a small tree. It commonly bears tri- or penta-foliolate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar. It is grown commercially as a crop in parts of Asia, Europe, North America and the West Indies [10]

The plant has pungent, bitter, acrid taste; heating, astringent, stomachic, anthelmintic; promotes the growth of hair; useful in disease of the eye, consumption, inflammation, leucoderma, enlargement of the spleen, bronchitis, asthma, biliousness, painful teething of children. The root is an antidote to snake venom. The root is considered tonic,

febrifuge and expectorant, otalgia, arthritis, dyspepsia, colic, rheumatism, leprosy, verminosis, flatulence, dysentery, urinary disorders, wounds, ulcers, bronchitis, cough, malarial fever, haemorrhoids, Dysmenorrhoea, leprosy, skin diseases and general debility. The plant is reported to have expectorant, carminative, digestive, anodyne, antiseptic, alterant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and tonic ^[11]

Trigonella foenum - graecum (Linn.)

Trigonella foenum - graecum (Linn.) belonging to the family Papilionaceae commonly known as Fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated throughout the country. A nearly smooth erect annual. Stipulets not toothed. Leaflets 2-2.5 cm long, oblanceolate – oblong, toothed. Flowers 1-2, axillary, sessile. Calyx-teeth linear. Corolla much exerted. Pod 5-7.5 cm long, with a long persistent beak, often falcate, 10-29 seeded, without transverse reticulations. Recently the WHO (World Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspects. Many developing countries all over the world have intensified their efforts in documenting the ethnomedicinal data and scientific research on medicinal plants. It is estimated that there are 250000 to 500000 species of plants on earth ^[12-13].

Azadirachta indica

All parts of the tree have been used medicinally for centuries. It has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties. The earliest Sanskrit medical writings refer to the benefits of Need's fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani medicine, and is now being used in pharmaceutical and cosmetics industries.

Medicinal uses

Abortifacient, analgesic, anthelmintic, antibacterial, ant yeast, antiulcer, antifertility, ant filarial, antifungal,

antihyperglycemic, anti-inflammatory, antiviral, antimalarial, diuretic, antinematodal, antipyretic, antispasmodic, insecticidal, antispermatogetic, antitumor, hypercholesteraemic, Hypoglycaemic, immunomodulator ^[3].

Materials and Methods

The Study was conducted at the department of pharmaceutics, Dayanand College of pharmacy, Latur. Maharashtra, India.

Collection of sample

The seeds of *Trigonella foenum graecum linn*, *Azadirachta indica*, *Vitex negundo Linn*. Leaves sample were collected from the local market of Latur, Maharashtra India. For authentication of plant, herbarium was prepared and sent to Dayanand science college, Latur.

Preparation of extract of plant material

The ethanol and aqueous extract are prepared of *Trigonella foenum graecum linn*, seed, *Azadirachta indica*, *Vitex negundo Linn*. leaves sample for which, weighed 15 gm of seeds of *Trigonella foenum graecum linn* are powdered, add 250 ml of water and ethanol separately to it, kept the mixture for 7 days and filtered it with muslin cloth, filtrate was allowed for hot extraction process on water bath at 40°C. ^[14]

The extract so obtain was used for qualitative analysis for the presence of Saponins, Flavonoids, Steroids, Terpenoids, Tannins, Alkaloids, Phenols, and Glycosides. ^[15]

Azadirachta indica, *Vitex negundo Linn*. leaves sample were cleaned to remove earthy matter and dried under shade. The powdered material was dried in an oven below 50°C. Dried plant material should be coarsely powdered and subjected for soxhlet extraction by using ethanol. Aqueous extract can be obtained by cold maceration process and the yield was calculated. Alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins and amino acids were qualitatively examined.

Table1: Preliminary phytochemical screening of the ethanol and aqueous extracts of *trigonella foenum graecum*, *Azadirachta indica*, *Vitex negundo Linn*.

Test	Fenugreek ethanol Extract	Fenugreek Aqueous extract	Neem ethanol Extract	Neem Aqueous Extract	Vitex nigundo ethanol Extract	Vitex nigundo Aqueous Extract
Carbohydrate	+	+	+	+	+	+
Protein/amino acid	+	+	+	+	+	+
Fats/ waxes	-	-	+	+	+	+
Glycoside	+	+	+	+	+	-
Flavonoides	-	-	+	+	-	+
Alkaloids	-	-	+	+	+	+
Terpenes	+	+	+	+	-	-
Steroids	+	+	+	+	+	-
Saponins	+	-	+	+	+	+
Phenolics /Tannins	-	-	+	+	+	+
Volatile oil	+	+	+	+	+	+

+ (presence) - (Absence)

Organism used

Microorganisms used for the study were obtained from the National collection of industrial microorganism NCIM (ATCC NO 2079) Pune Maharashtra, India. The test organisms included *staphylococcus aureus* which were used in the present study. The bacteria were grown in nutrient broth (Himedia, M002) at 37±1oC and maintained on nutrient agar at 40°C.

