Journal of Medicinal Plants Research Vol. 7(7), pp. 329-338, 17 February, 2013
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.286
ISSN 1996-0875 ©2013 Academic Journals

Full Length Research Paper

In vitro antibacterial efficacy of some important traditional medicinal plants in India against *Escherichia coli* and *Staphylococcus aureus* strains

Abdul Viqar Khan[1]*, Qamar Uddin Ahmed[2], Athar Ali Khan[1] and Indu Shukla[3]

[1]Department of Botany, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, 202002, India.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang Darul Makmur, Malaysia.
[3]Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, 202002, India.

Accepted 1 March, 2012

Historically, bacteria have been the cause of some of the *most* deadly diseases and widespread epidemics of human civilization. Many plants are known for their ethno-medicinally importance in the region of western Uttar Pradesh, India but their sensitivity against hospital isolated *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) human pathogenic bacterial strains has not been examined properly. *E. coli* and *S. aureus* are the two most common bacteria responsible for chronic infections among patients across the world. Hence, this communication emphasized upon the sensitivity of methanol (MeOH) and aqueous (AQ) extracts of 24 plant species against *S. aureus* ATCC 25953, *E. coli* MTCC 739 and four clinical isolates including *S. aureus* (Sa1), *S. aureus* (Sa2), *E. coli* (Ec1) and *E. coli* (Ec2) using disc diffusion and agar dilution method. Methanol extracts of the plants exhibited potent antibacterial activity against organisms taken into consideration. The results also support ethno-medicinal use of plants reported earlier. Present study revealed that studied plant extracts could be efficacious remedial herbal antibiotics, particularly both in controlling Gram-positive and Gram-negative human pathogens.

Key words: Ethnomedicinal plants, antibacterial activity, disc diffusion method, MIC.

INTRODUCTION

Historically, bacteria have been the main reason of some of the most fatal diseases and widespread epidemics of human civilization (Shinwari, 2010). Statistically, malaria and smallpox, diseases caused by other microorganisms, have killed more people than bacterial diseases, but diseases such as plague, cholera, typhus, diphtheria, tuberculosis, typhoid, pneumonia and dysentery have taken a large toll of humanity as well. In the initial period of the Twentieth Century, tuberculosis, diarrhea and pneumonia were the three chief causes of death among humans. Water purification, immunization (vaccination) and antibiotic treatment have reduced the morbidity and the mortality of bacterial disease in the Twenty-first Century, at least in the developed world where these are acceptable cultural practices. Notwithstanding, some bacterial diseases have been conquered, but many new bacterial pathogens have been documented in the past 30 years, and many "old" bacterial pathogens, such as *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), have emerged with new forms of virulence and new prototypes of resistance to antimicrobial agents. Great vigilance is warranted, and research and study are needed to control both old and new bacterial pathogens (Nabera, 2009; Shinwari et al. 2009).

S. aureus is a facultative anaerobic Gram-positive coccal bacterium. *S. aureus* infections are increasingly reported around the world (Nabera, 2009; Grundmann et

*Corresponding author. E-mail: viqarvicky@gmail.com. Tel: 09410211046.

al., 2006; Lescure et al., 2006; Stenhem et al., 2006; Laupland et al., 2003). In a recent analysis of US inpatients, nearly 400,000 inpatient admissions for *S. aureus* infection per year were reported in 2003 (Noskin et al., 2007; Fowler et al., 2005; Wisplinghoff et al., 2004). Infections due to *S. aureus* also impose a high and increasing burden on health care resources (Chu et al., 2005). The incidence of methicillin-resistant *S. aureus* (MRSA) has also increasingly become more common in most countries of the world. MRSA infections kill ~19,000 hospitalized American patients annually; this is similar to the number of deaths due to AIDS, tuberculosis, and viral hepatitis combined (Klevens et al., 2007).

