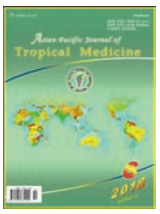

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Traditional medicines and their *in-vitro* proof against *Staphylococcus aureus* in Pakistan

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ABSTRACT

Objective: To gather the fragmented literature on ethnobotany, phytochemistry and *in-vitro* activities of medicinal plants of Pakistan being used against common infections caused by *Staphylococcus aureus* (*S. aureus*). **Methods:** A large number of published and unpublished research studies related to the ethnomedicinal, phytochemical and anti-*S. aureus* activity of medicinal flora of Pakistan published from 1990–2018 were reviewed using online bibliographic databases such as PubMed, Web of Science, Science Direct, ResearchGate and libraries. **Results:** *S. aureus* can cause many human ailments including endocarditis, staphylococcal scalded skin syndrome, septic arthritis, respiratory problems with an estimated infection rate of 25%–35% across the globe. This review comprised of 86 medicinal plants. Data showed that people mostly used leaves (50%) for the preparation of traditional medicines. Correlation analysis on the reviewed data revealed that methanolic extract concentrations of medicinal plants was highly significantly positive correlated (r=0.8; P<0.01) with the *S. aureus* zone of inhibitions. *S. aureus* reportedly showed complete resistant to the commonly used antibiotic erythromycin. Isolated compounds like altheahexacosanyl lactone, cinnamaldehyde, niloticane, gobicusin A, asparacosin A, muzanzagenin, isoagatharesinol, friedelin, inophynone and eugenol were active against *S. aureus*. This study provided *in-vitro* proof for the flora of Pakistan used against different infections caused by *S. aureus*. **Conclusions:** Antibacterial agents from natural sources could be more effective against bacterial pathogens and will be helpful in minimizing the adverse effects of synthetic drugs, and hence provides a base for the pharmaceutical industries.

1. Introduction

Medicinal plants constitute an important component of the pharmaceutical industries and primary healthcare system at local level. Approximately, 80% of the world populations depend on ethnomedicines for primary health care. Pakistan has reportedly over 1 000 medicinal plant species that are mostly being used by the rural population in various herbal remedies[1]. Main reasons for the ethnomedicinal uses are their availability, low cost, fewer side effects and associated ancestral experiences. The medicinal efficacy of such plants against pathogenic infections is due to the presence of

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phytoconstituents including alkaloids, glycosides, resins, flavonoids, triterpenes, phenolic acids, alcohols, carotenoids and mucilage.

Pathogenic bacteria are involved in causing serious infectious diseases that successively result in mortality and morbidity among the population, especially in developing countries. The pharmaceutical industries are keen to develop new drugs due to the constant emergence of microbial resistance to conventional medicines. As an example, the multi-drug resistance of *Staphylococcus aureus* (*S. aureus*) makes it one of the most stubborn pathogenic bacteria against antibiotic efficacy. *S. aureus* causes many human ailments including endocarditis, staphylococcal scalded skin syndrome, septic arthritis, and respiratory problems[2]. It is estimated that *S. aureus* has 25%–35 % infection rate worldwide[3]. In Pakistan, a high prevalence rate (42%–51%) has been observed for methicillin resistance in *S. aureus*[4]. Skin soft tissues' infections due to *S. aureus* are ranging from benign to the immediately life threatening[5]. *S. aureus* is one of the important causative agents of pneumonia, with 97% cases being reported in children from tropical and subtropical developing countries, and 50% from India, China and Pakistan[6].

Most of these infectious diseases are being treated by using ethnomedicinal recipes derived from plant species. A large number of *in–vitro* studies have been conducted on the medicinal flora of Pakistan against *S. aureus*. Out of these medicinal plants, certain species are therapeutically effective due to the presence of active phytochemicals. However, most of such data are available in individual research studies and can scarcely be found collectively. Hence as per our knowledge, this is the first attempt to review all the scattered literature on ethnobotany, phytochemistry and *in–vitro* activities of Pakistani medicinal plants locally being used against common infections caused by *S. aureus*.

2. Methodology

2.1. Literature selection for the current study

A large number of published and unpublished research studies related to the ethnomedicinal, phytochemical and anti-*S. aureus* activities of flora of Pakistan published between 1990–2018 were reviewed by using online bibliographic databases like Science Direct Navigator, Pub Med, Google Scholar, ResearchGate and libraries. Data was gathered on *in–vitro* activities of plants extracted by using different solvents including ethanol, chloroform, methanol, *n*–hexane and ethyl acetate. Extracts of different plant parts have been reported for *in–vitro* screening against *S. aureus*. However in this review, we focused only on those plants, whose extract concentrations were quantitatively listed in the literature, *i.e.*, milligrams of extracts dissolved in milliliters of the respective solvent. Therefore by keeping the above mentioned criteria, a total of 86 medicinal plants reportedly having anti-*S. aureus* activities were selected for this review. Furthermore, we documented all the available information on the phytoconstituents and ethnobotany of the selected plants in order to establish their relation with the anti-*S. aureus* activities. Plant databases such as "Flora of Pakistan"[7] and "The Plant List"[8] were used for taxonomic corrections of the reported plant species.

2.2. Tabulation, figures and data analysis

In this study, the selected literatures have been represented in tables and figures. Figure 1 shows the selection criteria for the documented plant species in this review that are extracted with different solvents in Pakistan. These plants have shown both qualitative and quantitative *in–vitro* activities against different bacteria. Further, the graph shows a comparison of a total number of plants to that of selected plants, which showed activities against *S. aureus*. Figures 2 and 3 show Pearson correlation coefficients for the determination of two-tailed significance between plant extracts of a particular solvent and inhibition zone of *S. aureus*. Table 1 contains ethnomedicinal data, while the remaining tables (2, 3 and 4) contain data on phytochemical constituents and anti-*S. aureus* activity of selected medicinal plants belongs to Lamiaceae, Solanaceae and Compositae families. Data on the phytochemical and anti-*S. aureus* activities of medicinal plants from the rest of families are mentioned in Table 5. Data on extract concentrations are presented in mg/mL, while zone of inhibition in mm.

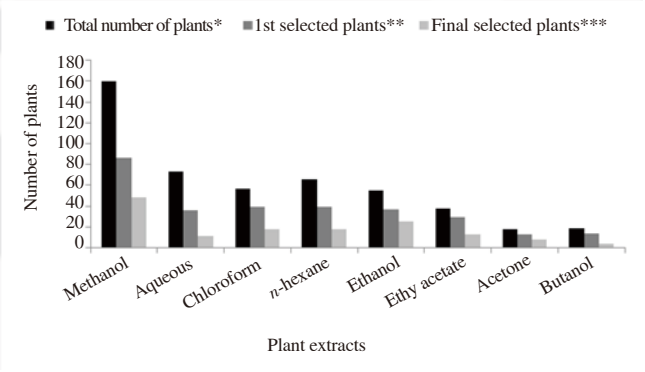

Figure 1. Plants extracted with different solvents.
*Total number of plants extracted with a particular solvent and used against all types of bacteria **Number of plants extracted a particular solvent and used against *S. aureus* and presenting both quantitative and qualitative data on inhibition. ***Number of plants used against *S. aureus* and presenting effective quantitative data on inhibition.

