Journal of Chemical and Pharmaceutical Research



J. Chem. Pharm. Res., 2011, 3(4): 777-789

ISSN No: 0975-7384 CODEN(USA): JCPRC5

Evaluation of antimicrobial activity of plant extracts on antibioticsusceptible and resistant *Staphylococcus aureus* strains

Firdaus Jahan, Rubina Lawrence*, Vinod Kumar and Mohd. Junaid

Department of Microbiology and Fermentation Technology, Jacob school of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India

ABSTRACT

Ethanolic leaf extracts of 5 medicinal plants traditionally used in medicine were studied for their antimicrobial activity against antibiotic-susceptible and resistant Staphylococcus aureus strains isolated from different clinical samples. The antimicrobial activity of plant extracts was determined by using agar well diffusion method. The plant extracts showed varied levels of antimicrobial activity against antibiotic-sensitive and resistant S. aureus isolates. Extracts of Syzygium cumini (Jamun) and Lawsonia inermis (Mehndi) showed good activity against most of the sensitive and resistant isolates whereas the extracts of Ficus religiosa (Peepal), Ocimum sanctum (Tulsi) and Zizyphus mauritiana (Ber) showed moderate activity against most of the sensitive and resistant isolates. The plant extracts showed variable initial MIC values against resistant and sensitive S. aureus isolates being minimum for S. cumini (1.2 mg/ml, 0.6 mg/ml) and L. inermis (0.6 mg/ml, 1.2 mg/ml). The final MIC and MBC values were observed to be either same or 2 to 4 fold higher than initial MIC. The present study thus suggests the use of these medicinal plants in the treatment of various diseases caused by drug resistant S. aureus strains.

Key words: Clinical samples, *Staphylococcus aureus*, antibiotic sensitivity, medicinal plants, plant extracts, antibacterial activity, MIC, MBC.

INTRODUCTION

In recent years, drug resistance to human pathogenic bacteria is being commonly reported from all over the world [1]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics [2]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms have increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [3].

Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms [4].

Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [5]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains [6].

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists [7]. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent [8].

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [9, 10]. They have antidiabetic, antioxidant, antibacterial, anti-inflammatory, anti-pyretic activities, gastro-protective effects and many more important medicinal properties. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens. According to WHO, medicinal plants would be the best source for obtaining a variety of drugs [11]. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity [12]. There are several reports on the antimicrobial activity of different herbal extracts [13-15]. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections [16, 17].

There has also been a considerable effort to discover plant-derived antibacterial active against methycillin-resistant *Staphylococcus aureus* (MRSA) strains, which have developed resistance to most antibiotics. Driven by this urgent need of numerous anti *S. aureus* plant-derived antibacterials with good MIC values have been identified in past decades by researchers. These evidences contribute to support and quantify the importance of screening natural products. The aim of the present study was to investigate the antibacterial activity of ethanolic leaf extracts of *Syzygium cumini, Ocimum sanctum, Lawsonia inermis, Zizyphus mauritiana* and *Ficus religiosa* against antibiotic resistant and sensitive *Staphylococcus aureus* strains isolated from different samples collected from patients having burn, wound or pus infections.

EXPERIMENTAL SECTION

Sample Collection

Hundred samples from patients having burn, wound and pus infections were collected from different hospitals of Allahabad region.

Isolation of Staphylococcus aureus

The collected samples were streaked onto Nutrient agar and on the selective media for *Staphylococcus aureus i.e.* Mannitol Salt agar and Blood agar media. The inoculated plates were then incubated aerobically at 37 °C for 24 - 48 h.

Identification

The bacterial growth obtained on the incubated plates was identified as *Staphylococcus aureus* on the basis of cultural, morphological and biochemical characteristics [18].

Antibiotic sensitivity test

Antibiotic sensitivity of the isolated *Staphylococcus aureus* strains was determined by Standard Disc Diffusion Method [19]. Different antibiotics (Hi-media, Mumbai) were used in the present work, *viz*. Amikacin (Ak), 10 μ g; Amoxycillin (Am), 30 μ g; Ampicillin (A) 10 μ g; Cefuroxime (Cu), 30 μ g; Chloroamphenicol (C), 30 μ g; Ciprofloxacin (Cf), 30 μ g; Clindamycin (Cd), 30 μ g; Erythromycin (E), 15 μ g; Gentamicin (G), (10 μ g); Kanamycin (K), 30 μ g; Methicillin (M), 30 μ g; Nalidixic acid (Na), 30 μ g; Netilmicin (Nt), 30 μ g; Tetracycline (T), 30 μ g and Vancomycin (Va), 30 μ g.