Management has been complicated by the emergence of methicillin resistance, and controversies in its antibiotic efficacious therapy (Chu et al., 2005; Mainous et al., 2006; Mongkolrattanothai et al., 2009; Haessler and Brown, 2009). In contrast, *Escherichia coli* is commonly present in the intestines of humans and animals. Most strains of *E. coli* are harmless, but O157:H7 is a key exception because this strain causes severe diarrhea leading to renal damage and other serious complications including death. In 1994, *E. coli* O157 became a nationally notifiable infection in United Sates, and by year 2000, reporting was compulsory in 48 states. An estimated 73,480 illnesses due to *E. coli* O157 infection occur each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (Rangel et al., 2005), and it is an important cause of acute renal failure in children (Neill et al., 1987; Siegler et al., 1994; Mead et al., 1999; Rangel et al., 2005).

Plants have been used to cure different diseases from ancient times. India is known for its traditional uses of plants; in order to promote the proper uses of herbal medicine and to determine their potential as a source of new drug, it is essential to study them in a more systematic and scientific manner (Asolkar et al., 1992; Cordell, 2000). Though they have medicinal and nutrients and are considered good for human consumption (Shinwari and Gilani, 2003) but there is no denial of the fact that they also cause toxicity (Gilani et al. 2007, 2010). During the last twenty five years, many developing countries have shown keen interest in investigating herbal repository as a source of new antibacterial agents.

Another reason for such research is development of drug resistance and appearance of undesirable side effects of certain antibiotics (Cox, 1994; Farnsworth, 1988; Jain, 1991, Khan, 2011a). In order to overcome the severe problems associated with chronic infections of *S. aureus* and *E. coli*, there is an urgent need to identify novel substances that are active towards such pathogens. Contrary to the synthetic drugs, plant constituents are not associated with many side effects and heal various infectious diseases effectively (Leven et al., 1979; WHO, 2004, Vanden and Vlietinck, 1991). Hence, this scientific communication is devoted to such an attempt to study the antibacterial activity of some ethnomedicinally important plant species growing in the region of Western Uttar Pradesh, India against different strains of *S. aureus* and *E. coli* with respect to finding out effective antibacterial agents in the form of herbal antibiotics.

MATERIALS AND METHODS

Plant material

All plant species were collected from different rural areas of five districts of Western Uttar Pradesh, India. The climate is dry in summer with a rise in temperature up to 42°C and cool in winter up to 6°C. Plant species were identified by Dr. Athar Ali Khan (Taxonomist), Department of Botany, Aligarh Muslim University Aligarh, U.P., India. Identified plant specimens were kept in the Herbarium of the same department and voucher specimen number of each plant was registered separately.

Uses in traditional medicine

Plants screened for sensitivity were selected on the basis of their ethnomedicinal uses recorded by the authors in the study area previously described (Khan, 2002).

Reported phytochemistry and pharmacology

***Achyranthes aspera* L.:** (1) Chemical constituents: Achyranthine, ecdyosterone, inkosterone, saponin A, B, C & D, betaine, ecdyosterone, inkosterone and harmones have been reported. (2) Pharmacology: *A. aspera* has been reported to show hypoglycemic, antifungal, antimicrobial; antiimplantation, aborticidal, antipyretic, antispasmodic, diuretic and purgative activities (Khan, 2002).

***Aegle marmelos* L.:** (1) Chemical constituents: Aegelenine, lupeol, aegelin, rutin, marmesinin, β-sitosterol, coumarin, xanthotoxol, scoparone, scopoletin, umbelliferone, marmesin, skimming and tembamide have been reported. (2) Pharmacology: *A. marmelos* has been reported to show hypoglycemic, spasmogenic; antibacterial and antifungal activities (Khan, 2002).