2.3. Methods and solvents used for plant extraction in the reported studies

In Pakistan, different techniques are being applied for the extraction of active metabolites from medicinal plants, which include infusion, maceration, soxhlet, decoction and percolation. The selection of a technique mainly depends upon the nature of solvent and metabolites solubility. Methanol, aqueous and ethanol are the preferable solvents used for plants extraction across the world[76]. This might be due to the solvent polarity as they can easily degrade the cell wall and extract polyphenols from the plant cells. Polyphenols are aromatic or saturated organic compounds in nature and carries sound antibacterial activities[77]. Out of 86 studied plants, 86 plants were

reportedly extracted with methanol (Figure 1). Similarly, 37 plants were reportedly extracted with ethanol, 39 plants with *n*–hexane, 39 plants with chloroform, 36 plants with water, 29 plants with ethyl acetate, 13 plants with acetone, and 14 plants were extracted with butanol (Figure 1).

Correlation analysis showed that increase in methanolic extract concentrations (mg/mL) had significantly increased the antibacterial activity against *S. aureus* at r=0.8; P<0.01 (Figure 2) and r=0.60; P<0.01 (Figure 3). In a similar way, increase in aqueous extract concentrations (mg/mL) had significantly increased the *S. aureus* inhibition zone at r=0.8; P<0.01 (Figure 2). Water is a universal solvent used for medicinal plants extraction. Chloroform has been proved as an important solvent for the extraction of different compounds such as isolation of aglycon of deoxy-niazimicine from *Moringa oleifera*[78]. Likewise, *n*–hexane is preferably used for the extraction of edible oils from natural sources[79]. Butanol is not the widely used organic solvent for extraction; however, considered best for the extraction of phenolic compounds[35].

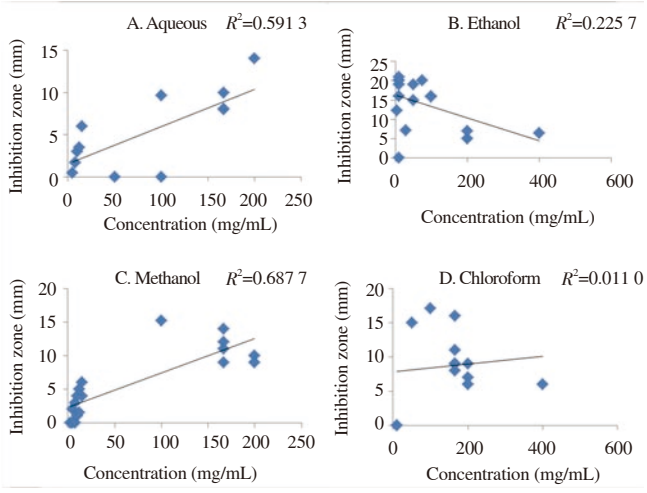

Figure 2. Relationship between extract concentrations and *S. aureus* inhibition zone of respective solvents.

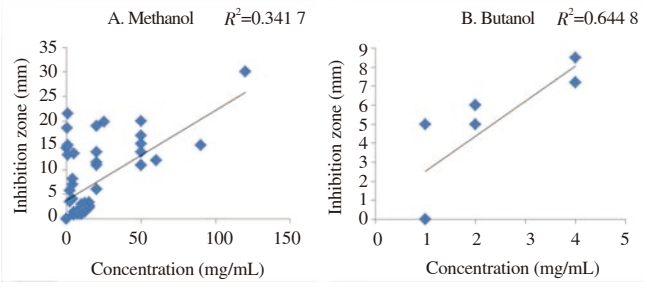

Figure 3. Relationship between extract concentrations and *S. aureus* inhibition zone of respective solvents dissolved in DMSO.

3. Results

3.1. Ethnobotany and anti–S. aureus activities of Pakistani medicinal plants

Pakistan is blessed with diversity of medicinal plants, which play a key role in safeguarding the human health since long. Approximately, 40 000 registered traditional practitioners in the country are using these plants (mono- and poly-herbal remedies) for providing primary health care facilities to a majority of rural population. At present, about 50% of the total plant-derived drugs are mono-herbal and the remaining 50% are poly-herbal[19]. Moreover, a number of modern allopathic drugs also belong to the plant origin.

This study presented 86 plant species with reported antibacterial activities against *S. aureus*. Lamiaceae and Solanaceae families have also been reported in the literature with maximum number of plants being tested for antimicrobial activity[80,81]. This is because most active compounds have been isolated from the oil of their plants, hence providing us with an opportunity to test the unexplored plant species (part wise) of these families against *S. aureus*.

Different plant parts such as rhizomes, roots, stems, barks, leaves, flowers, fruits and seeds are used traditionally in various herbal remedies. Leaves are the most commonly used part, because these are the sites of high photosynthetic activity and secondary metabolites, and they usually do not result in the fatality of mother plant (Table 1). Traditionally, these medicinal plant parts are not only used to treat infectious diseases but also other diseases like stomachache, fever, gastrointestinal disorders, skin problems and cleanser, wound healing, gallstones, and whooping cough[19,20,82]. These traditional practices are more common in rural areas having less access to physicians, while this knowledge comes to them from their ancestral strong beliefs on plant efficacy with fewer side effects. These medicinal plants also have *in–vitro* proofs for the activities like antispasmodic, anti-inflammatory, anticancer and antiparasitic[20,26,68] and could be very effective against multi-drug resistant microorganisms.

World Health Organization recognizes antibiotic resistance as a global concern, which resulted in more than 25 000 deaths per year in the European Union and costs more than 1.5 billion per year in healthcare expenses and productivity losses. Similarly in the USA, about 2 million people acquire serious infections caused by bacterial resistance to at least one recommended antibiotic. The emergence of antibiotic resistance and dearth of new antibiotics threaten the ability to treat patients with infectious diseases[83]. Starting with penicillin and methicillin, *S. aureus* has demonstrated a unique ability to quickly respond to each new antibiotic by the development of a resistance mechanism. Such mechanisms include enzymatic inactivation of the antibiotic (penicillinase and aminoglycoside-modification enzymes), alteration of the target with decreased affinity to antibiotic (notable examples being penicillin-binding protein 2a of methicillin-resistant *S. aureus*), trapping of the antibiotic (for mycin and possibly daptomycin), efflux pumps (fluoroquinolones and tetracycline), and resistance developed due to spontaneous mutations and positive selection[84]. Therefore, a dire need is to develop new medicines (synergistic or additive effects) from plants to cope up with the emerging global public health threat of antibiotic resistivity. This review provides detailed information on the pharmacological evidence of anti- *S. aureus* plant families, and baseline to pharmaceutical industries for the production of new antibiotics.

Table 1

Ethnomedicinal uses of Pakistani medicinal plants.

