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PAKISTANI ANTI-MASTITIS MEDICINAL PLANTS AND THEIR SCIENTIFIC VALIDATION AGAINST MULTIDRUG RESISTANCE MASTINOGEN *STAPHYLOCOCCUS AUREUS*

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Abstract

Mastitis is an infectious disease of livestock affecting agricultural economy especially in developing countries. Present review was designed to gather literature about the traditional uses of Pakistani anti-mastitis plants and their *In vitro* background against a common multidrug resistant mastitis causing bacteria *Staphylococcus aureus*. Different online search engines were used use to collect data such as Web of Science, Scopus, Google Scholar and Pub Med.Traditional healers of Pakistan used 38 plants for the treatment of mastitis in buffaloes and cows. Zingiberaceae, Asteraceae, Rutaceae and Solanaceae (3 plants each) were the most preferred plant families while the roots, fruits and seeds were the most commonly used parts for the treatment of mastitis in Pakistan. Thirteen plants were reported to be evaluated for their antibacterial activities. Different extracts had shown good activities against *S. aureus*, however, ethanolic, aqueous and methanolic extracts shown greater inhibition zone (10-25mm). Variety of phytochemical classes was identified from in-vitro tested plants such as saponins, glycosides, alkaloids, carbohydrates flavonoids and terpenoids. Literature review showed that *S. aureus* is resistant (60-100%) to commonly used antibiotics against mastitis. In Pakistan large number of plants are being used against mastitis but most of the regions are yet to explore. Moreover, many reported plants were found to be active against *S. aureus* while large number of plants is still unexplored. There is a dire need to expedite detailed ethnopharmacological studies on unexplored anti-mastitis plants for the replacement of current drugs with new plant based drugs.

Key words: Livestock, Mastitis, Ethnoveterinary plants, Pakistan.

Introduction

Mastitis is an infectious and costly disease of dairy animals affecting industrial economy around the globe especially in developing countries (Yousaf *et al*., 2012). It is an udder disease and change the color, odor and taste of the milk. The main causative agent of mastitis is *Staphylococcus aureus* (Rossi *et al*., 2011) while there are many other bacterial species involved in causing mastitis such as *Corynebacterium pyogenes, Pseudomonas mendocina, Klebsiella pneumoniae, Escherichia coli* and *Micrococcus pyogenes* (Yousaf *et al*., 2012; Dharajiya *et al*., 2012). As mastitis decreases the quantity and quality of milk, therefore the income of local farmers also decreases. Depletion in dairy sources results in loss of economics for example, worldwide about $35 billion and $2 billion in the United States (Kenyanjui *et al*., 2009; Mubarack *et al.,* 2011). Dairy animals not only helpful for food industries, infact large number of Pakistan inhabitants (75%) living in rural areas are dependent on agriculture (Dilshad *et al.,* 2010). Almost 30-35 million people living in rural areas of Pakistan have household holdings of 5-6 sheep/goat and 2-3 cattle/buffalo each family and their 30-40 percent income come from livestock (Iqbal *et al*., 2005). Pakistan is the5th largest milk producer (31 million tons of milk annually) country in the world (Kenyanjui *et al*., 2009; Hassan *et al*., 2014).Worldwide there are different antibiotics being used against mastitis and most of the mastinogens have now been resistant to many commonly used antibiotics (Roesch *et al*., 2006). Present of drug residues in milk possibly affects the health of consumers because it may interfere with intestinal flora, allergic reactions, resulting in ineffectiveness of antibiotic treatment (Bharti *et al*., 2012). Large number of Pakistani farmers cannot afford modern health facilities due to financial constraints which lead toward poor health of their livestock and economic loss. Ethnomedicines of plants are considered as best alternatives for the treatment of various diseases (Ali & Qaiser, 2009; Hamayun *et al*., 2006; Shinwari *et al*., 2006; Walter *et al*., 2011). Rural population of Pakistan is highly dependent on natural resources because of rich traditional knowledge transferred to them from their ancestors (Sarwat *et al*., 2012; Gul *et al*., 2012; Deeb *et al*., 2013). They are using different plants for the treatment of their livestock diseases especially mastitis for compensating their income and improving their livelihood (Sindhu *et al*., 2010).