***Aloe vera* L.:** (1) Chemical constituents: Acetylated mannans, polymannans, anthraquinone, C-glycosides, anthrones, anthraquinones, lectins, β-sitosterol, campesterol, lupeol, isoemodin, resin, glycoprotein have been reported. (2) Pharmacology: *A. vera* has been reported to show lymph juvenating, haemagglutinating, cancer agglutinating, antibacterial, antifertility, antifungal, antiulcer and antidiabetic activities (Khan, 2002; Boudreau and Beland, 2006; Vogler and Ernst, 1999; Eshun and He, 2004).

***Amaranthus spinosus* L.:** (1) Chemical constituents: Flavonoids, phenols, sterols, triterpenols, carotenoids, rutin, betacarotene, asprime, xylofuranosyluracil, hydroxycinnamates, betalains,betaxanthin, betacyanin cardenolids, ferredoxin, fatty acids, β-sitosterol, stigmastero, and spinoside l have been reported. (2) Pharmacology: *A. spinosus* has been reported to show antagonism of amphetamine hyperactivity in animals (Khan, 2002; Mathur et al., 2010).

***Argemone mexicana* L.:** (1) Chemical constituents: Sanguinarine, coptisine, isorhamentin 3-glycoside, cheilanthifolined, protopine and horsanguinarine have been reported. (2) Pharmacology: *A. mexicana* has been reported to show antifungal, antiviral, antibacterial and carcinogenic activities (Khan, 2002).

***Bacopa monnieri* (L.) Wettst.:** (1) Chemical constituents: Saponins, β-sitosterol, D-mannitol, stigmasterol, betulic acid,

stigmastanol, monnierin, bacoside A and B, nicotine, luteolin, bacogenin A_1-A_4, bacoside A_3-A and triterpenoid aglycone have been reported. (2) Pharmacology: *B. monnieri* has been reported to show anticonvulsant, tranquilizing, muscle relaxant, antiseptic, anticancer, and improvement in maize learning activities. (khan 2002)

***Calotropis procera* (Ait.) R.:** (1) Chemical constituents: Benzoyllineolone, benzoylisolineolone, evanidin, 3-rhamno-glucoside, cardenolites, choline, calotropine and calotropagenin have been reported. (2) Pharmacology: *C. procera* has been reported to show spasmogesic, vermicidal, anticancer, antiimplantation, antibacterial, antifungal and anti-inflammatory activities (Khan, 2002).

***Chenopodium album* L.**: (1) Chemical constituents: Ecdysteroids, β-ecdysone and polypodine B have been reported. (2) Pharmacology: *C. album* has been reported to show central nervous system depressant activity (Khan, 2002).

***Citrullus colocynthis* L. Schrad.**: (1) Chemical constituents: Elaterin, elatericin B, dihydroelatericin B, α-elaterin 2-D-glucopyranoside, choline, hepatacosan 1-ol, citrullonal, triterpenoids, 1, 11-undecanediol monoacetate and nonylhexadecanoate have been reported. (2) Pharmacology: *C. colocynthis* has been reported to show anti-inflammatory and antibacterial activities.

***Clerodendrum inerme* L. Gaertn.**: (1) Chemical constituents: 3-epicaryoptin and neolignan have been reported. (2) Pharmacology: *C. inerme* has been reported to show hypotensive and antifungal activities. (Khan, 2002).

***Cusuta reflexa* Roxb.:** (1) Chemical constituents: Cuscutin, cuscutalin, β-sitosterol, dulcitol, mannitol, kaempferol, myricetin, β-hydroxy olean-12-ene-tridecanoate and 3- β-hydroxy olean-12-ene-hepta decanoate have been reported. (2) Pharmacology: *C. reflexa* has been reported to show antiviral, relaxant, spasmolytic, choline like action and antifertility activities (Khan, 2002).

***Cycas rumphii* Miq.**: (1) Chemical constituents: Cycasin, β-glycosidase, amentoflavone, podocarpusflavone, A, 2-3-dihydromentoflavone, 2, 3-dihydrohinokiflavone, isoginkacin, and bilobetin have been reported (Khan et al., 2011b; Uddin et al., 2004).