Botanical name	Plant family	Location(s)	Part used	Ethnomedicinal efficacy	References
Acacia nilotica (L.) Delile	Leguminosae	Kohat	Leaves	Gum inflammation, diarrhea, leucorrhoea, cough, dysentery, sore throat and respiratory ailments	[9]
Aesculus indica (Wall. ex Camb.) Hook.	Sapindaceae	Lower barian taien valley	Leaves	Colic pain, rheumatism, skin diseases and vein complications	[10]
Althaea officinalis L.	Malvaceae	Muzaffarabad	Root, leaves, flower	Expectorant, demulcent and for curing burns, snake bite, respiratory diseases, rheumatism and kidney problems	[11,12]
Arisaema flavum (Forssk.) Schott	Araceae	Kaghan valley	Rhizome	Cough and cold, snake bites	[13]
Artemisia absinthium L.	Compositae	Kohat	Whole plant	Skin infections, cold, vermifuge, gastric disorders, carminative	[14]
Asparagus racemosus Willd.	Asparagaceae	Swat	Root	Use in stomach related problems and also diuretic	[15]
Azadirachta indica A.Juss.	Meliaceae	Faisalabad	Leaves	Antiseptic, digestive and skin problems, fever, cough	[16]
Calendula arvensis M.Bieb.	Compositae	Cherat, Mardan, Malakand, Kohat	Leaves	Wound healing, disinfectant, antispasmodic and diuretic	[17]
Calophyllum inophyllum L.	Clusiaceae	Karachi	Leaves	Expectorant and diuretic, seed oil is used for skin ailments	[18]
Calotropis procera (Aiton) Dryand.	Apocynaceae	Kohat	Stem, leaves	Cholera, dermatitis, asthma, earache, expectorant, gastrointestinal, poultice used for dog bitten wounds, ring worm disorders and antihelmentic.	[19-21]
Camellia sinensis (L.) Kuntze (black tea)	Theaceae	Kohat	Leaves	Digestive problems	[22,23]
Cannabis sativa L.	Cannabinaceae	Cherat, Mardan, Malakand, Kohat	Leaves	As a cooling agent, antiseptic, stimulant, tonic and urinogenital diseases	[20]
Cichorium intybus L.	Compositae	Sawabi, Gawadar	Whole plant	Whole plant is used for vomiting and diarrhea	[20]
Cistanche tubulosa (Schenk) Wight	Orobanchaceae	Karak	Whole plant	Whole plant is used for diarrhea	[24]
Cymbopogon citratus (DC.) Stapf.	Poaceae	Kohat	Leaves	Febrifuge and flu	[20]
Datura innoxia Mill.	Solanaceae	Mirpur AJK	Leaves, seeds	Anti-inflammatory and laxative	[19]
Debregeasia saeneb (Forssk.) Hepper & J.R.I.Wood (=*Debregeasia salicifolia* (D.Don) Rendle)	Urticaceae	Islamabad	Stem	Antiseptic, skin ailments, urinary system complaints, anticancer	[25]
Dodonaea viscosa (L.) Jacq.	Sapindaceae	Cherat, Mardan, Malakand, Kohat	Leaves	Rheumatism, swelling and burns	[20]
Elaeagnus umbellata Thunb.	Elaeagnaceae	Rawalakot (Azad Kashmir)	Flower	Stimulant, astringent and to treat pulmonary infections	[26]
Eucalyptus camaldulensis Dehnh.	Myrtaceae	Cherat, Mardan, Malakand, Kohat	Leaves	Flu and cold	[27]
Ficus carica L.	Moraceae	Cherat, Mardan, Malakand, Kohat	Leaves	Wound healing, respiratory problems, measles, constipation, dysentery, laxative and antiseptic purposes	[20,28]
Fumaria indica (Hausskn.) Pugsley	Papaveraceae	Kohat	NA	Whole plant is used as a blood purifier and febrifuge	[20]
Geranium wallichianum D. Don ex sweet	Geraniaceae	Abbottabad	Rhizome, leaves	Mouth ulceration and dysentery	[29]
Glycyrrhiza glabra L.	Leguminosae	Peshawar	Rhizome	Respiratory problems	[30]
Hyssopus officinalis L.	Lamiaceae	Azad Kashmir (AJK)	Leaves	Respiratory problems	[11]
Justicia adhatoda L.	Acanthaceae	Margalla Hills	Whole plant	Rheumatism, stomachache, scabies ear ailments, asthma, bronchitis, cough and as an antiseptic, insect repellent, expectorant and antispasmodic	[19,20]
Malva neglecta Wallr.	Malvaceae	Swat	Whole plant	Diarrhea, dysentery, skin diseases and purgative for young cattle	[19,31]
Mentha longifolia (L.) L.	Lamiaceae	Cherat, Mardan, Malakand, Kohat	Leaves	Diarrhea, dysentery, stomachache and carminative	[19]
Olea europaea L.	Oleaceae	Cherat, Mardan, Malakand, Kohat	Leaves	Bronchial asthma, urinary infections, gallstones, diuretic, skin cleanser, hypotensive, emollient, laxative, febrifuge	[27]
Paeonia emodi Royle	Paeoniaceae	Swat	Whole plant	Tonic, gastro problems tuberculosis and eye diseases	[19]
Phyllanthus emblica L.	Phyllanthaceae	Kohat, AJK	Whole plant	Whole plant is used for tuberculosis, diabetes, gonorrhea, dysentery, diarrhea, rheumatism jaundice, bronchitis, scurvy, asthma, cooling effect, carminative, diuretic, laxative	[32]
Pistacia chinensis subsp. integerrima (J. L. Stewart ex Brandis) Rech. f. (=*Pistacia integerrima* J. L. Stewartex Brandis)	Anacardaceae	Islamabad	Fruits	Fruit used for cough, asthma, body tonic and expectorant	[19]
Rydingia limbata (Benth.) Scheen & V.A.Albert (=*Otostegia limbata* (Benth.) Boiss.)	Lamiaceae	Abottabad, Cherat, Mardan, Malakand, Kohat	Aerial part	Aerial parts and leaves are used for curing gums diseases, jaundice, ophthalmia and act as an antiulcer, antispasmodic, antidepressant	[19]
Skimmia laureola Franch.	Rutaceae	Thandiani (Abottabad)	NA	Respiratory tract problems	[19]
Solanum surattense Burm. f.	Solanaceae	Mirpur AJK	Fruit	Foot cracks, asthma, cough, fever, chest pain and jaundice, expectorant, digestive, diuretic, rheumatism	[19]
Solanum virginianum L. (=*Solanum xanthocarpum* Schrad. Wendl.)	Solanaceae	Lahore	Whole plant	Root, stem, leaves and flower used in sore throat, respiratory and stomach disorder, Root paste used for gums ailments, snake and scorpion bite	[33]
Trachyspermum ammi (L.) Sprague (=*Carum copticum* L. Benth. & Hook .f)	Apiaceae	Kohat	NA	Used to cure digestive problems, kidney stone, and whooping cough	[34]
Viscum album L.	Santalaceae	AJK	Leaves, twigs	Used in diabetes, jaundice, digestive problems, common fever and asthma	[32]
Withania somnifera (L.) Dunal	Solanaceae	Cherat, Mardan, Malakand, Kohat, Mirpur	Fruits and seeds	Carminative, diuretic, ophthalmia, liver disorders, asthamatic problems, insomnia, rheumatism	[35]
Woodfordia fruticosa (L.) S.Kurz	Lythraceae	Kohat	Flowers	Flowers are used in diarrhea and dysentery	[9]
Ziziphus jujuba Mill. (=*Ziziphus vulgaris* Lam.)	Rhamnaceae	Mianwali	Leaves, fruits	Leaves are used for digestion. Also effective in cough	[11]

3.1.1. Family Lamiaceae

Methanolic extract of leaves of *Hyssopus officinalis* (*H. officinalis)* at increasing concentrations has shown increased *S. aureus* inhibition[85]. Phytochemicals like pinocamphone, isopinocamphone, linalool and 1,8-cineole isolated from the oil of *H. officinalis* have reportedly shown notable antibacterial activities. Pinocamphone compound can abundantly be found in the oil of *H. officinalis* and hence needs to be tested for minimum inhibitory concentration (MIC)[11]. According to Subhan *et al*.[27], ethanolic extract of *Mentha longifolia* has 12 mm inhibition at 10 mg/mL (Table 2), with MIC and minimum bactericidal concentration (MBC) values of 1.5 and 2.5 mg/mL, respectively. This activity may be due to the phytochemicals including oxygenated monoterpenes, isomenthone, 1,8-cineole, borneol, and piperitenone oxide[36]. Ethanolic and methanolic extracts of *Rydingia limbata* also showed good inhibitory activities, which may be because of phytochemicals like limbatolide B and limbatolide C, oleanolic acid and b-sitosterol[39,40]. Chloroform, *n*-hexane, ethyl acetate and methanolic extracts of *Teucrium stocksianum* were tested for their anti-*S. aureus* activities, out of which ethyl acetate has shown maximum inhibition (24 mm) at the 1 mg/mL concentrations[42]. Acetone extract of aerial parts of *Salvia cabulica* produced maximum inhibition zone (14 mm) against *S. aureus*, while petroleum ether, butanol, and aqueous extracts have shown no activity at 1 mg/mL[41].