Melted and cooled nutrient agar media was poured in sterile petridishes and swabbed with overnight culture of *S. aureus* strains. Under aseptic condition, antibiotics discs were placed on the surface of the inoculated plates. Following overnight incubation at 37 ± 0.2 °C, zone of inhibition (mm) for each drug was measured and values were compared with the NCCLS standards [19] to determine the sensitivity pattern of *S. aureus* strains.

Selection of plant material

Leaves of the plants viz. *Syzygium cumini* (Jamun), *Lawsonia inermis* (Mehndi), *Zizyphus* mauritiana (Ber), *Ocimum sanctum* (Tulsi) and *Ficus religiosa* (Peepal) were selected for the evaluation of their antimicrobial properties against the isolated *S. aureus* strains.

Preparation of plant extracts

Ethanolic leaf extracts of all the five selected plants were prepared [20]. 100 g of air dried and powdered leaves of each plant were soaked in 100 ml of 70% ethanol for 72 h. Each mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction each extract was passed through Whatman Filter Paper No. 1. The ethanolic filtrates obtained were concentrated at 30 °C and then stored at 4 °C. All the plant extracts were screened for their antimicrobial activity.

Antimicrobial assay

The antimicrobial activity of the plant extracts was evaluated using Agar Well Diffusion Method [21] with minor modifications. 0.1 ml of diluted inoculum (10^5 CFU/ml) of the *S. aureus* strains was swabbed on the Nutrient agar plates. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized cork borer (5 mm). Using a micropipette, 100 µl of the plant extracts were added to the wells made in the plate. The plates were incubated aerobically in an

upright position at 37 ± 2 °C for 24-48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the *S. aureus* strains. The test was performed in triplicates with controls.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The plant extracts that were found effective, as antimicrobial agent, were later tested to determine the MIC and MBC values for each strain. MIC was determined using broth dilution method. The extracts were diluted to give the final concentrations of 75, 37.5, 18.8, 9.4, 4.7, 2.4, 1.2, 0.6, 0.3, 0.15 mg/ml. 100 μ l of 10⁵ CFU/ml of the *S. aureus* strains was inoculated in tubes with equal volume of nutrient broth and plant extracts. The tubes were incubated aerobically at 37 °C for 24-48 h. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 h when compared with the control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 h at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC.

MBC value was determined by sub culturing the test dilution [which showed no visible turbidity] on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC.

RESULTS AND DISCUSSION

Isolation of Staphylococcus aureus strains from different clinical samples

In the present study, out of the 100 different clinical samples collected, 57 samples were found to be positive for *Staphylococcus* spp. Of these, 22 samples were found positive for *S.aureus* (38.59%). Burn samples were found to have higher incidence of *S. aureus* infection (45%), followed by wound (25%) and pus (20%) (Table 1). Similar findings were also reported by other workers where burn samples were found to show higher prevalence of *S. aureus* infection as compared to other samples [22-27]. In other studies, recovery rate of *S. aureus* was reported to be more from pus and wound samples in the absence of burn samples [28-31].

The susceptibility of burn wound to such colonization by bacteria results from several factors including the presence of coagulated proteins, the absence of blood-borne immune factors, and the avascularity of the burn wound. Burns provide a suitable site for bacterial multiplication and infection, mainly because of the larger area involved and longer duration of patient stay in the hospital [26]. Further, it was reported that the infection of methycillin resistant *Staphylococcus aureus* (MRSA) is more compounded in the burn patients as they are severely immuno-compromised and receive numerous antibiotics [32]. Moreover, care of these patients is often very labour-intensive, requiring many hours of hands-on contact. Ina study it was observed that Staphylococci can survive intracellularly in polymorphonuclear leucocytes (PMNs) [33]. However, in burn patients, PMNs bactericidal function is decreased allowing the organism to survive longer.