To best of our knowledge present review is the first attempt to gather fragmented literature on Pakistani anti-mastitis plants and their in-vitro validation against most common mastinogen Staphylococcus *aureus*. This review would disclose scientific gaps in present knowledge and help researchers to carry out future researches for the development of safe and novel plant based veterinary drugs for the treatment of mastitis.

Methodology: Present study was designed by gathering fragmented literature (mostly published) about Pakistani anti-mastitis plants, their scientific validation against most common mastinogen *Staphylococcus aureus*. Different search engines like Flora of Pakistan, Google Scholar, Web of Science and Pub Med were searched for the collection of our desired data. Search indicators such as anti-mastitis plants of Pakistan, anti-bacterial activities of medicinal plants, drug resistivity of *S. aureus* etc were used to collect data from search engines. Total 62 researches published in various journals were considered for this review. Different criteria's were followed for the selection of articles such as i) Detailed information about ethnoveterinary plants of Pakistan ii) those anti-bacterial studies in which the units of concentration (mg/ml) and inhibition zone (mm) were same iii) recent reports on *S. aureus* drugs resistance were included. Data was organized in the form of three tables using Microsoft Excel and Word 2007. Information about phytochemical compounds of anti-mastitis plants was also reported and mentioned.

Ethnoveterinary practices used to treat mastitis in Pakistan: In Pakistan agriculture is a main sector for income generation and dairy animals are playing an important role in growing agricultural economy. Pakistan is among leading countries for producing milk due to greater dependency of rural population on livestock (cows and buffaloes). In Pakistan dairy animals are exposed to numerous infections and diseases and mastitis is one of the most common diseases among them because the causal agent is commonly occurring bacteria *S. aureus* (Sharif & Muhammad, 2009). In Pakistan traditional medicines use is common because of low socioeconomic situation of local farmers and inhabitants. Local inhabitants of the rural regions cannot afford modern allopathic drugs and facilities because of low accessibility to the modern drugs in city areas and financial constraints (Hassan *et al*., 2014). Local farmers of Pakistan use large number of medicinal plants for treating their dairy animals. In present review total 38 anti-mastitis plants were reported (Table 1) to be used in Pakistan. Ethnoveterinary practices are also common in other regions of Pakistan but still there is a lack of proper scientific documentation due to negligence of ethnoveterinary medicines in Pakistan. Asteraceae, Rutaceae, Solanaceae and Zingiberaceae were reported to be more frequently used anti-mastitis plant families in Pakistan. Not only in Pakistan, infact throughout the world these families are frequently being used in ethnoveterinary practices (Dilshad *et al*., 2010; Yigezu *et al*., 2014) possibly because of higher abundance or potent therapeutic potential.

Different plant parts were reported to be used against mastitis but roots, seeds and fruit were most commonly used parts in Pakistan. Present findings are in contrast with several studies from other countries (Grade *et al*., 2009; Monteiro *et al*., 2011), however, higher utilization of roots in ethnoveterinary practices have also been documented in other regions of the world (Lulekal *et al*., 2014). Use of seeds and fruits does not endanger plants life but harvesting roots can cause plants death and considered as serious conservation issue. Farmers in Pakistan have rich traditional knowledge for formulating herbal recipes to treat mastitis. Local farmers have good knowledge about using appropriate amount of plant parts in formulations and the proper dosage (Table 1). Different additives or vehicles (honey, salt, water and flour) are being used in different recipes in order to reduce bitter taste of plant remedies and to confirm ample dose. Recovery time of ethnoveterinary medicines was reported to be range in5-7 days indicating strong healing properties and potential of anti-mastitis of Pakistan.