3.1.2. Family Solanaceae

In total, five plants belonging to the Solanaceae were tested for *in-vitro* activities against *S. aureus* (Table 3). Methanolic extract of *Solanum surrattense* showed increased inhibition with increasing concentration[43]. Out of different methanolic extract concentrations of *Solanum virginianum*, the chloroform showed maximum inhibitory zone (17.06 mm) at 100 mg/mL. Ethanol showed moderate inhibition at all concentration, *n*-hexane and aqueous at 50 and 100 mg/mL concentrations did not show any activity[48]. The phytochemicals isolated from *Solanum virginianum* including stearic acid, oleic acid, linoleic acid, A-solamargin, and coumarin may possess these antibacterial activities[49]. The antibacterial potential of stearic acid, oleic acid and linoleic acid has already been tested in a study[86]. Ethanolic extracts of *Withania somnifera* has greater inhibitory effect as compared to its methanol extract[27,43], and this inhibition might be due to the presence of active phytochemicals like withaferin, withanolides, and steroidal lactone[50]. Kharel *et al*.[87] studied the antimicrobial effect of these isolated phytoconstituents and found them active against many bacteria in the concentration equal to or higher than 2 mg/mL. Moreover, they found that the inhibition is increased with the concentration-dependent manner. At increasing concentrations, methanolic extract of *Datura innoxia* showed increasing *S. aureus* inhibition[43].

3.1.3. Family Compositae

Methanolic, ethanolic, *n*-hexane and acetone extracts of *Artemisia absinthium* and three widely used antibiotics were tested against *S. aureus*. The methanolic extract has shown maximum inhibition zone (13.66 mm) (Table 4); however, the bacterium showed resistance to erythromycin[9]. Myrcene, *trans*-thujone, *trans*-sabinyl acetate, chamazulene, nuciferolbutanoate, nuciferol propionate, caryophyllene oxide, monoterpene, esters, and sesquiterpenes (anti-*S. aureus* effect with MIC 0.10 mg/mL) are the main chemical components of *Artemisia absinthium* oil, which were tested in different biological activities and showed potential antibacterial effect against *S. aureus*[53,88]. Similarly, different extracts of *Cichorium intybus* was investigated against *S. aureus*, of which *n*-hexane showed good inhibition while chloroform did not show any inhibition at 20 mg/mL concentration[71]. The secondary metabolites like saponins, alkaloids, flavonoids, tannins and triterpenoids have been isolated from this plant, which carries antibacterial properties[68]. The ethanolic extract of *Calendula arvensis* showed 20 mm inhibition at 10 mg/mL concentration against *S. aureus*[27]. This activity may be due to the presence of sesquiterpene, glycosides, triterpene, saponin, and arvensoside A, B, C, as well as calandulasoids[54]. According to a study, saponins extracted from *Medicago* species showed antibacterial activity against *S. aureus*[89]. Similarly, another study proved that some triterpenes inhibited the growth of Gram positive and negative bacteria[34].

Table 2

Phytochemical and pharmacological data of effective Pakistani medicinal plants belong to Lamiaceae family against *S. aureus*.

Botanical name	Location(s)	Part used	Extracts	Concentration (mg/mL)	Inhibition zone (mm)	Phytoconstituents	References
Mentha longifolia L.	Cherat, Mardan, Malakand, Kohat, Nowshera	Leaves	Eth.	10 C	12	Oxygenated monoterpenes, borneol, isomenthone, 1,8-cineole, piperitenone oxide	[27,36-38]
Rydingia limbata (Benth.) (=*Otostegia limbata* (Benth.) Boiss.)	Abottabad, Cherat, Mardan, Kohat, Malakand	Aerial part	Eth.	8 C	18	Limbatolide A, limbatolide B and limbatolide C, oleanolic acid and b-sitosterol	[27,39,40]
			Meth.	8 C	16		
		Leaves	Eth.	10 C	19		
Salvia cabulica Benth.	Mach, Ziarat	Aerial parts	Dichloromethane	1 D	11	NA	[41]
			Acetone	1 D	14		
Teucrium stocksianum Boiss.	Parachinar Kurrum Agency	NA	Chloro.	1 D	12	NA	[42]
			n-Hex.	1 D	17		
			Ethyl ace.	1 D	24		
			Meth.	1D	13		

C=Extract concentration in their respective solvent; D=Extract dissolved in DMSO. Meth.=Methanol ; Eth.=Ethanol ; *n*-Hex.=*n*-Hexane; Ethyle ace.=Ethyle acetate; Chloro.=Chloroform; NA=not available.

3.1.4. Family Malvaceae

Chloroform extracts of *Malva neglecta* (*M. neglecta*) have maximum inhibition against *S. aureus*[90], which might be due to phytochemicals like flavonoids, hydroxyl cinnamic acids and phenol[91]. According to different studies, these phytochemicals have potentially shown antibacterial, antifungal and antiviral activities[92,93]. Walter *et al*.[11] studied the methanolic extract of *M. sylvestris* against *S. aureus* and compared the result with antibiotic ampicillin. The methanolic extract showed 3.1 and 2.1 mm inhibitory zones at a rate of 15 and 10 mg/mL, respectively. Several phytochemicals like malvone, terpenoids and phytoalexins have been isolated from *M. sylvestris*[94]. Likewise, methanolic extracts of *Althaea officinalis* at concentrations of 15 and 10 mg/mL have shown 2.7 mm and 2.3 mm inhibition zones, respectively against *S. aureus*[11]. However, in Iranian *Althaea officinalis*, the *n*-hexane extracts of flowers and roots produced 18.4 and 16.8 mm inhibition zone in *S. aureus*[95]. This inhibitory effect may due to chemical compounds like dihydrokaempferol 4'-*O*-glucoside, tiliroside, hypolaetin 8-*O*-gentiobioside, *n*-hexacos-2-enyl-1,5-olide (altheahexacosanyl lactone), 2β-hydroxycalamene (altheacalamene) and 5,6-dihydroxycoumarin-5-dodecanoate-6β-*D*-glucopyranoside (altheacoumarin glucoside)[96].

