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Antimicrobial potential of Pakistani medicinal plants against multi-drug resistance *Staphylococcus aureus*

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PEER REVIEW

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Comments

The article content is valid for publication because it addresses the importance of alternatives to control multiresistant *S. aureus*. This theme has been recurring in the literature because it is a current problem in both the field of animal and human health. So, it can be considered for publication due to its theoretical value.

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ABSTRACT

Objective: To determine resistance patterns of *Staphylococcus aureus* (*S. aureus*) isolated from different areas of Pakistan and to identify antimicrobial agents against multi-drug resistant *S. aureus* strains.

Methods: A total of 67 samples (sewerage, nasal and milk) were collected from different farm areas of Pakistan to identify local strains of *S. aureus*. Sixteen out of 67 samples were positive for *S. aureus*. Only 6 out of 16 *S. aureus* strains showed resistance to antibiotics. Then the antibacterial effect of 29 medicinal plants was evaluated on these *S. aureus* isolates and a standard *S. aureus* strain ATCC 25923. The solvents used for the extraction of plants were acetone, dimethyl sulfoxide and methanol. The *in vitro* antibacterial activity was performed using agar disc diffusion method. Moreover, minimum inhibitory concentration of effective medicinal plant extracts was identified through micro-dilution method to find out their 50% inhibitory concentration.

Results: Plant extracts of 5 medicinal plants (*Psidium guajava*, *Nigella sativa*, *Piper nigrum*, *Valeriana jatamansi*, and *Cucurbita pepo*) exhibited antibacterial activity against locally isolated multidrug resistant strains of *S. aureus*. The minimum inhibitory concentration of these extracts was ranged from 0.328 to 5.000 mg/mL.

Conclusions: Plant extracts of *Psidium guajava*, *Piper nigrum* seed, *Valeriana jatamansi*, *Cucurbita pepo* and *Nigella sativa* showed significant *in vitro* antibacterial activity and thus, such findings may serve as valuable contribution in the treatment of infection and may contribute to the development of potential antimicrobial agents against multi drug resistant strains of *S. aureus*.

KEYWORDS

S. aureus, MRSA, Antimicrobial agents

1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive, non-motile, non-spore forming, facultative anaerobe, catalase and coagulase positive bacteria with diameter of 0.5-1.5 μm and belongs to Staphylococcaceae family[1]. *S. aureus* is considered to be a major pathogen of both humans and animals and is causative agent of contagious bovine mastitis, a serious disease of livestock resulting in both reduced milk production and quality. *S. aureus* is found naturally on the skin, mucous membranes and in the nasopharynx of the human body. Some members are normal micro-flora of skin and mucous membranes; while other causes skin pustules, impetigo, suppuration, abscesses formation, septicemia, pneumonia, osteomyelitis, renal abscess, endocarditis, meningitis, gastroenteritis and toxic shock syndrome. Pathogenic *S. aureus* produces variety of extracellular enzymes and heat stable enterotoxins which

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cause food poisoning[2].

Multi-drug resistance in *S. aureus* strains is a serious cause of concern in dairy animals and humans. The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are drug inactivation, alteration of target site, alteration of metabolic pathway and reduced drug accumulation[3]. Strains of *S. aureus* that are resistant to β-lactam antibiotics are known as multidrug resistant *S. aureus*. Penicillin resistant strains of *S. aureus* have been observed to be multi-drug resistant against aminoglycosides, macrolides, quinolones and tetracycline, which are often used in the treatment of mastitis[4]. Penicillin resistant strains of *S. aureus* typically produce an enzyme, called a β-lactamase, which inactivated the β-lactam and deactivate penicillin. It becomes difficult to successfully treat mastitis because drugs are not able to penetrate to all infection sites and because the bacteria can avoid contact with antibiotics while residing inside leukocytes[5].

The development of antibiotic resistance is a multifactorial. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for different viral and bacterial diseases[6-11]. The current study made an attempt to find out novel anti-bacterial agents against multidrug resistant *S. aureus* from medicinal plant extracts. For this purpose, twenty nine Pakistani medicinal plants were collected and screened for their antibacterial activity against multi-drug resistant *S. aureus*. Moreover, minimum inhibitory concentrations (MIC) of these plant extracts were identified through micro-dilution method to find out 50% inhibitory concentration of extract. Hopefully the resulting herbal extracts with antibacterial activity will be helpful for future to study these extracts in detail, and also to find out new therapeutic agents for better management of infections with multi-drug resistance *S. aureus*.