Clinical	No. of	Samples positiv	ve for Staphylococcus	Samples positive for <i>Staphylococcus</i>									
Samples	samples		spp.		aureus								
		No.	%	No.	%								
Burn	60	40	66.67	18	45								
Wound	30	12	40	3	25								
Pus	10	5	50	1	20								
Total	100	57	57	22	38.59								

Table 1: Incidence of *Staphylococcus* spp. from different clinical samples

Antibiotic sensitivity of S. aureus isolates

The different strains of *S. aureus* isolated in the study showed variable response towards various antibiotics tested (Figure 1). On the basis of the antibiotic sensitivity pattern shown, the *S. aureus* isolates were divided into two categories: "Antibiotic-Resistant" and "Antibiotic-Sensitive" isolates. A total of 13/22 (59%) isolates were resistant to most of the antibiotics and were regarded as MDR strains. The remaining 41% isolates were found to be sensitive. This observation is comparable with the studies [28, 34] where 57.5% strains were identified as MDR strains. In contrast, a study reported a lower incidence of resistant strains (10%) [35]. The increasing trend in development of antibiotic resistance could be attributed to frequent, unnecessary and indiscriminate usage of antibiotics and longer duration of hospitalization [28, 36].

In the study, maximum antibiotic resistance was observed for nalidixic acid (100%), cefuroxime and kanamycin (81.82%) and amoxycillin (72.73%), followed by ciprofloxacin and ampicillin (63.64%), erythromycin, amikacin, clindamycin and methicillin (59.09%). For rest of the antibiotics the percentage resistance varied from 9.09 to 50%. In case of vancomycin all strains were found to be sensitive. Similar observations have been observed for methycillin[25], tetracycline [37], chloroamphenicol [30] and vancomycin [25, 26,27, 29, 38-41]. In contrast, lower percentage incidence of resistant *S. aureus* strains have been reported by many workers with respect to most of the antibiotics tested, with the exception of tetracycline, amoxycillin, clindamycin, amikacin and methicillin where higher percentage resistance was reported [25, 26,27, 29, 30,31,36, 37, 39, 42].

The variation in the antibiotic sensitivity pattern of isolated organisms may be due to several factors like differences in pH, nature and time of incubation, composition and nature of the culture media, size of inoculum, source of isolated organism and perhaps differences in strain activity [36].

The antibiotic resistant and sensitive *S. aureus* strains showed variable sensitivity patterns towards the leaf extracts of all the plants used (Table 2, 3). In case of *Lawsonia inermis*, among the resistant isolates R3 and among the sensitive isolates S1 showed maximum zone of inhibition corresponding to 18.33 mm and 19 mm, respectively. Similar observations have been reported by others [2, 43, 44]. Few workers have also reported good antibacterial activity of *L. inermis* using extracts prepared from other parts and different solvents [45-53]. The antimicrobial activity of *L. inermis* has been attributed to the presence of alkaloids, anthocyanin, phenols, xanthoproteins, flavanoids, carboxylic acid, coumarins and sterols [52].

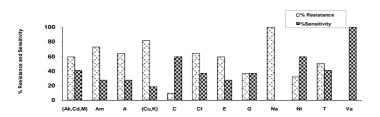


Figure 1: Percentage resistance and sensitivity of S. aureus isolates to various antibiotics

Antibacterial activity of leaf extracts of the selected plants

Syzygium cumini leaf extract showed maximum inhibition against *S. aureus* isolates R8 and S4 i.e. 18.66 mm and 19.33mm respectively. The observations were comparable with that of other studies [2, 54-56]. Meshram *et al* [57] studied antibacterial potential of ethanolic extract of *S. cumini* seeds powder against *S. aureus* and observed good antibacterial activity. The flavanoids and tannins present in the leaves are responsible for their antibacterial properties [2, 54].

Similarly, the leaf extract of Zizyphus mauritiana showed variable antimicrobial activity against the antibiotic resistant and sensitive S.aureus isolates. Similar antimicrobial activity of leaf extract of Zizyphus sp. has been previously reported [2]. Dubey et al [58], reported good antibacterial activity of aqeous, methanolic and saponin extracts of Zizyphus mauritiana barks against S.aureus and other human vaginal pathogens. Few studies have also reported antimicrobial properties of fruit and root extract of Zizyphus sp. [59-61]. Saponins, glycosides and flavanoids have been identified as antimicrobial agents in the plant [61].

In case of *Ficus religiosa*, R4 and S3 isolates showed maximum zones of inhibition, i.e. 15.33 and 12.33 mm. Other studies have reported similar findings [2, 59, 62]. The antimicrobial activity of *F. religiosa* is suggested to be due to the presence of glycosides, phenols and tannins [2].

Using leaf extract of *Ocimum sanctum*, maximum inhibition was observed for R4 and S3 isolates. As in the present study, various workers have reported antibacterial properties of *O. sanctum* [2, 43, 63, 64]. Phytochemical analysis of the plant revealed the antibacterial properties to be due to glycosides, phenols and tannins [2].