3.1.5. Family Apocynaceae

Stem and leaves extracts of *Calotropis procera* were reportedly tested *in-vitro* against *S. aureus*. The result showed that the butanolic extract of both stem and leaves have produced inhibitory zones 8.5 mm and 7.2 mm, respectively at 4 mg/mL[13]. *Calotropis procera* contains phytochemicals like alkaloids, flavonoids, tannins, steroids, triterpenoids and saponins[97]. Various kinds of leaves extracts of *Carissa spinarum* (*C. spinarum*) were also tested against *S. aureus*. All these extracts were found to be inactive except 20 mg/mL of chloroform extract dissolved in DMSO showed 10.2 mm anti-*S. aureus* inhibition[60]. According to Ahmed *et al*., ethanolic extracts of leaves and fruits of *C. spinarum* (3 mg/mL concentrations each) showed anti-*S. aureus* inhibitory effect of 23.1 mm and 19.3 mm, respectively[98]. Similarly in the same study, ethanolic extracts of leaves and fruits of *C. spinarum* (3 mg/mL concentrations each)

Table 3

Phytochemical and pharmacological data of effective Pakistani medicinal plants belong to Solanaceae family against *S. aureus*.

Botanical name	Location(s)	Part used	Extracts	Concentration (mg/mL)	Inhibition zone (mm)	Phytoconstituents	References
Datura innoxia Mill.	Mirpur AJK, Peshawar	Leaves	Meth.	2 D	24	NA	[43-45]
		Seeds	Meth.	2 D	18		
		Stem	Meth.	2 D	24		
		Root	Meth.	2 D	13		
			Aque	50 C	14		
Solanum nigrum L.	Muzaffargarh, Chakwal	Fruit	Water	20 C	14	Pentadecanoic acid,14-methyl-,methylester, 9,12-octadecadienoic acid, 9,12,15-octadecadienoic acid(2.2.2)-	[46,47]
			Meth.	5 C	10		
				15 C	13		
Solanum virginianum L. (=*Solanum xanthocarpum* Schrad. Wendl.)	Lahore, Muzaffargarh	Whole plant	*n*-Hex.	50 D	11.96	Stearic acid, oleic acid, linoleic acid, A-solamargin, coumarin	[33,46,48,49]
			Chloro.	50 D	14.96		
			Eth.	5 D	12.32		
			Aque.	5 D	15.04		
			Water	20 C	14		
			Meth.	20 C	14		
Withania somnifera (L.) Dunal	Mardan, Malakand, Kohat, Mirpur, Charsadda	Leaves	Eth.	10 C	21	Withaferin, withanolides, steroidal lactone	[27,43,50,51]
Withania coagulens /(L.) Dunal	Khyber Pakhtunkhwa	Root	Meth.	0.9 C	13	NA	[52]
		Leaves	Meth.	0.9 C	14		

C=Extract concentration in their respective solvent; D=Extract dissolved in DMSO. Meth.=Methanol; Eth.=Ethanol; *n*-Hex.=*n*-Hexane; Chloro.=Chloroform; NA=not available.

Table 4

Phytochemical and pharmacological data of effective Pakistani medicinal plants belong to Compositae family against *S. aureus*.

Botanical name	Location(s)	Part used	Extracts	Concentration (mg/mL)	Inhibition zone (mm)	Phytoconstituents	References
Artemisia absinthium L.	Kohat	Whole plant	Meth.	50 D	13.66	Myrcene, *trans*-thujone, *trans*-sabinyl acetate, monoterpene esters and sesquiterpenes	[9,53]
			Eth.	50 D	11.33		
			n-Hex.	50 D	12		
			Acetone	50 D	11		
Calendula arvensis M.Bieb.	Cherat, Mardan, Malakand, Kohat	Leaves	Eth.	10 C	20	Sesquiterpene, glycosides, triterpene, saponin, arvensoside, calandulasoids	[17,27,54]

C=Extract concentration in their respective solvent; D=Extract dissolved in DMSO. Meth.=Methanol; Eth.=Ethanol ; *n*-Hex.=*n*-Hexane.

Table 5

Phytochemical and pharmacological data of effective Pakistani medicinal plants against *S. aureus*.

Botanical name	Family name	Location(s)	Part used	Extracts	Concentration (mg/mL)	Inhibition zone (mm)	Phytoconstituents	References
Acacia nilotica (L.) Delile	Leguminosae	Kohat, Islamabad, Faisalabad	Leaves	Meth.	50 D	15.33	Niloticane	[34,51,55-57]
				Eth.	50 D	21		
				Acetone	50 D	21		
				Eth.	15 C	24		
				Chloro.	12.5 C	10		
			Bark	Meth.	1 C	11		
				Chloro.	15 C	14		
					12.5 C	10		
Acacia modesta L.	Leguminosae	Khyber Pakhtunkhwa	Bark	Meth.	0.9 C	14	NA	[52]
			Leaves	Meth.	0.9 C	18		
Glycyrrhiza glabra L.	Leguminosae	Peshawar, Lahore	Leaves	Meth.	15 D	2.4	Glycyrrhizin	[11,38,58,59]
			Root	Eth.	10 C	10		
Aesculus indica (Wall. ex Camb.) Hook.	Sapindaceae	Lower barian taien valley	Leaves	Meth	20 D	14.5	Tannin, saponins, flavonoids, steroids, terpenoids	[60,61]
				Chloro.	20 D	13		
				Ethyl ace.	20 D	12		
				Meth.	20 D	11		
				Aque.	20 D	13		
Dodonaea viscosa (L.) Jacq.	Sapindaceae	Cherat, Mardan, Malakand, Kohat, MuzzafarAbad	Leaves	Eth.	10 D	16	Tannins, saponins, flavanoids, terpenoids	[36,62,63]
			Arial parts	Eth.	3.2 D	12		
Elaeagnus angustifolia L.	Elaeagnaceae	Bannu	Whole plant	Meth.	1 D	15	Phenol, rutin, gallic acid, chlorogenic acid, hydroxybenzoic acids, hydroxy cinnamic acids, hydroxybenzoic acids	[64,65]
				n–Hex.	1 D	17		
				Ethyl ace.	1 D	25		
				Aque.	1 D	20		
Hippophae rhamnoides L.	Elaeagnaceae	Gilgit	Berries	Hex.	2 C	15.46	NA	[66]
					6 C	22.93		
				Chloro.	2 C	14.69		
					6 C	23.37		
				Aque	2 C	13.53		
					6 C	18.01		
			Leaves	Hex.	2 C	14.70		
					6 C	24.93		
				Chloro.	2 C	14.63		
					6 C	20.95		
				Aque	2 C	12.76		
					6 C	17.23		
Viburnum grandiflorum Wall. ex DC. (=*Viburnum foetens* Decne.)	Adoxaceae	Muzaffarabad	Leaves	Eth.	10 D	21.5	NA	[67]
				Meth.	10 D	26.33		
Viburnum nervosum D. Don	Adoxaceae	Muzaffarabad	Leaves					
	Eth.	10 D	21.33	NA				
				Meth.	10 D	19.16		
Punica granatum L.	Lythraceae	Kohat	NA	Meth.	50 D	20	NA	[9,68]
				Eth.	50 D	19		
				Acetone	50 D	19		
Woodfordia fruticosa (L.) S.Kurz	Lythraceae	Kohat	NA	Meth.	50 D	20	α-Pinene, β-selinene, β-caryophyllene, 2,6-dimethyl-1,3,5,7- octatetraene, γ-curcumene , germacrene D, caryophylleneoxide	[9,69]
				Eth.	50 D	17		
				n– Hex.	50 D	12		
				Acetone	50 D	16.5		
Arisaema jacquemontii	Araceae	Swat	Tubers	Meth.	1C	22	Alkaloids, saponins, tannins, sterols, flavonoids, protein, carbohydrates and fats	[70]
					3 C	40		
				n–Hex.	1 C	25		
					3 C	39		
				Ethyl ace.	1 C	35		
					3 C	45		
				Butanol	1 C	25		
					3 C	30		
Carissa spinarum L. (=*Carissa opaca* Stapf ex Haines)	Apocynaceae	Islamabad, Lahore, Margala Foothills Islamabad	Leaves, fruit	Chloro.	20 D	10.2	Phenolic compounds, flavonoids (orientin, isoquercetin,myricetin and apigenin)	[60,71-73]
				Eth.	3 C	23.1		
				Eth.	3 C	19.3		