***In vitro* activities of anti-mastitis plants against *Staphylococcus aureus*:** Pakistani farmers strongly believe in high effectiveness of traditional medicines because of fewer side effects than modern allopathic drugs. Present review reported 38 medicinal plants from Pakistan but only 13 plants have been reportedly evaluated throughout the world for their *In vitro* authentication against most common mastinogen *S. aureus*. *In vitro* investigated plants shown good activities against *S. aureus* (Table 2). Interestingly, many parts of plant have been reported to be used in extract formation; however, roots, seeds, fruits and whole plants were commonly used giving an indication about the reliability and potency of traditional medicines practices. Present review showed that different extract of plants were used at different concentrations (mg/ml) against *S. aureus*, however, aqueous, methanol and ethanol extracts are most widely used extraction techniques. Present findings are in line with other studies in which methanolic and ethanolic extracts are most preferred extraction techniques (Ncube *et al*., 2008) because these solvents have polar nature and easily degrade cell wall and release polyphenols from the cell of plants (Shinwari *et al*., 2013). Among all reported extracts, ethanolic and methanolic extracts had shown good inhibition zones ranging from 15-25 and 10-20 mm respectively against *S. aureus* (Agrawal *et al*., 2013; Shiri *et al*., 2013; Doss *et al*., 2012; Gull *et al*., 2012). Moreover, other solvent extraction techniques such as ethyl acetate, benzene and chloroform etc have shown satisfactory activities against reported bacteria. No detailed phytochemical studies (pure compounds) on *In vitro* studied plant parts were reported, however several classes of compounds have been reportedly isolated from the studied plant parts such as glycosides, flavonoids, terpenoids, alkaloids, steroids, saponins, and carbohydrates that are responsible for anti-bacterial activities. Moreover, plant extracts had shown more potent effects than antibiotics against mastitis. There is a dire need to expedite *In vitro* studies as well as clinical trials on ethnoveterinary anti-mastitis plants that could lead toward the screening of novel compounds and development of veterinary drugs.

Table 1. Ethnomedicinal uses of Pakistani medicinal plants against Mastitis.

Botanical name	Local name	Area	Part used	Recipes	Dairy animal	References
Allium cepa L. Liliaceae	Piaz	Faisalabad	Bulb	500-1000 g piaz alone or in combination with ajvain	Cow	(Bilal *et al*., 2009)
Allium sativum L. Alliaceae	Lehson	Sargodha	Rhizome	250g, grinded powder with butter is given orally for almost 7 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Amaranthus graecizans L. Juncacaeae	Kahan	Karak	Whole plant	100 g of plant is taken and grind to make fine powder and mixed with sugar and given for one week	Cattles	(Shah *et al*., 2012)
Amomum subulatum Roxb. Zingiberaceae	Baree Ilaichee	Sargodha	Fruit	25g, given orally for 3 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Brassica campestris L.Brassicaceae	Sarsoon	Sargodha	Seeds oil	500ml, given orally for 10 days.	Cows and buffaloes	(Dilshad *et al*., 2010)
Capparis decidua (Forssk.) Edgew. Capparidaceae	Karir	Sargodha	Fruit	50g, administered orally for 3 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Capsicum annuum L. Solanaceae	Lal mirch	Sargodha	Fruit/ whole plant	50g, administered orally for 8 days	Cows and buffaloes	(Dilshad *et al.,* 2010)
Capsicum frutescens L. Solanaceaea	Surkh mirch	Faisalabad	Fruit	125g fruit pulverized cooked in water given*PO* 4- 5 days	Livestock	(Deeba *et al.,* 2009)
Centratherum anthelmisticum L Asteraceae	Kali Zeeri	Sargodha	Seeds	50g of seeds are mixed with wheat flour and administered orally for 5 days	Cows and buffaloes	(Dilshad *et al.,* 2010)
Citrullus colocynthis L. Cucurbitaceae	Indryan/Kor tuma	Sargodha	Fruit	2-3 pieces given orally daily for 5 days	Cows and buffaloes	(Dilshad *et al*., 2010; Khatibi &Teymorr 2011)
Citrus limon (L.) Burm. f Rutaceae	Khatian	Sargodha	Fruit	250g, cut the fruit and place it in dew drops for a complete night and dust common salt before given, it should be given orally for 5 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Citrus limon L. Osbeck*Rutaceae*	Nimbu	Himalaya	Fruit	Fruit juice is mixed with sugar and this pasteis fed to animals and applied topically (to themammary glands) for 10–15 days	Livestock	(Abbasi *et al*., 2013)
Citrus reticulata Blanco.Rutaceae	Malta	Bannu	Leaves	Fresh heated leaves decoction is prepared and apply externally, while the dried leaves decoction is given orally.	Cattles	(Khan *et al*., 2013)
Cuminum cyminum L. Asteraceae	Sufaid zeera	Sargodha	Seeds	1 kg, given orally for 6 days.	Cows and buffaloes	(Dilshad *et al*., 2010)
Curcuma longa L. Zingiberaceae	Haldi	Sargodha	Roots	25g, proper grinding of roots with sugar and administered orally for 7 days.	Cows and buffaloes	(Dilshad *et al*., 2010)
Foeniculum vulgare Mill Apiaceae	Saunf	Sargodha	Seeds	50g, roasted seeds mixed in vegetable oil (125ml) and drenched for 4 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Galium aparine L. Rubiaceae	Banafsha	Sargodha	Vine	500g, given as decoction drench for 3 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Gossypium hirsutum L. Malvaceae	Paiway/ waraiwain	Sargodha	Flowers	250g, of flowers are boiled in about 1L water and drenched for 3 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Lepidium sativum L. Brassicaceae	Halia	Sargodha	Seeds	500g, of seeds put in 2L of milk and boiled, givenorally for 8 days	Cows and buffaloes	(Dilshad *et al*., 2010)