It was observed that all the plants used showed inhibition against the antibiotic and sensitive S. aureus isolates. Zones of inhibition were observed to range from 14.0 - 18.33 mm for *L. inermis*, 13.33 - 18.66 mm for *S. cumini*, 10.0 - 12.66 mm for *Z. mauritiana*, 9.66 - 15.33 mm for *F. religiosa* and 10.66 - 15.66 mm for *O. sanctum*. On comparing the data, *S. cumini* was found to be more effective against the resistant isolates while *F. religiosa* was least effective. In a similar study, *L. inermis* showed maximum antibacterial activity against resistant *S. aureus* isolates while *O. sanctum*, *F. religiosa*, *Zizyphus* spp. and *S. cumini* were found to exhibit moderate activity [2].

Among the sensitive isolates the zones of inhibition varied from 12.66 - 19.0 mm for *L. inermis*, 10.66 - 19.33 mm for *S. cumini*, 10.0 - 12.33 mm for *Z. mauritiana*, 10.0 - 12.33 mm for *F. religiosa* and 10.0 - 13.66 mm for *O. sanctum*. *S. cumini* was found to be more effective as compared to other extracts while *Z. mauritiana* showed least activity against the antibiotic sensitive isolates. As in the present study, in a study, it was reported that the sensitive *S. aureus* strains to be more susceptible to *S. cumini* extract as compared to the resistant isolates [54], while *O. sanctum* showed greater inhibitory action against resistant isolates as compared to sensitive isolates [43].

The variation in the antibacterial activity of the plant extracts from other studies can be attributed media to inoculum size, type of used. type of solvent used for extraction, extraction procedure, incubation time and temperature, part of the plant used and its time of collection, method of extraction procedure, incubation time and temperature, part of the plant used and its time of collection, method of antibacterial assay and strain activity.

Minimum inhibition concentration [MIC] and Minimum Bactericidal concentration [MBC] of the plant extracts

The minimum inhibitory and bactericidal concentrations of each extract against resistant and sensitive *S. aureus* isolates were evaluated in the present study (Table 4, 5). In case of *L. inermis,* initial MIC ranged from 0.6 - 4.7 mg/ml for the resistant isolates and from 1.2 - 4.7 mg/ml for the sensitive isolates Final MIC was generally 2 - 4 fold higher than the initial MIC and MBC was either the same or 2 fold higher than final MIC. In contrast to the present study, in a study conducted by Muhammad and Muhammad [50], *S. aureus* was found to be inhibited at a higher concentration.

The initial MIC for *S. cumini* was observed to range from 1.2 - 9.4 mg/ml for the resistant and 0.6 - 4.7 mg/ml for the sensitive *S. aureus* strains. Final MIC was generally 2 - 4 fold higher than initial MIC and MBC was either same or 2 - 4 fold higher than final MIC. As compared to the present study, lower MIC values have been reported by some workers, i.e. 0.3 mg/ml [54] and 0.2 mg/ml [56].

Plant Extracts			Zone	of inhibi	tion (mr	n) again	st antibi	otic-resi	stant S. d	<i>aureus</i> is	solates		
Plant Extracts	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13
Lawsonia inermis	15.33	12.66	18.33	18	17	16.66	17.33	18	17	15.66	14	14.33	16
Syzygium cumini	10.66	10.66	18.33	15.66	15.66	15.33	16.33	18.66	15.66	11.33	13.33	15.66	15.33
Zizyphus mauritiana	10	10.66	12	12.66	11.33	11.66	12.33	11	12.33	11.33	10.66	11.33	11.33
Ocimum sanctum	11	11.33	12.66	15.66	14.66	12	13.66	12	12	10.66	11	11.33	10.66
Ficus religiosa	10	10.33	11.66	15.33	13.33	11.66	12	12	11.33	9.66	10.66	10.66	11

 Table 2: Antibacterial activity of the leaf extracts (ethanolic) of the selected plants against "Antibiotic-Resistant" S. aureus isolates

In case of Z. *mauritiana* the initial MIC corresponded to 2.4 - 18.8 mg/ml for resistant and 4.7 - 18.8 mg/ml for sensitive strains. The final MIC values were 2 - 4 fold higher than initial MIC while MBC was either same or 2 fold higher than final MIC. In a study, almost similar MIC

values of *Zizyphus* sp. have been reported against *S. aureus* [25 mg/ml] [59]. However, Kubmarawa *et al* [61], reported a lower MIC value (1.0 mg/ml).