***Dactyloctenium aegyptium* L. Wild.:** (1) Chemical constituents: Cynogenic glycosides and oxalic acid have been reported (Khan, 2002).

***Eclipta alba* L. Hassk.:** (1) Chemical constituents: Stigmasterol, α-terthienylmethanol, β-amyrin, wedelolactone and luteolin-7-glucoside reported. (2) Pharmacology: *E. alba* has been reported to show anticancer, antiviral, spasmogenic, nematicidal, haemostatic, antihepatitis and hypotension activities. (Khan, 2002)

***Euphorbia hitra* L.:** (1) Chemical constituents: Leucoyanidol, choline, shikimic acid, l-inositol 1-hexacosanol, 24 menacycloartenol, tenol, cycloartenol, β-sitosterol and euphorbol-hexacosonate have been reported. (2) Pharmacology: *E. hirta* has been reported to show proteolitic, antiviral, spasmogenic, anticancer, antibiotic and anti-dysentery activities (Khan, 2002; Hussain et al., 2009).

***Euphorbia thymifolia* L.:** (1) Chemical constituents: Hexacosanol, deoxyphorbol-OAc, epitaraxerol, n-hexacosanol and euphorbol have been reported. (2) Pharmacology: *E. thymifolia* has been reported to show insecticidal, antibacterial and antifungal activities (Khan, 2002).

***Melia azedarach* L.:** (1) Chemical constituents: Bakayanin, quercitrin, rutin, backalactone 6 β-hydroxy-4-stigmastem-3-one, 6 β-hydroxy-4-campesten-3-one, 4, 5-dihydroxy-7-O-α-L-rhamnopyranosyl-(1◊4)-β-D-glucopyranoside, cystine, serine, arginine, glycine, glutamic acid, threonine, methionine, leucine, lycine and proline have been reported. (2) Pharmacology: *M. azedarach* has been reported to show insecticidal, antibacterial, anthelmentic, central nervous system depressant, mild analgesic, anticancer, antibacterial, antispasmodic and antiviral activities.

***Malvastrum coromandelianum* L. Garcke.:** (1) Chemical constituents: β-phenyl ethylamine, N-methyl-β-phenyl ethylamine, dotriacontane, dotriacontanol, β-sitosterol, stigmasterol and campesterol have been reported. (2) Pharmacology: *M. coromandelianum* has been reported to show hypotensive activity in animals (Khan, 2002, Adnan et al., 2010).

***Nerium indicum* Mill.**: (1) Chemical constituents: Plumericin, α-amyrin, α-sitosterol, kaempferol, plumieridine, digitalinum, odorobioside D, G and K, strospheside, odorbioside D, odorotrioside K, odoroside G, oleandrin, gitoxigenin, digitoxigenin, rutin, gluco-oleandrin, decacetylo leandrin, adynerin, neriantin, neritaloside, nerviin, folinervin, quercitrin and adigoside have been reported. (2) Pharmacology: *N. indicum* has been reported to show cardiotonic, inotropic, bradycardiac antifungal, antipyretic, anti-inflammatory, emetic, cardiokinetic and diuretic activities (Khan, 2002).

***Oxystelma esculentum* (L.*f.*) Sm.**: (1) Chemical constituents: Cardenolides, flavonoids, phenolics, sterols and triterpenoids have been reported. (2) Pharmacology: *O. esculentum* has been reported to show anti-ulcer and antibacterial activities (Kirtikar and Basu, 1935; Pandya and Anand, 2011).

***Solanum nigrum* L.: (1)** Chemical constituents: Riboflavin, nicotinic acid, β-carotene, β-sitosterol, solamargine, solasonine α and β-solanigrine, solasodine and diosgenine have been reported. (2) Pharmacology: *S. nigrum* has been reported to show antibacterial, central nervous system depressant, hypotensive, vasodilation and diuretic activities (Khan, 2002; Yousaf et al., 2010).