Table 1. (Cont'd.).

Botanical name	Local name	Area	Part used	Recipes	Dairy animal	References
Linum usitatissimum L. Linaceae	Alsi	Sargodha	Seeds	25g of seeds are mixed with 3-4 *Citrus limon*extract and add sugar, administered orally for 5 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Narcissus tazetta Linn Amaryllidaceae	Gul-e-Nargis	Allai valley	Roots	10 gm of roots is grind and make powder mix with salt and given to cattle's two time in a day for the period of 10 days	Cattles	(Haq, 2012)
Nigella sativa L. Ranunculaceae	Kaoolnji	Sargodha	Seeds	50g of seeds are boiled in either 250 ml or 2L of and drenched for three days in winter season.	Cows and buffaloes	(Dilshad *et al*., 2010)
Oryza sativa L. Poaceae	Chawal/Moonji	Sargodha	Seeds	500g boiled in 2L milk + sugar 500g and administered orally for 8 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Piper nigrum L. Piperaceae	Kali mirch	Faisalabad	Pepper corn	Pepper corn 30g + *Capsicumannuum* L. fruit 125 g+ *Capsicum frutescens* L. fruit125 g Grated given *PO* f or 6-7 days	Livestock	(Deeba *et al*., 2009)
Bistorta amplexicaul L. Polygonaceae	Anjbar	Sargodha	Bark	125g, of bark is boiled in 250 ml to 1L water and given orally for 4 days.	Cows and buffaloes	(Dilshad *et al*., 2010)
*Protulaca oleracea*Linn. Portulacaceae	Loonrak	Dera Ghazi Khan	Whole plants	Given orally	Livestock	(Gulshan*et al*., 2012)
Rosa indica L. Rosaceae	Gulab	Sargodha	Petals	750g of petals are boiled in I L of milk and drenched regularly for for 7 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Saccharum officinarum L. Poaceae	Kamad	Sargodha	Extract	2 L, drenched daily for 7 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Sesamum indicum L. Pedaliaceae	Meetha tael	Sargodha	Seed oil	250 ml, mixed oil in 1.5L of milk whey, and given orally for 7days	Cows and buffaloes	(Dilshad *et al*., 2010)
Sorghum halepense (L.) Pers Poaceae	Barron gass	Poonch valley, Azad Kashmir	Root	Root decoction is mixed with mud of pound and pasted on teats of cattle to cure mastitis	Cow and buffaloes	(Khan *et al*., 2012)
Thymus serpyllum L. Lamiaceae	Sattar, Jangli Podina	D.I.Khan	Leaves	Its paste applied to udder	Livestock	(Marwat *et al*., 2009)
Trachyspermum ammi L.Apiaceae	Ajvain	Faisalabad	Seeds	80-100 g seed are taken and decoction is given to cattle's	To treat mastitis	(Bilal *et al*., 2009)
Trichodesma indicum L. Boraginaceae	Kalar booti	Poonch valley, Azad Kashmir	Roots	Root decoction is used	Cow and buffaloes	(Khan *et al*., 2012)
Trigonella foenumgraceum L. Papilionaceae	Matheray	Sargodha	Seeds	25g, paste is made with handful of wheat flour and vegetable oil and given orally for 5 days.	Cows and buffaloes	(Dilshad *et al*., 2010)
Triticum aestivum L. Poaceae	Gandam/kanakHarmal	Sargodha	Fruit + Stem crushing (Hay)	50 g + 2 kg, fumigation of harmal by putting it on fired hay under the affected udder for 4 days	Cows and buffaloes	(Dilshad *et al*., 2010)
Vernonia anthelmintica Wild. Asteraceae	Kali zeri	Faisalabad		Seed 30 g+ Burntmilk fat 60 gMixture given for 2 days	Livestock	(Deeba *et al*., 2009)
Withania somnifera L. Solanaceae	Aksan	Himalaya	Root	200 g fresh roots are crushed and paste isapplied topically up to a week	Livestock	(Abbasi *et al*., 2013)
Zingiber officinale Roscoe Zingiberaceae	Sund	Sargodha	Rhizome	125g, grinded finely with sugar, given orally for 5 days	Cows and buffaloes	(Dilshad *et al*., 2010)