 Table 3: Antibacterial activity of the leaf extracts (ethanolic) of the selected plants against "Antibiotic-Sensitive" S. aureus isolates

S.No.	Plant Extracts	Zone	of inhibi	ition (m	m) again	ist antibi	iotic-sen	sitive S.a	<i>ureus</i> is	olates
		S1	S2	S3	S4	S5	S6	S7	S8	S9
1	Lawsonia inermis	12.66	18.33	17.66	12.66	16.66	14.66	19	14.66	15.33
2	Syzygium cumini	10.66	16.33	18.33	11	16	12.33	19.33	14	13
3	Zizyphus mauritiana	10	11.66	12.33	10	11.66	11	12	11.33	11
4	Ocimum sanctum	10	12.66	13.66	10	12.66	10.33	13	11	11.33
5	Ficus religiosa	10	11.66	12.33	10	11.66	11	12	11.33	11

In case of *F. religiosa*, the initial MIC value ranged from 4.7 - 18.8 mg/ml for resistant and from 2.4 - 18.8 mg/ml for sensitive isolates. The final MIC values were 2 - 4 fold higher than initial MIC while MBC was either same or 2 fold higher than final MIC. Valsaraj *et al* [59] reported similar MIC values (25 mg/ml) while Ahmad and Beg [2] reported inhibition of *S. aureus* at a higher concentration (150 mg/ml).

The initial MIC of *O. sanctum* ranged from 2.4 - 9.4 mg/ml and 4.7 - 18.8 mg/ml for resistant and sensitive strains, respectively. Final MIC was generally 2 fold higher than initial MIC while MBC was either same or 2 fold higher than final MIC. Lower MIC values for *Ocimum* spp. was reported by Adiguzel *et al* [65] (0.25 mg/ml) and Akinvemi *et al* [66] (0.02 mg/ml). Further, Akinvemi *et al* [66] also reported a lower MBC value [0.03 mg/ml].

The variations in the result may be due to the solvent used, incubation temperature and duration, media used for making dilutions, amount of inoculum added, plant species and parts used and moreover, methods used for determining MIC and MBC values.

	MIC and MBC values (mg/ml) of the plant extracts against antibiotic resistant <i>S.aureus</i> isolates																																						
Plant		R1		R2			R3			R4			R5			R6			R7		R8		R9			R10			R11			R12			R13				
Extracts	Initial MIC [ma/m]]	Final MIC [mg/ml]	MBC [mg/ml]	Initial MIC [mø/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [ma/m1]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mo/ml]	Final MIC [mg/ml]	MBC [mg/ml]	Initial MIC	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mø/ml]	Final MIC [mg/ml]	MBC [mg/ml]	Initial MIC [mo/m1]	Final MIC [mg/m]]	MBC [mg/m]]	Initial MIC	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mø/m]]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mo/m1]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mø/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mø/ml]	Final MIC [mg/ml]	MBC [mg/ml]
Lawsonia inermis	2.4	9.4	9.4	4.7	9.4	18.8	0.6	2.4	2.4	1.2	2.4	2.4	1.2	2.4	4.7	1.2	2.4	4.7	1.2	2.4	2.4	1.2	2.4	2.4	1.2	2.4	4.7	1.2	4.7	9.4	4.7	9.4	9.4	2.4	4.7	9.4	2.4	4.7	4.7
Syzygiumc umini	9.4	18.8	18.8	9.4	18.8	18.8	1.2	2.4	2.4	1.2	4.7	9.4	2.4	4.7	4.7	2.4	4.7	4.7	1.2	2.4	4.7	1.2	2.4	2.4	2.4	4.7	4.7	4.7	9.4	18.8	4.7	9.4	9.4	2.4	4.7	9.4	2.4	4.7	4.7
Zizyphusm auritiana	18.8	37.5	37.5	9.4	37.5	37.5	4.7	9.4	9.4	9.4	18.8	18.8	9.4	18.8	37.5	9.4	18.8	18.8	2.4	9.4	9.4	9.4	18.8	18.8	9.4	18.8	37.5	9.4	18.8	18.8	9.4	37.5	37.5	9.4	18.8	18.8	9.4	18.8	18.8
Ocimum sanctum	9.4	18.8	18.8	9.4	18.8	18.8	4.7	9.4	18.8	2.4	4.7	9.4	2.4	9.4	9.4	4.7	9.4	18.8	4.7	9.4	9.4	9.4	18.8	8 18.8	9.4	18.8	37.5	9.4	18.8	18.8	9.4	18.8	18.8	9.4	18.8	37.5	9.4	18.8	18.8
Ficusreligi osa	18.8	37.5	37.5	18.8	37.5	37.5	9.4	18.8	18.8	4.7	9.4	9.4	4.7	9.4	9.4	9.4	18.8	18.8	9.4	18.8	18.8	9.4	18.8	8 18.8	9.4	18.8	18.8	18.8	37.5	37.5	9.4	37.5	37.5	9.4	37.5	37.5	9.4	18.8	37.5