2. Materials and methods

2.1. Sample collection

Different samples (milk, sewerage, and nasal) were collected for isolation of *S. aureus*. A total of 66 samples were collected, out of which 11 buffalo nasal samples were collected from University of Veterinary and Animal Science (UVAS), Lahore, 25 sewerage samples were from different areas of Lahore, and 30 milk samples of cows and buffalo were collected from different farms of Punjab. Milk and sewerage samples were stored at −20 °C, while nasal swabs were kept in normal saline at 4 °C.

2.2. Plant collection and extraction

The plant materials from 29 plants were collected from different areas of Pakistan on the basis of un-documented reports (Table 1). The plants were identified by Pakistani plant taxonomist. The fresh plants were dried under shade at room temperature (20-25 °C) for 7 d. The dried plants were soaked in different solvents and extracts were filtered on the following morning. Extracts of each plant were concentrated at temperature between 30 to 40 °C and were evaporated to dry and then stored in refrigerator. About 20 mg of each dried plant extract was dissolved in 500 μL of respective solvent to make the final concentration of 40 mg/mL.

2.3. Antimicrobial susceptibility activity

The antimicrobial susceptibility testing was performed using Kirby-Bauer modified disc diffusion method[12]. At least three to five well-isolated colonies were selected from an agar plate and transferred into a tube containing 4 to 5 mL of trypic soy broth. The broth culture was incubated at 37 °C for overnight in shaker. To achieve the turbidity equals to 0.5 McFarland standards, 50 μL of overnight culture was added in 5 mL of trypic soy broth and incubated at 37 °C for 1.5 h. Spectrophotometer was used to measure the optical density. In case of *S. aureus* the optical density should between 0.3-0.6. Muller-Hilton agar plates were prepared for disc assay. The disc diffusion technique and zone interpretation of each antimicrobial agent was used in accordance with National Committee for Clinical Laboratory Standards (NCCLS) guidelines[13]. The ampicillin (10 μg), tetracycline (30 μg), kanamycin (10 μg), erythromycin (15 μg) and oxacillin (1 μg) were used for drug susceptibility testing. The plates were then incubated at 37 °C for 19 h and the results were interpreted according to NCCLS standards for antibiotics susceptibility testing.

2.4. Antimicrobial analysis of plants extracts

The antimicrobial activity was determined on multi-drug resistant *S. aureus*. Standardized inoculums were prepared. Sterile filter paper discs previously soaked in a known concentration of extract (40 mg/mL) was carefully placed at the center of the labeled plate. The plates were incubated aerobically at 37 °C in incubator and examined for zones of inhibition after 19 h.

2.5. Determination of MIC

MIC is the minimum concentration that can inhibit the growth of a microorganism after overnight incubation. The

Table 1

Plants selected for antibacterial activity against multidrug resistant *S. aureus*.