***Trifolium alexandrinum* L.:** (1) Chemical constituents: Kaempferol, quercetin, velutin, trifolexin, quercetrin, chalcanol glycosides, formononetin, genistein, biochenin A, coumarin, bersimoside, dehydroazukisaponin, β-sitosterol, proteins, galactomannan, oleic acids, linolenic acid, palmitoleic acid, caproic acid, myristic acid, lauric acid, and xanthosine have been reported. Pharmacology: *T. alexandrinum* has been reported to show antibacterial activity (Khan et al., 2012).

Preparation of methanol (MeOH) extract

Plant extracts were prepared as described by Harbone (1988). Shade dried leaves of each plant was pulverized in an electric grinder. Powder so obtained was stored in a dessicator. 500 g plant powder each was macerated with 95% methanol in a round bottom flask at room temperature for about 24 h. Mother liquor (crude MeOH extract) was filtered out and residual plant material was again macerated with methanol for another 24 h. The process was repeated four times to ascertain the maximum yield of methanol (MeOH) extract of each plant. The MeOH extracts were evaporated to dryness at 35°C under reduced pressure using rotary evaporator (Buchi, Switzerland) and kept in the chiller at -18°C till further use.

Preparation of aqueous (AQ) extract

Shade dried leaves (500 g) of each plant was pulverized and

poured with double-distilled water in a closed round bottom flask, and left for 72 h at room temperature. The flask was then refluxed over hot water bath for 1 h and the mother liquor was filtered. The process was repeated for 4 times. The filtrate, thus obtained, was evaporated to complete dryness under reduced pressure. The residues thus obtained were stored in labeled sterilized screw capped bottles at -18ºC. All extracts were frequently checked for sterility by streaking on nutrient agar plates.

Bacterial susceptibility test

Disk diffusion method was used to determine susceptibility of plant extracts (Bauer et al., 1966; Colle and Marr, 1989). Standardized inoculums (1 to 2×10^7 CFU/ml 0.5 McFarland standard) were introduced on the surface of the plates containing Mueller Hinton agar (MHA), which was spreaded evenly with a glass spreader. A sterile disk (6 mm in diameters) previously soaked in a known concentration of extract (20 mg/ml/disc) was placed at the centre of the labeled seeded plate. The plates were incubated aerobically at 37°C and examined for the zone of inhibition after 24 h.

Antibiotics

Standard sensitivity disc of cholaremphenicol, ciprofloxacin and gentamycin (30 µg/ml/disc each) (Span Diagnostics Limited, Surat, Gujrat, India) were used as positive control to test the sensitivity profile against the reference bacteria.

Determination of minimum inhibition concentration (MIC)

MIC was measured by agar dilution method (Colle and Marr, 1989; EUCAST, 2000) by diluting the plant extracts in 10% Di-Methyl Sulphoxide (DMSO) using various concentrations, including, 200, 100, 50, 25 and 12.5 mg/ml, respectively. Equal volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1 ml of standard inoculums (1 to 2×10^7 CFU/ml) was added to each tube.

Tubes were incubated aerobically at 37°C for 18 to 24 h; two control tubes were maintained for each test batch. Those containing antibiotic control (tube containing plant extract and growth medium) and organism control (tube containing growth medium, physiological saline and the inoculums). The lowest concentration of extract that produced no visible growth (no turbidity) when compared with the control tube was regarded as MIC.

Test microorganisms

In the present study, the following bacterial strains were used: (Standard strains): *S. aureus* ATCC 25953*, E. coli* MTCC 739; (Clinical isolates): *S. aureus* (Sa1), *S. aureus* (Sa2), *E. coli* (Ec1) and *E. coli* (Ec2). The strains were maintained on nutrient agar slope at 4°C and sub cultured before use. The tested bacterial strains were procured from Microbiology Department, Jawaharlal Nehu Medical College, Aligarh Muslim University, Aligarh-202002, U.P., India.