Table 4: MIC and MBC values of leaf extracts (ethanolic) of the selected plants against "Antibiotic-Resistant" S. aureus isolates

					М	IC an	d ME	BC va	lues (mg/m	l) of 1	the pl	ant ex	tract	s aga	inst a	ntibio	otic-se	nsitiv	e S. a	ureus	isola	tes				
Plant Extracts		S1		S2			S3				S4			S5			S6			S7			S8		S9		
	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/m]]	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]]	MBC [mg/ml]	Initial MIC [mg/ml]	Final MIC [mg/m]	MBC [mg/m]]
Lawsonia inermis	4.7	9.4	18.8	1.2	2.4	4.7	1.2	2.4	4.7	2.4	9.4	9.4	1.2	2.4	4.7	1.2	4.7	4.7	1.2	2.4	4.7	2.4	4.7	9.4	2.4	4.7	4.7
Syzygium cumini	9.4	18.8	18.8	9.4	18.8	18.8	1.2	2.4	2.4	1.2	4.7	9.4	2.4	4.7	4.7	2.4	4.7	4.7	1.2	2.4	4.7	1.2	2.4	2.4	2.4	4.7	4.7
Zizyphus mauritiana	9.4	18.8	18.8	9.4	18.8	18.8	1.2	2.4	2.4	1.2	4.7	9.4	2.4	4.7	4.7	2.4	4.7	4.7	1.2	2.4	4.7	1.2	2.4	2.4	2.4	4.7	4.7
Ocimum sanctum	9.4	18.8	18.8	9.4	18.8	18.8	1.2	2.4	2.4	1.2	4.7	9.4	2.4	4.7	4.7	2.4	4.7	4.7	1.2	2.4	4.7	1.2	2.4	2.4	2.4	4.7	4.7
Ficus religiosa	9.4	18.8	18.8	9.4	18.8	18.8	1.2	2.4	2.4	1.2	4.7	9.4	2.4	4.7	4.7	2.4	4.7	4.7	1.2	2.4	4.7	1.2	2.4	2.4	2.4	4.7	4.7

Table 5: MIC and MBC values of leaf extracts (ethanolic) of the selected plants against "Antibiotic-Sensitive" S. aureus isolates

CONCLUSION

Antibiotic-resistant bacteria continue to emerge rapidly, causing a problem in the treatment of diseases caused by them. In the past decades as well as in the present study, *Staphylococcus aureus*, which is a predominant organism of burn and wound infections, showed increased resistance to commonly used antibiotics. The plants used in the present study showed promising antibacterial activity against the resistant *S. aureus* strains. Thus, the study suggests the use of these plants in the treatment of various diseases caused by resistant bacteria. Further, the potential of these plants must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria.

Acknowledgement

The authors are grateful to Dr. Ebenezer Jeyakumar, Department of Microbiology, Sam Higginbottom Institute of Agriculture, Technology and Sciences (formerly Allahabad Agricultural Institute) for providing valuable help, suggestions and advice in carrying out this research work.

REFERENCES

[1] KJV Piddock, R Wise. J. Antimicrob. Chemother. 1989, 23, 475-483.

[2] I Ahmad, AZ Beg. J. Ethnopharmacol. 2001, 74, 113-123.

[3] ML Cohen. Science. 1992, 257, 1050-1055.

[4] H Harbottle, S Thakur, S Zhao, DG White. Anim. Biotechnol. 2006, 17, 111-124.

[5] A Coates, Y Hu, R Bax, C Page. Nat. Rev. Drug Discov. 2002, 1, 895-910.

[6] LC Braga, AAM Leite, KGS Xavier, JA Takahashi, MP Bemquerer, E Chartone-Souza, AMA Nascimento. *Can. J. Microbiol.* **2005**, 51, 541-547.