Species	Family	Vernacular names	Part used	Local uses
Cinnamomum zeylanicum (DARM)	Lauraceae	Cinnamon	Bark	Analgesic, antibacterial, antifungal, antioxidant, antispasmodic, anti-viral, aphrodisiac, aromatic
Acacia nilotica (ANI)	Mimosaceae	Indian Gum Arabic tree	Root	Used for the treatment of stomatitis, wounds, hemorrhage, ulcers, dysentery, diarrhea, skin diseases, burning sensation, cough, and dental caries
Santalum album (SAM)	Santalaceae	Indian Sandalwood	Flower	Useful in skin diseases
Azadiracta indica (AZD)	Meliaceae	Neem Indian Lilac	Leaf	Anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive, sedative, blood purifying properties
Taraxacum officinale (T.off)	Asteraceae	Dandelion	Leaf	Used for gallbladder, diabetes, Loss of appetite and act as mosquito repellent
Diospyros peregrine (DPSM)	Ebenaceae	Paniccha Panichi	Seed	Chronic dysentery, menorrhagia and excessive salivation
Artemisia absinthium linn (AFM)	Asteraceae	Common Wormwood	Leaf	Tonic, stomachic, febrifuge, anthelmintic
Berberis lyceum (ZSM)	Berberidaceae	Ishkeen Kushmul Zarch	Flower	Used in the treatment of eye complaints, menorrhagia, chronic diarrhoea and piles
Tamarindus indica (IND)	Fabaceae	Ambli	Flower	Cathartic, astringent, febrifuge, antiseptic, refrigerant
Nymphaea alba (N.ALB)	Nymphaeaceae	White Lotus	Leaves	It acts as astringent antiseptic, anesthetic, anaphrodisiac and sedative
Fagonia cretica (FCWM)	Zygophyllaceae	Dhamsha	Whole plant	It has medicinal potential, especially against cancer and tumors
Nardostachys jatamansi (NJRM) (NJRD)	Valerianaceae	Sambul lateeb Balchar	Root	Effective against kidney stones, jaundice, removes blood impurities, nervous headache, convulsions, respiratory and digestive diseases, skin conditions, typhoid, gastric disorders
Cucurbita pepo (CPLM)	Cucurbitaceae	Pumpkin Kaddoo	Leaves	It is used in treating bladder disorders, stomach upsets, intestinal worms, bed-wetting, rheumatism, benign prostatic hyperplasia, burns, and wounds
Piper nigrum (PNSM)	Piperaceae	Black pepper Kali mirch	Seed	It possesses anti-tumourigenic, immunostimmulatory, stomachic, carminative, anticholestrolemic and again known for its strong phytochemical activities
Trianthema portulacastrum (TPWM)	Aizoaceae	Bishkapra	Whole plant	Analgesic, purgative, stomachic, used for the treatment of anemia, bronchitis, piles, inflammation, liver troubles, asthma, itch, chronic ulcer, night-blindness, diseases of blood and skin
Syzygium cumini (SCLM)	Myrtaceae	Jamun	Leaves	Plant pacifies diarrhea, diabetes, leucorrhoea, fever, skin diseases and general debility
Vitis vinifera (VVLM)	Vitaceae	Grape seed	Leaves	It helps in burning sensation, constipation, hemorrhoids, anemia, skin diseases, colic, flatulence, jaundice, vomiting, splenomegaly, arthritis, and amenorrhea
Musa paradisiacal (MPTM)	Musaceae	Banana		It provides large amounts of vitamins B-6 and C
Moringa oleifera (MOLM) (MOPM)	Moringaceae	Sahajna	Leaves Pods	Anti-inflammatory, cancer treatment, gout treatment, alzheimer prevention, relief for gastric ulcers, control of herpes symptoms, high cholesterol prevention, powerful natural antibiotics, HIV and AIDS treatment
Valeriana jatamansi (VJAM) (VJRM)	Valerianaceae	Samayo Sugandhawal	Root	Used as a sleeping and calming agent, used for sedative, mild anodyne, hypnotic, spasmolytic, carminative, hypotensive; Indicated for hysterical states, excitability, insomnia, hypochondriasis, migraine, cramp and rheumatic pain
Citrus sinensis (CSLM)	Rutaceae	Orange peel	Leaves	It strengthens the immune system, aid digestion and lowers the risk of food-borne pathogens
Chenopodium album(CAWM)	Chenopodiaceae	Fat hen	Whole plant	Acts as anthelmintic, antiphlogistic, antirheumatic, mildly laxative, odontalgic
Terminalia arjuna (TAFM) (TAFD) (TABM)	Combretaceae	Arjuna	Flower Leaves Bark	Analgesic and anti-inflammatory properties. Cardiomyopathy like myocardial Infarction, angina, coronary artery disease, heart failure, hypercholesterolemia, hypertension.
Punica granatum (POM)	Lythraceae	Anaar	Fruit coat	It has immuno-stimulantory, anti-oxidant, anti-inflammatory anti-diabetic and anticancer
Nigella sativa (KAL)	Ranunculaceae	Kalonji	Seed	Use to relieve the symptoms of asthma, bronchitis, and coughing, to treat abscesses and tumors of the abdomen, eyes, and liver
Psidium gaujava (GUA)	Myrtaceae	Amrood	Leaves	Use against cancer, bacterial infections, inflammation and pain
Momordica charantia (BGM)	Cucurbitaceae	Karela	Leaves	Act as antihelmintic, antimalarial, antiviral,anticancer and cardioprotective
Avicennia marina (AMLC)	Avicenniaceae	Mangrove	Leaves	Used for the treatment of ulcers
Grewia asiatica (GALM)	Malvaceae	Falsa	Leaves	The leaves are used as an application to pustular eruptions

lower the MIC, the better is the antimicrobial agent. MIC was determined by micro-dilution method using serially diluted (10 folds) plant extracts according to the NCCLS 2000[13].