Table 2. *Invitro* screening of anti-mastitis plants against *Staphylococcus aureus*.

Plant species	Part used	Extract	Phytochemistry	Concentration (mg/ml)	Inhibition (mm)	Reference
Allium cepa	Bulb	Chloroform	Alkaloids, Saponins, Terpenoids, Reducing sugars	100	14	(Yousufi, 2012; Jadon &Dixit, 2014)
		Ethanol		100	14	
		Aqueous		100	8	
Allium sativum	Bulb	Pure juice	Alkaloids, Saponins, Flavonoids, Terpenoids, Cardiac glycosides, Resins	200	13	(Jadon &Dixit, 2014)
		Aqueous decoction		200	9	
	Bulb	Aqueous		100	19.3	(Gull *et al*., 2012)
		Ethanol		100	12.6	
		Methanol		100	11	
	Bulb	Chloroform		100	15	(Yousufi, 2012)
		Ethanol		100	15	
		Aqueous		100	19	
Amomum subulatum	Oil extract	Water	Proteins, Crude fiber, Starch, Volatile and non-volatile ether, Alcohol	750,000	14	(Ritender *et al*., 2013; Gopal *et al*., 2012)
Brassica campestris	Roots	Benzene	NA	100	10	(Agrawal *et al*., 2013)
		Petroleum ether		100	15	
		Chloroform		100	10	
		Ethyl acetate		100	10	
				50	10	
		Methanol		100	10	
		Ethanol		50	15	
				100	20	
		Aqueous		50	10	
				100	10	
	Stems	Benzene		50	10	
				100	10	
		Petroleum ether		100	10	
		Chloroform		100	10	
		Ethyl acetate		50	10	
				100	15	
		Methanol		50	15	
				100	15	
		Ethanol		50	20	
		Aqueous		50	10	
				100	10	
	Leaves	Benzene		50	10	
				100	10	
		Petroleum ether		50	10	
				100	10	
		Chloroform		100	10	
		Ethyl acetate		50	15	
				100	15	
		Methanol		50	10	
				100	15	
		Ethanol		50	20	
				100	20	
		Aqueous		50	10	

Table 2.(Cont'd.).