[7] E Kafaru. . In *Essential Pharmacology*; IstEd. Elizabeth Kafaru Publishers: Lagos, Nigeria, **1994**.

[8] JEC Betoni, RP Mantovani, LN Barbosa, LC Di-Stasi, A Fernandes. *Mem. Inst. Oswaldo Cruz.* 2006, 101, 387-390.

[9] MA Iyenger. Study of crude drug, 2nd ed. College of Pharmaceutical Sciences, Manipal, **1985**, 13-78.

[10] RN Chopra, SA Nayer, IC Chopra. Glossary of Indian medicinal plants, 3rd ed. Council of Scientific and Industrial Research, New Delhi.**1992**, 7-246.

[11] PRV Santos, ACX Olivera, TCB Tomassini. Rev. Farm Bioquim. 1995. 31, 35-38.

[12] SB Lee, KH Cha, SN Kim, S Altantsetseg, S Shatar, O Sarangerel, CWNho. J. Microbiol. 2007, 45, 53-57.

[13] S Bonjar. J. Ethnopharmacol. 2004, 94, 301-305.

[14] B Islam, SN Khan, I Haque, M Alam, M Mushfiq, AU Khan. J. Antimicrob. Chemother. 2008(in press).

[15] HJ de Boer, A Kool, A Broberg, WR Mziray, I Hedberg, JJ Levenfors. *J. Ethnopharmacol.* **2005**, *96*, 461-469.

[16] A Brantner, E Grein. J. Ethnopharmacol. 1994, 44, 35-40.

[17] MN Somchit, I Reezal, IE Nur, AR Mutalib. J. Ethnopharmacol. 2003, 84, 1-4.

[18] JG Holt, PHA Sneath, NS Mair, ME Sharpe. Bergey's Manual of Systematic Bacteriology, **1986**, Volume 2, pp 1013-1035.

[19] AW Bauer, WM Kirby, JC Sherris, Turck M. Am. J. Clin. Pathol. 1966, 45, 149-158.