2.6. Statistical method

All statistical analysis was done using SPSS software (version 11.0, SPSS Inc). Data is presented as mean±SE.

3. Results

3.1. Antimicrobial susceptibility

Disc diffusion assay was performed on 17 isolates of *S. aureus*. Due to the relatively small size, no separate analysis was undertaken for sewerage, milk and nasal isolates of *S. aureus*. In this study *S. aureus* were found to be highly resistant to ampicillin (100%), tetracycline (53%) followed by kanamycin (41%), erythromycine (30%), and oxacillin (5%) as shown in Table 2. The *S. aureus* isolates from milk samples (M, A, RM12, 1MB, 2MB and 3MB) had more resistance to the antimicrobial agents as compared to the resistance of sewerage and nasal isolates.

3.2. Antimicrobial analysis of plants extracts

Antimicrobial analysis of plant extracts were checked on multidrug resistant *S. aureus* (A, RM12, M, 1MB, 2MB, 3MB, NS2, NS4, NS6, NS8, NS9, NS10, NS11, RM4, RM10, RD24) and ATCC (25923) strains. These extracts produced a clear zone of inhibition around disc impregnated with extract as shown in Figure 1.

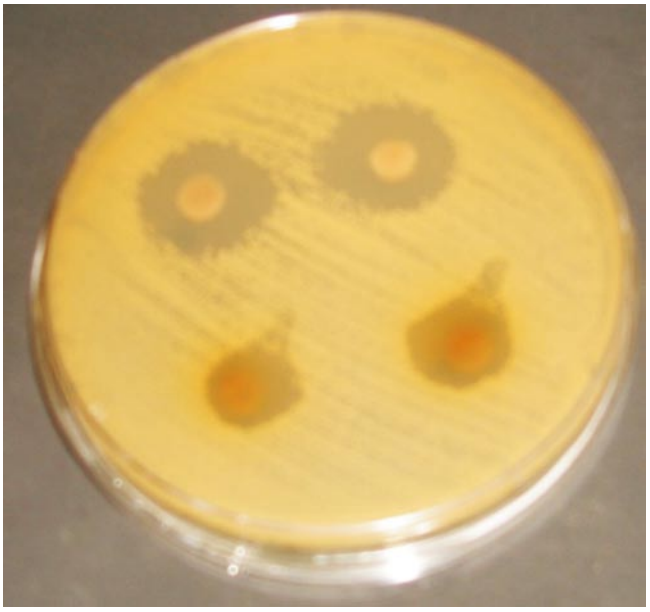

Figure 1. Effect of *P. guajava* (GUA), *V. jatamansi* (VJAM), and *N. sativa* (KAL) on multidrug resistant *S. aureus*.
Discs impregnated with extracts shows zone of inhibition of *S. aureus*. Above two inhibition zones shows antibacterial effect of *P. guajava* and *V. jatamansi* while bottom shows the effect of *N. sativa*.

Twenty nine plant extracts were checked but only few extracts exhibited inhibition of *S. aureus*. Maximum inhibition zone was observed in methanolic extract of *Psidium guajava* (*P. guajava*), *Nigella sativa* (*N. sativa*), *Citrus sinensis* (*C. sinensis*), *Valeriana jatamansi* (*V. jatamansi*), and *Cucurbita pepo* (*C. pepo*). Other extracts such as *Acacia nilotica* (*A. nilotica*) and *Santalum album* (*S. album*) extracts gave zone of inhibition greater than 10 mm, while all other extracts showed minimum zone of inhibition or no zone as shown in Table 3.

All the multidrug resistant strains and standard ATCC (25923) were tested for MIC against these five potent plant

Table 2
Multidrug resistant *S. aureus* identified by Disc diffusion.