Statistical analysis

The variation between experiments was estimated by standard deviations and statistical significance of changes was estimated by students t-test method.

RESULTS

All plant species are used traditionally in the region of Western Uttar Pradesh, a northern province of India for various diseases. Table 1 demonstrates antibacterial potential of the twenty four (24) plant species investigated against test pathogens taken into account. The MIC values of the crude leaves extracts (MeOH and AQ) against bacterial strains along with their traditional medicinal uses are listed in Table 2. It is revealed that MeOH extracts of the plants demonstrated antimicrobial activity at low concentration as demonstrated in Table 2 [MIC values ranging from 12.5 mg/ml (lowest) up to 100 mg/ml (highest)] as compared to the AQ plant extracts [MIC values ranging from 50 mg/ml (lowest) up to 200 mg/ml (highest)].

MeOH extracts of twenty one (21) plant species showed potential antimicrobial activity against *S. aureus* strains except *A. spinosus*, *C. album* and *S. cordata.* MeOH extracts of fourteen (14) plant species significantly inhibited the growth of *E. coli* (Gram-negative bacteria). AQ extracts of the plants showed weak antimicrobial action. It was also observed that MeOH and AQ extracts displayed antibacterial activity against both types of pathogens (Tables 1 and 2). Plant extracts of *A. spinosus* and *C. album* did not demonstrate antibacterial activity against tested pathogens.

DISCUSSION

Owing to the fact that methanol is a versatile polar organic solvent for the extraction of biologically active constituents and most of the active substances are easily extracted with it (alkaloids, carbohydrates, terpenoids, glycosides, amino acids, tannins, saponins, flavonoids, higher phenolic oligomers, anthocyanins) therefore, the MIC values were observed minimum in the MeOH extracts as compared to the AQ extracts**.** Moreover, MeOH extract is a mixture of many biological active components, it could also be assumed that antibacterial action may be synergistic and not due to the efficacy of one single substance present in the extract (Sule et al., 2011).

The aforementioned results explicitly revealed that previously mentioned plant extracts could prove to be efficacious remedial herbal antibiotics, particularly both in controlling Gram-positive and Gram-negative human pathogens. The results also strongly confirm the utility of plants in many ethno-medicinal uses reported earlier. It was also discerned that plant species which are not reported previously for their antibacterial activity (marked with*) against hospital isolates, also possess antibacterial potential. This has been reported by the authors in other cases (Walter et al., 2011).

Table 1. Antibacterial potential of crude plant extracts against test strains (Disc Diffusion Assay).

Plant species	Solvent	Sa (ST)	Ec(ST)	*Sa*1	*Sa*2	*Ec*1	*Ec*2
Achyranthes aspera L.	Methanol	9.0	8.5	8.4	8.4	8.1	8.1
	Aqueous	6.5	N	6.5	6.4	N	N
Aegle marmelos (L.) Correa	Methanol	8.8	9.6	8.2	N	8.4	8.2
	Aqueous	N	N	N	N	N	N
Aloe vera (L.) Burm. F.	Methanol	10.4	N	8.4	8.42	N	N
	Aqueous	6.5	N	N	N	N	N
Amaranthus spinosus	Methanol	N	N	N	N	N	N
	Aqueous	N	N	N	N	N	N
Argemone mexicana L.	Methanol	7.4	N	6.4	N	N	N
	Aqueous	N	N	N	N	N	N
**Bacopa monnieri* (L.) Wettst.	Methanol	9.0	N	6.4	N	N	N
	Aqueous	N	N	N	N	N	N
Calotropis procera (Ait.) R.	Methanol	N	9.0	N	N	8.2	8.2
	Aqueous	N	N	N	N	6.3	N
Chenopodium album L.	Methanol	N	N	N	N	N	N
	Aqueous	N	N	N	N	N	N
Citrullus colocynthis (L.)Schrad.	Methanol	7.4	7.0	6.4	8.4	7.4	7.4
	Aqueous	N	N	N	N	N	N
**Clerodendrum inerme* (L.) Gaertn.	Methanol	10.6	11.1	8.4	7.8	8.2	8.4
	Aqueous	6.5	N	N	N	N	N
Cusuta reflexa Roxb.	Methanol	11.2	10.2	8.4	8.4	8.2	N
	Aqueous	7.0	N	N	N	N	N
**Cycas rumphii* Miq.	Methanol	6.8	7.0	6.5	6.4	6.6	6.5
	Aqueous	N	N	N	N	N	N
**Dactyloctenium aegyptium* (L.)Wild.	Methanol	7.0	N	6.5	6.5	N	N
	Aqueous	N	N	N	N	N	N