Antibacterial activity of plants extract

The antiacne activity of all plants was determined against *staphylococcus aureus* at three different concentrations of each extract by the agar well diffusion method. Well diffusion method was measured the zone of inhibition to know the antimicrobial activity of fenugreek. All procedures involved in this preparation were done under strict aseptic conditions to avoid contamination of the extracts. All glassware including

beaker, volumetric flask, dropper, measuring cylinder, pipette, conical flask, laboratory glass bottles were autoclaved at 121°C for 20 minutes prior to use.

Preparation of different concentration of extracts

Three different concentrations of aqueous and ethanolic extracts of all the plants 100mg/ml, 200mg/ml, 300mg/ml were prepared by using DMSO.

Preparation of culture media

Dehydrated media and standard antimicrobial drugs (discs) were purchased from Hi-Media Laboratories Ltd, India. All the media were prepared in sterile glass petriplates (4 mm thickness) according to the manufacturer's instructions.

Screening of antibacterial activity of the Plant extracts

Antibacterial test was performed by agar well diffusion method. Agar surface of each plate was streaked by using sterile cotton swab with the reference bacterial strain. Agar plate was punched with a sterile cork borer of 4 mm size and 100 µL of each extract taken in the concentration of 100 mg/mL, 200 mg/mL, 300 mg/mL. The plates were allowed to stand for 30 min. Then the plates were incubated at 37°C for 48 hrs under aerobic conditions. Only DMSO served as control [16]. The zone of inhibition formed around the wells was measured. Plates were prepared in triplets and the mean diameter of the zone of inhibition was noted. [17-18]

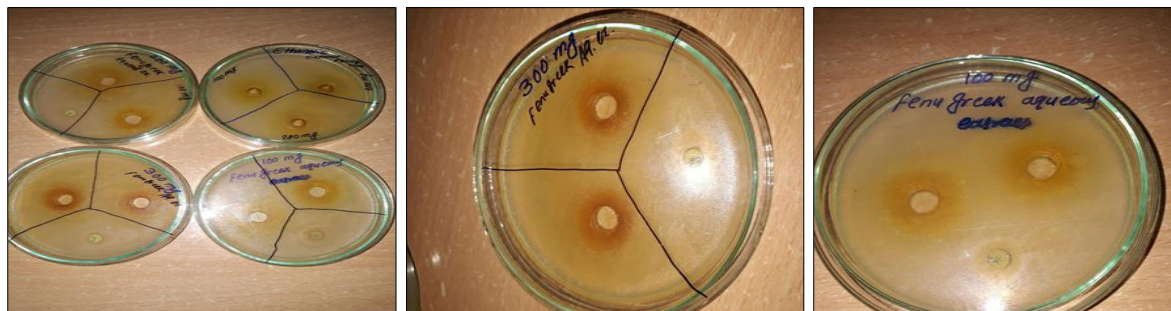


Fig 1: Antibacterial activity of plant extract.

Result and Discussion

The plant materials were extracted by using soxhlet apparatus the phytochemical screening of obtained extract was performed to know the presence of various secondary metabolites and is tabulated as table 1.

The antibacterial susceptibility was measured by using agar well diffusion method. Single strains were tested against *Staphylococcus aureus* ATCC NO 2079. *Azadirachta indica* ethanolic extract showed the zone of inhibition of 10.05 ± 0.25 , 13.3 ± 1.1 , 17.8 ± 0.9 and at a concentrations of 100 mg/mL, 200 mg/mL, 300 mg/mL. The *Azadirachta indica* aqueous

extract showed the zone of inhibition of 15.75 ± 1.15 , 18.65 ± 0.75 , 31.15 ± 4.15 at a concentration of 100 mg/mL, 200 mg/mL, 300 mg/mL. Tetracycline disc (30 µg/mL) was used as positive control and the zone of inhibition shown by standard is represented in figure 2.

Similarly the zone of inhibition of *Trigonella foenum graecum* and *Vitex nigundo* extract showed zone of inhibition, which is represented as figure 2. Extracts were dissolved in (DMSO) dimethyl sulphoxide. 100µL of each sample of various concentrations were loaded.

Antimicrobial activity of Aqueous and Ethanol Extracts of plants

Plant extract	Staphylococcus aureus		
	100mg/ml	200mg/ml	300mg/ml
<i>Azadirachta indica</i> , (leaves) ethanol extract.	42.5±0.03	40.0±0.03	42.5±0.04
<i>Azadirachta indica</i> , (leaves) aqueous extract.	17.5±0.09	25.1±0.08	32.5±0.06
<i>Trigonella foenum graecum</i> linn, (seed) ethanol extract.	20.5±0.04	26.0±0.03	31.3±0.06
<i>Trigonella foenum graecum</i> linn, (seed) aqueous extract.	28.0±0.01	32.0±0.03	30.0±0.07
<i>Vitex nigundo</i> , (leaves) ethanol Extract	27.5±0.08	32.5±0.06	42.5±0.09
<i>Vitex nigundo</i> , (leaves) Aqueous Extract	17.5±0.02	19.5±0.01	20.5±0.00
Standard Tetracycline disc	22.5±0.08	25.5±0.06	27.5±0.09