Table 5

Phytochemical and pharmacological data of effective Pakistani medicinal plants against *S. aureus*. (Continued)

Botanical name	Family name	Location(s)	Part used	Extracts	Concentration (mg/mL)	Inhibition zone (mm)	Phytoconstituents	References
			Whole plant	Meth.	30 D	11		
Geranium wallichianum D. Don	Geraniaceae	Abbottabad	Rhizome, leaves	Meth.	1 D	14.33	β-sitosterol , β-sitosterol-glactoside, stigmasterol, 2,4,6 trihydroxyethylbenzoat	[29]
				Ethyl ace.	1 D	22.33		
Ziziphus mauritiana Lam.	Rhamnaceae	Khyber Pakhtunkhwa	Bark	Meth.	0.9 C	10	NA	[74]
			Leaves	Meth.	0.9 C	12		
Pistacia chinensis subsp. integerrima (J. L. Stewart ex Brandis) Rech. f.(=*Pistacia integerrima* J. L. Stewartex Brandis)	Anacardaceae	Islamabad	Stem	Crude	20 D	23	NA	[60,75]
				n–Hex.	20 D	12		
				Chloro.	20 D	11.66		
				Ethyl ace.	20 D	13.3		
				Meth.	20 D	11.5		

C=Extract concentration in their respective solvent; D=Extract dissolved in DMSO. Meth.=Methanol; Eth.=Ethanol; *n*–Hex.=*n*–Hexane ; Ethyle ace.=Ethyle acetate; Chloro.=Chloroform; NA=not available.

showed inhibitory effects in the range between 14.5 to 18.5 mm against four different methicillin-resistant *S. aureus* (MRSA). Sahreen *et al*. have isolated flavonoids, orientin, isoquercetin, myricetin, and apigenin belongs to phenols from this plant, which may be responsible for anti- *S. aureus* activity[72].

3.1.6. Family Lythraceae

Shinwari *et al*.[9] studied the antibacterial effects of alcoholic, *n*–hexane and acetone extracts of *Punica granatum*, and these results were compared with widely used antibiotics. All these extracts show strong inhibitory effect except *n*–hexane. Methanolic extract dissolved in DMSO at 50 mg/mL has shown optimum anti-*S. aureus* inhibition zone (20 mm) in comparison to gentamicin antibiotic (24 mm). Similarly, among the three tested antibiotics, *S. aureus* showed resistance to erythromycin. Various extracts of *Woodfordia fruticosa* were reportedly tested against *S. aureus*, of which ethanolic extract came up with maximum inhibitory effect (17 mm)[69]. These results are comparable to the tested antibiotics, in which gentamicin showed highest inhibition (24 mm) against *S. aureus*. The phytochemical study showed that the oil of *Woodfordia fruticosa* is a rich source of α-pinene, β-selinene, 2,6-dimethyl-1,3,5,7-octatetraene, β-caryophyllene, γ-curcumene, germacrene *D*-caryophylleneoxide compounds belongs to class momoterpenes[69]. Compound α-pinene extracted from Iranian *Stachys schtschegleevii* showed inhibition zone of 7 mm against *S. aureus* at 10 mg/mL concentration[99].

3.1.7. Family Adoxaceae

Different leaf extracts such as petroleum ether, chloroform, ethanol and methanol of two plants *Viburnum grandiflorum (V. grandiflorum)* and *Viburnum nervosum* were reportedly tested against *S. aureus*. Petroleum ether and chloroform extract of both plants did not show any inhibition zone while ethanol showed (21.5 and 21.33 mm) inhibition and methanol (26.33 and 19.16 mm) inhibition at 10 mg/mL concentration, respectively[67]. These results are comparable to the anti-*S. aureus* activities produced by antibiotics erythrocin (28.17 mm) and ciprofloxacin (24.33 mm). Overall, the reported findings are in line with the study conducted by Uddin *et al*.[55], who found 20 mm inhibition zone in *S. aureus* being produced by the methanolic extract of *V. grandiflorum* stem. *V. grandiflorum* holds various phytochemical constituents that belong to class steroids, terpenoids, anthraquinones, saponins and glycosides. Further chemical investigations of these phytochemicals and their detailed *in–vitro* activities would help in the identification of active compounds and mechanism of action against *S. aureus*.

3.1.8. Family Leguminosae

Alcoholic, *n*–hexane and acetone extract of *Acacia nilotica* were compared with the commonly used antibiotics. All the extracts showed potential antibacterial activities except *n*–hexane extract[9]. Similarly, among the three tested antibiotics, *S. aureus* showed resistance to erythromycin. Niloticane isolated from *Acacia nilotica* is responsible for its anti-*S. aureus* activity with MIC values recorded as 8 μg/mL[100]. Mahesh and Satish tested the methanolic extract of *Acacia nilotica* leaves (10 mg/mL concentration), which produced 15 mm inhibition against *S. aureus*[101]. The methanolic extract of *Glycyrrhiza glabra* tested at different concentrations has shown an increasing trend in *S. aureus* inhibition at rising methanol concentrations[25]. In another study, Ateş and Turğay[102] found 7 mm/20 μL inhibition against *S. aureus* by the alcohol, ethyl acetate, acetone and chloroform extracts of *Glycyrrhiza glabra*.

3.1.9. Family Sapindaceae

Different extracts of *Aesculus indica* were reportedly tested for *in–vitro* activity against *S. aureus*. Crude extract was the most active with an inhibition zone of 14.5 mm among all the tested extracts. The *n*–hexane fraction did not show any activity, while chloroform and aqueous showed the same activity, i.e., 13 mm inhibition. Moreover, ethyl acetate and methanol showed inhibition zones of 12 and 11 mm, respectively[60]. Flavonoids, terpenoids, and steroids isolated from *Aesculus indica* reportedly carry diverse pharmacological properties[61]. Terpenoids isolated from *Aesculus indica* have shown potent inhibitory effect against *S. aureus* with MIC values ranges from 4–256 μg/mL[103]. Flavonoids and tannin has also been tested for anti-*S. aureus* activity[104,105]. Ethanolic extracts of *Dodonaea viscosa* leaves (10 mg/mL) and aerial parts (3.2 mg/mL) showed 16 and 12 mm inhibition, respectively in *S. aureus*; however, aqueous extract was found inactive[27,62]. Tannins, saponins, terpenoids and flavonoids isolated phytochemicals may be supportive in increasing the antibacterial effect of this plant[63]. Four kaempferol methyl ethers were isolated from the leaves extracts of South African Dodonaea viscose. These compounds are (i) 4'-*O*-methylkaempferol

(3,5,7-trihydroxy-4'-methoxyflavone), (ii) 5,7,4'-trihydroxy-3,6-dimethoxyflavone, (iii) 5,7-dihydroxy-3,6,4'-trimethoxyflavone (santin), (iv) 5-hydroxyl-3,7,4'-trimethoxyflavone, and (v) 3,4',5,7-tetrahydroxyflavone (kaempferol). Among all these compounds, iii and v were observed most active at a rate of 63 μg/mL concentrations in producing MIC in *S. aureus*[106]. However, testing the effective and toxicity doses of these compounds need to be carried out.