Plant species	Part used	Extract	Phytochemistry	Concentration (mg/ml)	Inhibition (mm)	Reference
Capparis decidua	Root	Ethyl acetate	Alkaloids, Sterols,Sitosterol, Spermidine alkaloid, Isocodonocarpine	10	10.33	(Sharma &Kumar, 2009; Rathee *et al*., 2010; Verma *et al*., 2011)
	Stem	Ethyl ether	Acyclic terpenoids, Alkaloids, Fatty acids, Sterols	10	14.66	(Rathee *et al*., 2010)
		Ethyl acetate		10	18.66	
	Fruit	Ethyl acetate	Stachydrine alkaloid	10	15.66	(Rathee *et al*., 2010)
Citrullus colocynthis	Fruit	Hydro methanol	Alkaloids, Iridoids, Steroids	200	0	(Hussain *et al*., 2011; Khatibi &Teymorr, 2011)
Cuminum cyminum	Seed	Ethanol	Carbohydrates, Protein, Vitamins, Minerals, Aldehydes, Alkyl derivatives, Tannins, Mucilage, Oleoresins, Gum, Malates	0.5	22	(Shiri *et al*., 2013; Nadeem &Riaz, 2012)
Curcuma longa	Dry plant	Aqueous	Carbohydrates, Flavonoids, Tannins, Saponins	400	11	(Al-Daihan *et al*., 2013)
				400	11	
		Methanol		400	15	
Peganum harmala	Seed	Ethanol	Alkaloids, Flavonoids, Anthraquinones, Triterpenes and sterols, Reducing compounds, Tannins, Saponins, Volatile oil	0.5	25	(Shiri *et al*., 2013; Benbott *et al*., 2013)
Sesamum indicum	Leaves	Ethanol	Sesamin, Sesamolin, Stigmasterol, β-Sitosterol, Stigmasterol-3-O-β-D-glucoside, Ferulic acid, Rhamnetin, Verbascoside, kaempferol-3-O-β-D-glucuronide, Mequelianin (quercetin-3-O-β-D-glucuronide), Sesamin, Sesamolin	400	0	(Ogunsola &Fasola 2014; Khaleel *et al*., 2007)
Trichodesma indicum	Whole plant	Methanol	Alkaloids, Flavonoids, Steroids, Glycosides, Proteins, Tannins, Phenolic compounds, Triterpenoids	100	10	(Mubarack *et al*., 2012; Kannadhasan *et al*., 2013)
				200	14	
	Whole plant	Water		100	0	(Gull *et al*., 2012)
				200	0	
		Methanol		100	16	
				200	24	
Withania somnifera	Leaves	Aqueous	Glycosides, Alkaloids, Phytosterol, Fixed oil, Protein, Phenolic compounds, Flavonoids	100	13	(Ani *et al.,* 2014)
		Ethanol		100	15	
		Acetone		100	16	
Zingiber officinale	Rhizome	Ethanol	Saponins, Flavonoids, Terpenoid	50	15.11	(Jadon &Dixit, 2014; Shekhan &Hussaini, 2012)
				100	15.77	
				200	17.66	
				400	17.55	
	Dry plant	Aqueous	Alkaloids, Carbohydrates, Steroids, Saponins	400	10	(Al-Daihan *et al*., 2013)
		Methanol		400	12	
		Chloroform		100	22	
	Rhizome	Ethanol	NA	100	18	(Yousufi, 2012)
		Aqueous		100	10	

Table 3. Drug resistivity potential of *Staphylococcus aureus*.

Bacteria	Antibiotics	Resistivity (%)	Reference
Staphylococcus aureus	Penicillin	90.5	(Kawsar *et al*., 2008)
	Ampicillin	90.5	
	Erythromycin	52	(Brinda *et al*., 2010)
	Polimixin B	64	
	Tylosin	56	
	Oxacillin	52	
	Ampicillin	68	
	Novobiocin	40	
	Amoxiclav	48	
	Kenamycin	28	
	Cephalothin	8	
	Tetracycline	60	
	Oxacillin	100	(Perwaiz *et al*., 2007)
	Erythromycin	95	
	Gentamicin	93	
	Penicillin	100	
	Amikacin	54	
	Cephalexin	100	
	Chloromphenicol	58.04	(Adamu *et al.*, 2010)
	Cefotaxime	85.71	
	Benzyl Penicillin	100	(Sina *et al*., 2013)
	Tetracycline	60	
	Penicillin	67.9	(Daka *et al*., 2012)
	Ampicillin	67.9	
	Oxacillin	60.3	
	Ampicillin	82.2	(Kahsay *et al*., 2014)
	Amoxicillin	82.2	
	Erythromycin	95.5	
	Gentamicin	87.7	
	Penicillin G	82.2	
	Cotrimoxazole	97.2	
	Penicillin	83	(Jayatilleke & Bandara 2012; Saravanan *et al*., 2013)
	Amoxicillin	100	
	Ampicillin	100	
	Penicillin G	100	
	Rifampicin	100	
	Methicillin	100	
	Ampicillin	100	(Bukhari *et al*., 2011)
	Gentamicin	97.6	
	Tetracycline	100	
	Oxacillin	100	
	Erythromycin	98.3	
	Ciprofloxacin	75.8	
	Penicillin G	100	
	Cephalothin	92.4	