Conclusion

The plant extract was tested against Methicillin Resistant *Staphylococcus Aureus* (MRSA). DMSO was used as a control drug. Highest zone of inhibition was recorded against *S. aureus*, 42.5 ± 0.04 at the concentration of 300mg/ml by the ethanolic extract of *Azadirachta indica* and *Vitex nigundo*. Antibacterial activity of the plant is remarkable considering the importance of this organism in the treatment of bacterial skin infections as cellulitis and impetigo. The result of the present study showed the presence of wide spectrum of antibacterial activities against Methicillin Resistant *Staphylococcus Aureus* (MRSA). Therefore it can be used as antibacterial supplement and for the development of new therapeutic agent.

References

- Sandra Lawton, Impetigo: treatment and management, Nursing Practice Review Dermatology, Nursing Times www.nursingtimes.net. 2014; 110(11):18-20.
- Sarah Mc Sweeney-Ryan, MD, Megan Sandel, MD: The Health Care of Homeless Persons - Part I – Impetigo, 63-65.
- Luciana Baptista Pereira, Impetigo – review: An Bras Dermatol. 2014; 89(2):293-9.
- Sandra Lawton, Impetigo: treatment and management, Nursing Practice Review Dermatology, Nursing Times 2014, 110(11), 18-20. www.nursingtimes.net
- Dr. Amanda Oakley, Management of impetigo, BJP issue 19, 8-11.

6. Khalifa May' out Albuainain¹, Abduljabbar Abdullah Aljuwayd, Ahmed Abdullah Almarzouq, Zainab Ali A. Abu AlSaud, Nouf Abdullah Maziad Alkhattaf, Eman Ahmed Alabdrabalrasol¹, Nedaa Ahmed Zamzamy, Abbas Hassan Al-Lashit, Mahdi Hussain Al-Saad, Causes and Management of Cellulitis, The Egyptian Journal of Hospital Medicine. 2018; 70(12):2148-2151.
7. Dinesh Kumar Chellappan, Mayuren Candasamy, Cellulitis – Current Management Approach Through Complementary and Alternative Medicine, Advances in Pharmacoepidemiology & Drug Safety. 2016; 5(5):2167-1052.
8. Bassam Abdul Rasool Hassan, Medicinal Plants (Importance and Uses) Pharmaceutica Analytica Acta 2012, 3:10.
9. Muhammad Shahzad Aslam, Muhammad Syarhabil Ahmad, and Worldwide Importance of Medicinal Plants: Current and Historical Perspectives: Recent Advances in Biology and Medicine. 2016; 2:88-93.
10. Refaz Ahmad Dar, Mohd Shahnawaz, Parvaiz Hassan Qazi, General overview of medicinal plants: A review: The Journal of Phytopharmacology. 2017; 6(6):349-351.
11. Ladda PL, agdum CSM, Vitex Negundo Linn. Ethnobotany, Phytochemistry and Pharmacology- A Review, International Journal of Advances in Pharmacy, Biology and Chemistry, Ijapbc. 2012; 1(1):230-35.
12. Pradeep Singh, Garima Mishra, Sourabh Srivastava, Shruti Srivastava, Sangeeta KK, Jha RL. Khosa Phytopharmacological Review of *Vitex _egundo* (Sambhalu, *Pharmacologyonline*. 2:1355-1385.
13. Rashmi Yadav, Rahul Kaushik, Dipeeka Gupta, the Health Benefits of Trigonella Foenum-Graecum: A Review, International Journal of Engineering Research and Applications (Ijera). 1(1)032-035.
14. Ritika, Trigonella *Foenum-Graecum* L. A Review of Its Ethnobotany, Pharmacology and Phytochemistry, Risem 16.
15. Sharma V, Singh P, Rani A, Antimicrobial Activity of Trigonella foenum-graecum L. (Fenugreek): European journal of experimental biology. 2017;7(1):4.
16. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, tenth Ed. Nirali Prakashan, Pune, 2003, 149-158.
17. Meena Shamrao Deogade, Tarulata Pandya, Kethamakka Shivarama Prasad, Kunal Kale, Nilima Tankhiwale, Antimicrobial Activity of Vitex Negundo Linn. (Nirgundi) Leaves Extract: J. res. tradit. Medicine. 2016 2(4):99-102.
18. Dahlan WS. Yip, Pin KY, Formulation and Characterization of an Anti-Bacterial Cream Using Hemptedu Bumi (*Andrographis Paniculata*), International Journal of Pharmaceutical Sciences and Research. 2014; 5(9):3633-364.
19. Raja Ratna Reddy Y, Krishna Kumari C, Loganathan O, Mamatha S, Damodar Reddy C. Antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark and seed extracts, International Journal of Research in Phytochemistry & Pharmacology. 2013; 3(1):1-4.
20. Luciana Baptista Pereira, Impetigo – review: An Bras Dermatol. 2014; 89(2):293-9.