- [20] PI Alade, ON Irobi. J. Ethnopharmacol. 1993, 39, 171-174.
- [21] GRM Perez, JG Avila, MA Zavala, GS Perez, GC Perez. 1990. Phytomed. 3: 186
- [22] MT Cafferkey, R Hone, FR Falkiner, CT Keane, H Pomeroy. J. Med. Microbiol. 1983, 16(2), 117-127.
- [23] P Aravind, PU Krishnan, DPC Path, H Srinivasa, V Joseph. McGill J. Med. 2000, 5, 80-84.
- [24] R Kaushik, S Kumar, R Sharma, L Pawaindra. Burns. 2001, 27(6), 595-597.
- [25] AS Samy, IS Sayed, R Mohammad, B Ahmad, M Modather, AZ Hosam. *Egypt. J. Plast. Reconstr. Surg.* 2003, 27(1), 1 10
- [26] JLS Macedo, JB Santos. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 2005, 100(5), 535-539.
- [27] FA Orrett, M Land. Bio. Med. Central. Infect. Dis. 2006, 6, 83.
- [28] PP Sunderrajan, W Kale. J. Postgrad. Med. 1984, 30, 33-37.
- [29] SS Tahnkiwale, S Roy, SV Jalgaonkar. Ind. J. Med. Sci. 2002, 56, 330-334.
- [30] A Shittu, J Lin, D Kolawole. Wounds. 2006, 18(4), 77-84.
- [31] S Mulla, M Patel, L Shah, G Vaghela. Ind. J. Crit. Care Med. 2007, 11(2), 99-101.
- [32] JL Hunt, GF Purdue, DW Tuggle. Am. J. Surg. 1988, 156: 524.
- [33] AJ Steer, GB Hill, APR Wilson. J. Antimicrob. Agents. Chemother. 1995, 36, 851.
- [34] SV Kulkarni, BL Jahagirdar, SR Sengupta. Proceedings of 3rd All India Annual Congress of Medical Microbiologists, Bombay. **1979**, pp. 25-28.
- [35] RA Bhujwala, LN Mohapatra. Ind. J. Pathol. Bacteriol. 1972, 15, 66-72.
- [36] G Bhat, CN Nagesha, CN Pirvathi, KM Joseph, PG Shivananda. *Ind. J. Dermatol. Venereol. Leprol.* **1990**, 56, 371-374.
- [37] IE Al-Saimaru, SS Bakr, KE Al-Hamdi. Int. J. Dermatol. 2006, 4(2), 1531-3018.
- [38] BW Marbiel, L Cristina, TT Tessa, C Cynthia, CS Mediadora, AV Melecia. *Phil. J. Microbiol. Infect. Dis.* **1999**, 28(4), 128-132.
- [39] S Vidhani, Pl Mehndirratta, MD Mathur. Ind. J. Med. Microbiol. 2001, 19(2), 13-16.
- [40] L Savas, N Duran, Y Onlen, N Savas, S Ocak, NE Iris. *Eur. J. Gen. Med.* 2005, 2(1), 20-26.
 [41] AO Shittu, J Lin. *Bio. Med. Central Infect. Dis.* 2006, 6, 125.
- [42] PI Nwanze, LM Nwaru, S Oranusi, U Dimkpa, MU Okwu, BB Babatunde, TA Anake, W Jatto, CE Asagwara. *Sci. Res. Essay.* **2007**, 2(4), 112-116.
- [43] F Aqil, MSA Khan, M Owais, I Ahmad. J. Basic Microbiol. 2004, 45(2), 106-114.
- [44] F Aqil, I Ahmad. Methods Find Exp. Clin. Pharmacol. 2007, 29(2), 79-92.
- [45] F Malekzadeh. Appl. Microbiol. 1968, 16[4], 663-664.
- [46] S Satish, KA Raveesha, GR Janardhana. Lett. Appl. Microbiol. 1999, 28, 145-147.
- [47] GHS Bonjar. Asian J. Plant. Sci. 2004, 3(3), 310-314.
- [48] B Babu, VK Jisha, CV Salitha, S Mohan, AK Valsa. Ind. J. Microbiol. 2002, 42, 361-363.
- [49] OA Habbal, AA Al-Jabri, AH EL-Hag, ZH Al-Mahrooqi, NA Al-Hashmi. Saudi. Med. J. 2005, 26(1), 69-72.
- [50] HS Muhammad, S Muhammad. Afr. J. Biotechnol. 2005, 4(9), 934-937.
- [51] MAA Saadabi. Res. J. Biol. Sci. 2007, 2(4), 419-423.
- [52] K Sudharameshwari, J Radhika. Afr. J. Trad. Complement. Alter. Med. 2007, 4(2), 199-204.
- [53] P Arun, KG Puroshottam, JJ Jayarani and V Kumari. Int .J. Pharmatech Res. 2010, 2(2), 1178 1181.
- [54] GGF Nascimento, J Locatelli, PC Friestas, GL Silva. Braz. J. Microbiol. 2000, 31(4).
- [55] N Rajakaruna, CS Harris, GHN Towers. Pharmaceut. Biol. 2002, 40, 235-244.
- [56] GF Oliveira, NAJC Furtado, AA Filho, CHG Martins, JK Bastos, WR Cunha, ML Silva. *Braz. J. Microbiol.* **2007**, 38, 381-384.

[57] GA Meshram , SS Yadav, D Shande, B Patil and DJ Singh, *Phar. Sci. Res.* 2011, 3(2), 1060-1063.

[58] R Dubey, K Dubey, C Sridhar, and N Jayaveera. Int. J. Pharmaceut. Sci. Res. 2011, 2(3), 659-663.

[59] R Valsaraj, P Pushpangadan, UW Smitt, A Adsersen, U Nyman. J. Ethnopharmacol. 1997, 58: 75-83.

[60] D Dahiru, JM Sini, AL John. Afr. J. Biotechnol. 2006, 5(10), 941-945.

[61] D Kubmarawa, GA Ajoku, NM Enwerem, DA Okorie. Afr. J. Biotechnol. 2007, 6(14), 1690-1696.

[62] F Aqil, I Ahmad. World J. Microbiol. Biotechnol. 2003, 19(6), 653-657.

[63] I Ahmad, Z Mehmood, F Mohammad. J. Ethnopharmacol, 1998, 62, 183-193.

[64] B Joshi, GP Sah, BB Basnet, M Bhatt, D Sharma, K Subedi, J Pandey, and R Malla. J Microbiol & Antimicrobials. 2011, 3(1), 1-7.

[65] A Adiguzel, M Gulluce, M Sengul, H Ogutcu, F Sahin, I Karaman. *Turk. J. Biol.* 2005, 29, 155-160.

[66] KO Akinvemi, O Oladopo, CE Okwara, CKA Fasure. *BMC Complement. Altern. Med.* 2005, 5, 6-13.