Multidrug resistance potential of *Staphylococcus aureus*: Mastitis is very infectious disease and therefore use of antibiotics is common against it (Tenhagen *et al*., 2006). Variety of pathogens from mastitis infected milk have been isolated but *S. aureus* is the most commonly occurring bacteria reported in most of the studies (Rakhshandeh *et al*., 2011; Ritender *et al*., 2013). Different antibiotics such as chloromphenicol, gentamicin, tetracycline, pencillin, erythromycin, ampicillin, amoxicillin etc have been used widely for curing mastitis (Table 3). Erythromycin, tetracycline and gentamicin are not only being used against mastitis, infact also used in human medicines (Roesch *et al*., 2006). The use of antibiotics have been increasing for last 10-15 years, however, *S. aureus* developed resistance against variety of antibiotics (Shea, 2003). Recently resistance potential of *S. aureus* has extensively been studied and discussed in present review. Studies presented in this review showed 60-100% resistivity of *S. aureus* against most frequently used antibiotics such as erythromycin, tetracycline, amoxicillin, chloromphenicol, gentamicin and pencillin in cows for mastitis (Jayatilleke & Bandara, 2012; Saravanan *et al*., 2013; Kahsay *et al*., 2014). Present review showed that some antibiotics like kenamycin, tylosin, novobiocin, polimixin, amoxiclav, amikacin and cephalothin have good inhibitory actions against *S. aureus* but unfortunately these antibiotics have not been yet studied in detail (Perwaiz *et al*., 2007; Brinda *et al*., 2010). An emerging resistant potential of pathogens against antibiotics as well as high cost and side effects on the living system shifted researchers and farmers attention toward ethnoveterinary medicines. Therefore, traditional ethnoveterinary medicines and practices gained lot of importance in past few years due to the discovery of many potent ethnoveterinary drugs (Hassan *et al*., 2014).

Conclusions and future recommendations

Pakistan contains variety of plants used against mastitis and local farmers are highly dependent on ethnoveterinary medicines for the improvement of their livestock health and for generating their incomes. Present review reported 38 medicinal plants of Pakistan being used by local farmers of the remote regions for the treatment of their livestock; however, only 13 plants have been validated scientifically so far. Different plant extracts have shown good activities against *S. aureus* among which ethanolic, aqueous and methanolic extracts were more potent, other extracts should also been given focus in future studies which could lead the extraction of some new and different compounds. *S. aureus* has shown highest resistance against most common antibiotics, however, also showed sensitivity to some of the drugs such as polimixin, kenamycin, tylosin, amoxiclav etc which should be given focus in future studies. There are very limited studies on isolation of pure compounds and direct testing of those compounds against bacterium which needs study in future. *In vivo* trails are necessary for the further validation of ethnoveterinary medicines and to ensure the safety aspects. In spite of strong ethnoveterinary medicinal system of Pakistan and extensive use of traditional medicines against mastitis, very limited number of studies has been reported so far on ethnoveterinary plants. Due to strong drug resistivity potential of *S. aureus* it is imperative to expedite detailed scientific studies on unexplored plants that could be useful for the identification of potential medicinal plants, extraction of novel compounds and the development of new plants based veterinary drugs with high efficacy and low cost. This could be major breakthrough in increasing agricultural economy of Pakistan as well as other countries of the world.

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