3.1.10. Family Elaeagnaceae

Species belong to genus *Elaeagnus* are important medicinal plants being commonly used by the local people for various human infectious diseases including the one caused by *S. aureus*. *Elaeagnus angustifolia* (*E. angustifolia*) extracts were tested against *S. aureus* and it was found that ethyl acetate extract has shown maximum inhibition (25 mm), methanol showed minimum inhibition (15 mm), while chloroform showed no inhibition activity at each 1 mg/mL concentrations dissolved in DMSO. The phytochemicals of *E. angustifolia* are acid esters including phenol, rutin, gallic acid, chlorogenic acid, hydroxybenzoic acids, hydroxycinnamic acids and hydroxybenzoic acids (strong anti-*S. aureus* inhibition at 500 μg/mL) can have better antibacterial effect than extracts[64]. Similarly, different extracts of flower, leaves and berry of *E. umbellate* were reportedly tested *in-vitro* against *S. aureus*. Out of these, ether extract of flower and aqueous extract of berry possesses highest inhibition zones, *i.e.*, 14 mm each at 200 mg/mL concentrations. Rutin, gallic acid, chlorogenic acid, hydroxybenzoic acids and hydroxycinnamic acids are the reported chemical compounds isolated from plants[26]. These *in-vitro* activities support the validation of ethnomedicinal uses of both *Elaeagnus* species against *S. aureus* diseases. Different extracts of berries and leaves of *Hippophae rhamnoides* have shown good activity with increasing concentration and effective at very low concentration against *S. aureus*[66].

3.1.11. Other plant families

A number of plant extracts of families other than listed above have also reportedly shown good anti-*S. aureus* activities. As an example, methanolic extract of root of *Asparagus racemosus* (Asparagaceae) has shown significant antibacterial effect with increasing concentration[15]. Gobicusin A, asparacosin A and muzanzagenin are steroidal saponins compounds being isolated from *Asparagus racemosus* were tested for anti-*S. aureus* effect and showed 0.05 mg/mL MIC each, while isoagatharesinol showed slightly lower MIC value of 0.12 mg/mL in comparison to the antibiotic streptomycin by showing 0.01 mg/mL MIC[107]. In a study, it was revealed that muzanzagenin isolated from *Asparagus africanus* exhibit anti-leishmanial and anti-plasmodial activity[108]. Hence, all of these compounds (asparacosin A, gobicusin A, isoagatharesinol, and muzanzagenin) have the potential biological activities and may further be evaluated against different strains of *S. aureus* and other bacteria.

Different extracts of leaves of *Calophyllum inophyllum* (Clusiaceae) and isolated compounds were tested for *in-vitro* activity against *S. aureus* and found that all the extracts and compounds have shown good inhibitory effect except butanol and canophyllic acid[18]. Friedelin and inophynone compounds belongs to terpenes group being isolated from *Calophyllum inophyllum* have shown 6.60 and 4.51 mm inhibition zones, respectively against *S. aureus*. In a study by Yasunaka *et al*., friedelin being isolated from *Calophyllum brasiliense* has shown MIC of >512 μg/mL against both methicillin-sensitive and methicillin-resistant *S. aureus*[109].

Ethanolic extract of leaves of *Cannabis sativa* (Cannabinaceae) produced 20 mm zone of inhibition against *S. aureus* at 10 mg/mL[27]. Cannabinoid, a compound isolated from *Cannabis sativa* and show good inhibition at 0.5–2 μg/mL[110,111]. Methanolic extract of *Berberis aristata* (Berberidaceae) have shown good inhibition and different classes of compounds are present in these extract are alkaloids, flavonoids, terpenoids, saponins, and glycosides[112]. Different extracts of *Aerva javanica* (Amaranthaceae) have shown inhibition against *S. aureus* but methanolic extract show good inhibition[113]. Various extracts of *Cinnamomum verum* (Lauraceae) (*C. verum*) including methanolic, ethanolic, *n*-hexane and acetone and three antibiotics were tested against *S. aureus* for activity. All the extracts remain inactive except *n*-hexane which showed 13 inhibition zone at 50 mg/mL concentration while among three antibiotics, Erythromycin did not show any inhibition against *S. aureus*[9]. The isolated phytoconsitituents eugenol, monoterpenes, protocatechuic acid, urolignoside, cinnamaldehyde, benzaldehyde, and sesquiterpenes might be responsible for the antibacterial effect of this plant[110]. *C. verum* is rich in essential oils (mainly cinnamaldehyde and eugenol), which can inhibit the microbial growth. A study has shown that the essential oil of *C. verum* exhibit a strong antibacterial activity against *S. aureus* mainly because of cinnamaldehyde, which is the major component of the oil (73.35%). Cinnamaldehyde is reportedly effective against a wide range of bacteria both Gram-positive and Gram-negative[114]. Another study revealed that eugenol isolated from *Syzygium aromaticum* and cinnamaldehyde isolated from *C. verum* have shown significantly higher inhibition zones (>20 mm) against *S. aureus* with MIC 0.31 mg/mL[115,116].

Crude ethanolic extract of *Ficus carica* leaves at 10 mg/mL showed 20 mm inhibition zone against *S. aureus*. Leaf extract of *Ficus carica* had the highest total phenolic contents[(52.296±5.232) and (48.973±2.015) mg gallic acid equivalent/g of dry plant extract respectively] and flavonoids [(14.388±0.333) and (14.136±1.082) mg quercetin equivalent/g of dry plant extract]. These extracts showed bactericidal activity and moderate antifungal activity[117]. Phytochemicals like steroids, alkaloids, furano coumarins, flavonoids (rutin, quercetin, and luteolin), coumarins, saponins and terpenes are isolated[118]. Different fractions of rhizome and leaves of *Geranium wallichianum* (Geraniaceae) have been reportedly tested against *S. aureus*. Only methanolic (1 mg/mL) and ethyl acetate (1 mg/mL) extracts have shown anti-*S. aureus* activity, *i.e.*, 14.33 and 22.33 mm, respectively, while the rest extracts have shown no antibacterial activity. β-sitosterol, β-Sitosterol-glactoside, stigmasterol, 2,4,6-trihydroxyethylbenzoate are some of the isolated phytochemicals from *Geranium wallichianum*[29,37]. Methanolic extract of *Paeonia emodi* (Paeoniaceae) roots showed 15 mm inhibition at 5 mg/mL concentration[119] that may be due to the presence of paeonins A and B, and monoterpenegalactosides[120]. *Quercus floribunda* (Fagaceae) is a medicinal plant that is

traditionally used to treat asthma, diarrhea, and gonorrhea. Its methanolic extract at 25 mg/mL showed highest anti-*S. aureus* inhibition (19.8 mm). Quercetin, gallic acid, rutin and alkaloids are some of the reported phytochemicals isolated from this plant[121]. Quercetin has reportedly shown strong anti-*S. aureus* activities[122].