Sample	Ampicillin	Tetracycline	Kanamycin	Erythromycine	Oxacillin
A	17.00±2.00 (R)	12.00±0.30 (R)	12.00±1.00 (R)	13.00±2.00 (R)	10.00±0.70 (R)
RM12	9.00±2.00 (R)	0 (R)	10.00±0.50 (R)	0 (R)	20.00±0.00 (S)
M	16.00±3.00 (R)	13.00±0.50 (R)	12.00±1.00 (R)	0 (R)	14.00±0.00 (S)
1MB	14.00±3.00 (R)	13.00±0.50 (R)	14.00±0.50 (IR)	17.00±2.00 (IR)	15.00±0.00 (S)
2MB	18.00±0.00 (R)	12.00±1.00 (R)	14.00±0.00 (IR)	17.00±2.00 (IR)	16.50±0.70 (S)
3MB	9.50±0.70 (R)	13.00±0.00 (R)	13.00±0.70 (R)	14.00±1.00 (IR)	16.50±0.70 (S)
ATCC(25923)	24.50±4.90 (R)	15.50±4.90 (S)	13.00±2.80 (S)	16.50±0.70 (IR)	19.50±0.70 (S)
NS2	10.50±0.70 (R)	14.00±0.00 (S)	13.00±4.24 (S)	20.00±0.00 (S)	11.50±0.70 (S)
NS4	11.50±0.70 (R)	13.50±3.50 (S)	13.00±4.24 (S)	25.00±0.00 (S)	19.50±0.70 (S)
NS6	18.00±2.82 (R)	14.50±0.70 (S)	15.50±3.50 (S)	15.50±3.50 (IR)	14.50±0.70 (S)
NS8	14.50±0.70 (R)	0 (R)	18.00±0.00 (S)	24.00±0.00 (S)	12.50±0.70 (S)
NS9	22.00±2.82 (R)	16.50±3.50 (S)	20.00±2.82 (S)	26.00±0.00 (S)	14.50±0.70 (S)
NS10	18.00±1.41 (R)	0 (R)	19.50±2.12 (S)	27.00±1.41 (S)	16.50±2.10 (S)
NS11	24.50±2.12 (R)	17.00±1.41 (S)	18.00±1.41 (S)	26.00±1.41 (S)	15.50±1.40 (S)
RM4	18.00±7.00 (R)	19.00±1.41 (S)	23.5±2.12 (S)	16.00±1.41 (IR)	25.00±0.70 (S)
RM10	17.00±1.41 (R)	21.50±2.15 (S)	22.00±1.41 (S)	31.00±0.00 (S)	19.00±1.41 (S)
RD24	11.00±1.00 (R)	15.00±1.00 (S)	24.30±1.15 (S)	0 (R)	20.00±0.00 (S)

R: resistant; S: sensitive; IR: intense resistance.

Table 3

Zone of inhibition of plant extracts against multidrug resistant *S. aureus* (mm).

Plant extracts	Bacteria sample						
	A	M	RM12	1MB	2MB	3MB	ATCC (25923)
P. guajava	14.00±1.41	15.00±1.41	16.50±2.12	12.50±0.70	19.00±1.41	14.50±0.70	0
N. sativa	14.00±1.14	13.50±0.70	14.00±1.14	15.50±0.70	15.50±0.70	15.50±0.70	15.00±0.00
C. sinensis	7.50±0.70	10.00±0.00	0	0	0	0	8.00±1.41
V. jatamansi	10.00±0.70	15.50±0.70	11.50±2.12	11.50±0.70	10.00±0.00	10.00±2.80	14.50±0.70
C. pepo	0	14.50±0.70	11.00±1.41	0	0	0	13.50±0.70
A. nilotica	9.50±2.00	11.50±0.70	10.50±0.70	11.50±0.70	10.50±0.70	0	16.00±1.41
S. album	0	11.50±0.70	11.00±1.41	0	0	0	13.00±2.82

extracts: *N. sativa* (KAL), *P. guajava* (GUA), *C. pepo* (CPLM), *V. jatamansi* (VJAM) and *Piper nigrum* (PNSM) (*P. nigrum*) to find out the 50% inhibition of *S. aureus*. The results of present study showed that the methanolic extract of *N. sativa* (KAL), resulted in 50% inhibition of ATCC (25923), A, 1MB and 2MB *S. aureus* strain at a concentration of 0.625 mg/mL, while RM12 and 3MB gave LD_{50} at a concentration of 2.5 mg/mL and 0.328 mg/mL respectively.

The results of this research represented that the methanolic extract of *P. guajava* (GUA) gave 50% inhibition of M and 2MB strains of *S. aureus* at a concentration of 0.625 mg/mL, RM12 and A strains resulted in 50% inhibition at a concentration of 0.325 mg/mL, while 1MB and 3MB *S. aureus* strains showed LD_{50} at 1.250 mg/mL.

In this experiment the methanolic extract of *C. pepo* gave LD_{50} of M and A stains of *S. aureus* at the concentration of 1.250 mg/mL and 5.000 mg/mL and RM12 resulted in 50% inhibition at a concentration of 0.625 mg/mL.

Methanolic extract of *P. nigrum* (PNSM) exhibited LD_{50} of only ATCC (25923) at a concentration of 0.328 mg/mL. while *V. jatamansi* (VJAM) extract showed 50% inhibition of RM12, ATCC (25923) and A at a concentration of 5.000 mg/mL, and M gave LD_{50} at concentration of 0.625 mg/mL as shown in Table 4.

Table 4

MIC values of different plants against *S. aureus* isolates.

Sample	MIC (mg/mL)				
	N. sativa	*P. guajava*	*C. pepo*	*P. nigrum*	*V. jatamansi*
ATCC (25923)	0.625	–	–	0.328	5.000
M	–	0.625	1.250	–	0.625
RM12	2.500	0.325	0.625		5.000
A	0.625	0.325	5.000		5.000
1MB	0.625	2.500			
2MB	0.625	0.625			
3MB	0.328	2.500			

4. Discussion

Staphylococcus is a group of bacteria that can cause a number of diseases as a result of infection of various tissues of the body. Over 30 different types of staphylococci can infect humans, but most infections are caused by *S. aureus*. In the majority of cases, the bacteria do not cause disease. However, damage to the skin may allow the bacteria to overcome the natural protective mechanisms of the body, leading to infections. *S. aureus* causes one of the most common types of chronic mastitis. Once established, *S. aureus* infections do not respond well to antibiotic therapy. Recently published work has shown that 3% of all animals are infected with *S. aureus*[14]. However, *S. aureus* represents 10 to 12 percent of all clinical mastitis infections[5].

In the present study, *S. aureus* strains originated from bovine mastitis were identified phenotypically by some conventional tests. The primary tool for controlling staphylococcal mastitis is antimicrobial therapy. Therefore antimicrobial susceptibility tests were performed according to NCCLS standards to analyze the resistance pattern of *S. aureus*.

Second part of this study was designed to find out the antibacterial potentials of Pakistani herbal plants against multidrug resistant *S. aureus*. For this purpose, six multidrug resistant *S. aureus* strains were isolated from nasal, milk samples. The aim of study was to find out antibacterial agents from indigenous medicinal plants.

As herbal medicines are being used for centuries and botanicals have also become a main point for the identification of new active compounds to treat diseases. Therefore, the focus of this study was to get some knowledge of local plants which are being used for medicinal purposes in Pakistan. Active compounds, derived from plant extracts, are of continuing interest to the pharmaceutical industry.

Anti-bacterial activity of collected herbal extracts was tested against *S. aureus*. Methanol extracts of 29 different plants were screened for their anti-bacterial activity. Out of them, only five herbal extract (*P. guajava*, *N. sativa*, *P. nigrum*, *V. jatamansi* and *C. pepo*) were found to exhibit potential antibacterial activity against *S. aureus*. Other extracts such as *A. nilotica* and *S. album* also gave antibacterial activity against *S. aureus* to some extent.

Guava leaf tea of *P. guajava* Linnaeus is commonly used as a medicine against gastroenteritis. Popular Brazilian medicine has long made use of native plants for the treatment of gastrointestinal diseases, as in the case of *P. guajava*[15]. Chaidate *et al*. found that *P. guajava* leaves

extract kills the resistant strains of *S. aureus* using micro-broth dilution method and guava leaf extract was able to inhibit all bacterial strains in this study at minimum inhibitory concentration (MIC) between 0.2-51.2 mg/mL[16]. In the present study, the antimicrobial activity of *P. guajava* showed the inhibitory effect at a concentration of 40 mg/mL on all six multidrug resistant strains of *S. aureus* isolated from milk *i.e.* M, A, RM12, 1MB, 2MB and 3MB. While methanolic extract of *P. guajava* did not show inhibitory effect on standard stain of *S. aureus* ATCC (25923). The MIC value of methanolic extract of *P. guajava* was in between 0.3-2.5 mg/mL.