3.2. Mechanisms of antimicrobial actions

Phytoconstituents may exhibit different action mechanism against bacteria ranging from interference with cell membranes, loss of cellular constituents, enzymes damage, and disturbance of the genetic material. Overall, the action mechanism is considered to be damaging cytoplasmic membrane, disturbing proton motive force, electron flow, active transport mechanisms and coagulation of cell composition. Phenolics class of compounds destabilized and permeabilized cytoplasmic membrane and inhibits enzyme activity by the oxidized products, *e.g.*, reaction of quinines with amino acids and proteins. Phenols can also inhibit the synthesis of nucleic acids of both Gram-negative and Gram-positive bacteria. Types of microorganisms, its cell membrane structure and composition are considered important in the susceptibility to antimicrobials. The passage via outer membrane in Gram-negative bacteria is dependent on the chemical nature of antimicrobial product and regulated by the occurrence of hydrophilic channels, known as porins. Phytochemical constituents of *Moringa oleifera* can cause enzyme inhibition in bacteria (sortase inhibitory effect), replication of DNA, action by the bacterial toxin and bacterial cell lysis. Tannins are polyphenols having the ability to restrain the proliferation of bacterial cells through blocking the essential enzymes of microbial metabolism. Saponins might act as altering the permeability of cell walls and can combine with the cell membranes to bring out certain changes in cell morphology that may lead to cell lysis. An example of herbs including tarragon and thyme contain caffeic acid that is efficient against bacteria, fungi, and viruses. Catechol and pyrogallol both are hydroxylated phenols, which are toxic to certain microorganisms. Catechol possesses two hydroxyl groups while pyrogallol contains three. The sites and number of hydroxyl groups on the phenol group are believed to add in the microbial toxicity. This is being evident when increasing hydroxylation was related to increased microbial toxicity[123].

3.3. Toxicology

The toxicology of some isolated compounds and their associated plant species are being discussed in this sub-section. *Acacia nilotica* is one of the beneficial medicinal plants; however, its acute toxicity in rats has been proved. Eight percent of the extract was given to the animal for 4 weeks and a significant reduction in body weight, hemoglobin and cholesterol were observed[124]. Thus, the toxicity of the plant may be due to its frequently occurring compound such as niloticane. Furthermore, eugenol is a compound whose amount is approximately 86% in the essential oils of *C. verum* leaves[125]. According to the U.S. Food and Drug Administration, this compound is considered safe[126]. However, it has severe toxic effects on animals and human beings. As an example, it killed wet human dental tissues[127], broke DNA in human VH10 fibroblasts and caused cytotoxicity in malignant HepG2 hepatoma cells, malignant Caco-2 colon cells, and nonmalignant human VH10 fibroblasts[128]. Similarly, the compound decreased body weight with increased salivation, and abnormal breathing patterns in rats[129]. Similarly, asparacosine A, a compound belonging to the plant species A. racemosus, has reportedly produced cytotoxicity in different human cells in cultures including Lu1, Col2, LNCaP, HUVEC, and KB at the IC_{50} values between 10.7 μg/mL to>20 μg/mL[130]. Additionally, the plant extract caused teratological disorders *i.e.* desorption of fetuses and gross malformations in rats at 100 mg/kg/day for 60 days[131]. Thus, the toxicity of *Asparagus racemosus* might be due to asparacosine A and some other compounds such asisoagatharesinol, gobicusin A and muzanzagenin. Furthermore, altheahexacosanyl lactone is the frequently occurring compound in *Althaea officinalis*. According to a study, 10 g of the *Althaea officinalis* extract caused cytotoxicity and changes in blood biochemical parameters of common carp (*Cyprinus carpio*)[132]. Likewise, friedelin and inophynone, compounds of the species *Calophyllum inophyllum*, might also be harmful for animals as the unrefined oil of the plant has been proved for its toxic effects. There was a significant difference in the plasma cholesterol levels of the rats fed with *Calophyllum inophyllum* oil when compared to the control. Hence, more studies to be undertaken in order to understand the toxicological nature of plant species and their compounds in the processes of identification of safe drugs.

4. Conclusions and future recommendations

The review collects the traditional uses of medicinal plants against various diseases caused by *S. aureus*. The study also reviewed the *in-vitro* anti-*S. aureus* activities of various extracts of the reported medicinal plants along the active phytochemicals possessed by these plants. Overall, the *in-vitro* reports of different studies support the traditional uses of medicinal plants in the world in general and Pakistan in particular for the treatment of different ailments caused by *S. aureus*. However, there are certain lope holes in the previous studies, which are needed to be addressed for a complete set of knowledge on this topic. As an example in traditional uses, there is limited data available on doses range of medicinal plant, the period of intake and recovery against a certain disease caused by *S. aureus*. This kind of information is extremely important for the comparison of ethnomedicinal data with pharmacological and toxicological dataset of a plant species.

Almost all the discussed families showed good inhibition against the *S. aureus*. However, the frequently used anti-*S. aureus* families were Lamiaceae and Solanaceae. One of the possible reasons is the availability of a high number of plant species in these two families. Another reason could be higher ethnopharmacological efficacy of the members of these two families. Therefore, more ehtnopharmacological data on the plant species in these families should be explored for novel results.

Although most of the plants have shown good inhibition zone against *S. aureus* but there is little data on their MIC and MBC

values, so there is need to find these values. Species such as *A. racemosus*, *E. angustifolia*, *Teucrium stocksianum*, *Geranium wallichianum*, *Salvia cabulica*, *Quercus floribunda*, *Carissa spinarum* and *Cistanche tubulosa* may be given preference for further investigation related to anti-*S. aureus* activities. Plant families including Asparagaceae, Elaeagnaceae, Lamiaceae, Geraniaceae, Fagaceae, Apocynaceae and Orobanchaceae should be subjected to phytochemical and pharmacological investigations against *S. aureus*.

In Pakistan, methanol and ethanol solvent is mostly preferred for plants extraction process, which might be due to its significant results against *S. aureus*. Moreover, leaves are major plant part used in extraction and show good inhibition. Similarly, phytochemical classes like alkaloids, flavonoids, saponins and tannins should also be evaluated for anti-*S. aureus* activities. Moreover, majority of the plants has been observed with less number of pure phytochemical compounds oriented anti-*S. aureus* activities. At present, only some of the isolated compounds from the reported plants such as altheahexacosanyl lactone, cinnamaldehyde, niloticane, gobicusin A, asparacosin A, muzanzagenin, isoagatharesinol, friedelin and inophynone, eugenol have shown anti-*S. aureus* activities. These compounds should be carried forward for further investigations including, toxicology, *in-vitro*, *in-vivo* and mechanism of actions against *S. aureus*.

Other unexplored plants should also be investigated to the compounds level for anti-*S. aureus* activities. This is the essential part of the pharmacological activities, which will lead us to the evaluation of those phytoconstituents that are still unknown and could serve as potent antimicrobial agents. Such antibacterial agent from natural sources could be more effective against bacterial pathogens and will help to minimize the adverse effects of synthetic drugs and would provide a base for the pharmaceutical industries. Additionally, isolated phytochemical compounds may also be subjected to the development of several derivates, which can be tested *in-vitro* and *in-vivo* against *S. aureus*.

Another important aspect reportedly missing is related to the documented medicinal plants *in-vivo* studies on different animal models, which should also be considered in future studies. Clinical trials should also be taken into account along with absorption and metabolism of compounds that would allow in calculating the amount of compounds reaches the site to be treated. Mechanism of action of the plant extracts or isolated compounds on *S. aureus* would also bring the ethnopharmacological studies a bit further. Moreover, there is a dire need for testing the toxicities of the documented plants at various levels.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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