N. sativa is a herbaceous plant found in the Middle East, Europe and Western and Middle Asia. Its seeds have a great medicinal potential and have been reported to exhibit many pharmacological effects that include anti-parasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities[17]. Hannan *et al.* found that strains of methicillin-resistant *S. aureus* were sensitive to *N. sativa* extract at a concentration of 4 mg/disc while the extract had an MIC range of 0.2-0.5 mg/mL. Our study was therefore designed to evaluate this aspect of *N. sativa*. The results of disc diffusion assay demonstrated that strains of multi-drug resistant *S. aureus* were completely inhibited at 40 μg/mL on 6 mm disc and gave MIC value between 0.3-2.5 mg/mL.

C. pepo (pumpkin) belongs to the family Cucurbitaceae which includes cucumbers, melons, squash and gourds. Pumpkin is used as an emollient to soften the dryness of the skin and pimples spots. It is used in treating bladder disorders, stomach upsets, intestinal worms. Chonoko and Rufai 2011 described that antibacterial activity of *C. pepo* showed that its ethanol extract was active against *S. aureus* and *Salmonella typhi* (7mm)[19]. The methanol extract was active against *S. aureus* but inactive against *Salmonella typhi*. This study sought to screen *C. pepo* to determine the antibacterial activity of the plant extracts against *S. aureus*. The results of antibacterial activity showed that methanolic extract of *C. pepo* was active against three mastitis strains of *S. aureus* (M and RM12 and A) but has not shown any zone of inhibition against ATCC (25923) strain which is of clinical origin. The zone of inhibition for M and RM12 and A were (14.50±0.70) mm, (11.00±1.41) mm and (13.50±0.70) mm respectively and exhibited MIC value at a concentration between 0.625-5.000 mg/mL. Saponins were present in the extract which has been found to be used as anti-inflammatory and anti-oxidant agents.

V. jatamansi is also known as *Valeriana wallichii* is an indian plant which belongs to the family Valerianaceae. This plant is never used as antimicrobial against *S. aureus*. Here we used methonolic extract of axial part of this plant against multidrug resistant *S. aureus* and it gave a very good zone of inhibition. The zone of inhibition for mastitis *S. aureus* isolates *i.e.*, A, M, RM12, 1MB, 2MB, 3MB and clinical isolated *S. aureus* ATCC (25923) was (10.00±0.70) mm, (15.50±0.70) mm, (11.50±2.12) mm, (10.00±0.70) mm, (10.00±0.00) mm, (10.00±2.80) mm and (14.50±0.70) mm respectively and showed MIC values in between 0.625-5.000 mg/mL.

P. nigrum is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever. It has been shown to have antimicrobial activity. Chaudhry, Tariq *et al.* investigated the effect of *P. nigrum* on clinically isolated strains of *S. aureus* and found that both aqueous and methanolic extract of *P. nigrum* gave 23 mm zone of inhibition at the concentration of 10 μL/disc[18]. And their results are completely overlapping the results of this research. In this study only ATCC (25923) stain gave zone of inhibition (21.00±2.00) mm, and a very little zone was appeared in M bacteria *i.e.* (9.00±1.41) mm. P. nigrum extract resulted in 50% inhibition at a concentration of 0.328 mg/mL.

On the basis of results presented herein, it is theoretically possible that extracts of *P. guajava*, *P. nigrum* seed, *V. jatamansi*, *C. pepo* and *N. sativa* may play a role in the treatment *S. aureus* infection. This data also suggest that therapeutic induction of extracts might represent an alternative approach for the treatment of mastitis and nosocomial infections caused by *S. aureus* or this study may leads to the development of more potent drugs for *S. aureus* infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

S. aureus multidrug resistant is a serious problem in the concern of mastitis treatment. Several medicinal plants are capable of inhibit microbial growth and are frequently used by popular knowledge to treat infections.

Research frontiers

The present work highlights the emergence of multi-resistant *S. aureus* in mastitis cases and demonstrates the efficacy of plant extracts against these isolates.

Related reports

A number of medicinal plants are usually tested for their antimicrobial potential against mastitis pathogens, specially *S. aureus*. An example is the work of Kalayou *et al.* (2012) doi: 10.1016/S2221-1691(12)60088-4. However, the emerging of multi-resistant *S. aureus* increases the need for new effective drugs against these agents.

Innovations and breakthroughs

In the present study, authors have demonstrated the antimicrobial activity of some plant extracts selected due to the popular knowledge about their medicinal properties against multi-resistant *S. aureus*.

Applications

This scientific study suggest the use of the plants *P. guajava*, *N. sativa*, *P. nigrum*, *V. jatamansi* and *C. pepo* as sources of antimicrobial agents against some strains of multiresistant *S. aureus*.

Peer review

The article content is valid for publication because it addresses the importance of alternatives to control multiresistant *S. aureus*. This theme has been recurring in the literature because it is a current problem in both the field of animal and human health. So, it can be considered for publication due to its theoretical value

References

[1] Thwaites GE, Edgeworth JD, Gkrania-Klotsas E, Kirby A, Tilley R, Török ME, et al. Clinical management of *Staphylococcus aureus* bacteraemia. *Lancet Infect Dis* 2011; **11**: 208-222.

[2] Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005; **5**: 751-762.

[3] Li XZ, Nikaido H. Efflux-mediated drug resistance in bacteria: an update. *Drugs* 2009; **69**: 1555-1623.

[4] Wang Y, Wu CM, Lu LM, Ren GW, Cao XY, Shen JZ. Macrolide-lincosamide-resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Vet Microbiol* 2008; **130**: 118-125.

[5] Tenhagen BA, Hansen I, Reinecke A, Heuwieser W. Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *J Dairy Res* 2009; **76**: 179-187.

[6] Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz J Microbiol* 2000; **31**: 247-256.

[7] Littleton J, Rogers T, Falcone D. Novel approaches to plant drug discovery based on high throughput pharmacological screening and genetic manipulation. *Life Sci* 2005; **78**: 467-475.

[8] Ashfaq UA, Javed T, Rehman S, Nawaz Z, Riazuddin S. Inhibition of HCV 3a core gene through silymarin and its fractions. *Virol J* 2011; **8**: 1-7.

[9] Ashfaq UA, Masoud MS, Nawaz Z, Riazuddin S. Glycyrrhizin as antiviral agent against hepatitis C virus. *J Transl Med* 2011; **9**: 112.

[10] Rehman S, Ashfaq UA, Riaz S, Javed T, Riazuddin S. Antiviral activity of *Acacia nilotica* against hepatitis C virus in liver infected cells. *Virol J* 2011; **8**: 220.

[11] Djeussi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complement Altern Med* 2013; **13**: 164.

[12] Andrews JM, BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method (version 8). *J Antimicrob Chemother* 2009; **64**: 454-489.

[13] NCCLS. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards*. 5th ed. Wayne, PA, USA: NCCLS; 2000.

[14] Schukken YH, Gonzalez RN, Tikofsky LL, Schulte HF, Santisteban CG, Welcome FL, et al. CNS mastitis: nothing to worry about? *Vet Microbiol* 2009; **134**: 9-14.

[15] Vieira RH, Rodrigues DP, Goncalves FA, Menezes FG, Aragao JS, Sousa OV. Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Rev Inst Med Trop Sao Paulo* 2001; **43**: 145-148.

[16] Inchaisri C, Ajariyakhajorn K, Samngamnim S. Antibacterial activity of some herb extracts against bovine mastitis pathogens. The 4th Chulalongkorn University Veterinary Annual Conference 2005: 60th Veterinary Anniversary Building, Chulalongkorn University; 2005 Feb 15.

[17] Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* 2003; **17**: 299-305.

[18] Chaudhry NM, Tariq P. Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. *Pak J Pharm Sci* 2006; **19**: 214-218.

[19] Chonoko UG, Rufai AB. Phytochemical screening and antibacterial activity of *Cucurbita pepo* (Pumpkin) against *Staphylococcus aureus* and *Salmonella typhi*. *Bayero J Pure Appl Sci* 2011; **4**(1): 145 - 147.