Table 1. Contd.

Eclipta alba (L.) Hassk	Methanol	9.0	8.2	8.4	8.4	6.7	7.3
	Aqueous	6.5	N	N	N	N	N
**Emblica officinalis* Webster	Methanol	10.6	9.0	8.2	8.8	N	7.1
	Aqueous	8.4	N	7.0	6.8	N	6.1
Euphorbia hitra L.	Methanol	6.6	N	6.5	6.5	N	N
	Aqueous	N	N	N	N	N	N
Euphorbia thymifolia L.	Methanol	8.6	N	6.4	6.5	N	N
	Aqueous	N	N	N	N	N	N
**Malvastrum coromandelianum* (L.) Garcke	Methanol	N	N	N	N	N	N
	Aqueous	N	N	N	N	N	N
Melia azedarach L.	Methanol	10.6	10.0	8.4	8.0	8.0	N
	Aqueous	7.0	N	N	6.4	N	N
Nerium indicum Mill.	Methanol	6.2	8.2	N	N	6.7	7.5
	Aqueous	N	N	N	N	N	N
**Oxystelma esculentum* (L.*f.*) Sm.	Methanol	10.6	9.0	N	N	7.7	7.1
	Aqueous	N	N	N	N	N	N
**Sida cordata* L.	Methanol	N	9.1	N	N	N	N
	Aqueous	N	N	N	N	N	N
Solanum nigrum L.	Methanol	7.8	N	N	N	N	N
	Aqueous	N	N	N	N	N	N
**Trifolium alexandrinum* L.	Methanol	8.6	N	6.5	6.4	N	N
	Aqueous	N	N	N	N	N	N
Chloramphenicol	----	14.0	16.0	10.0	12.0	12.0	12.0
Ciprofloxacin	----	20.0	23.0	20.0	20.0	21.0	20.0
Gentamycin	----	22.0	23.0	21.0	21.0	22.0	22.0

Sign (N) represents no antibacterial activity; Chloramphenicol: 30 mg/disc; Ciprofloxacin: 30 mg/disc; Gentamycin: 30 mg/disc, Microorganisms: Standard Strains: {Sa (ST)} S.aureus ATCC 25953, {Ec (ST)} E. coli MTCC 739 Clinical isolates: Staphylococcus aureus (Sa1), Staphylococcus aureus (Sa2), Escherichia coli (E c1) and Escherichia coli (Ec2). Values are the mean of replication of three experiments. Plant extract: (20 mg/ml/disc), Inhibition zone in (mm).

Table 2. Antibacterial efficacy of some important traditional plants in India against *E. coli* and *S. aureus* strains (minimum inhibitory concentration[MIC]).

Plant species	Family	Plant Parts	Plant extracts	Ethnomedicinal uses (References)	MIC (mg/ml)		MIC (mg/ml)	
					*Sa*1	*Sa*2	*Ec*1,	*Ec*2
Achyranthes aspera L. [VS; NO:AVK012]	Amaranthaceae	L	Methanol Aqueous	Antiseptic, antipyretic (Asolkar et al.,1992; Khan, 2002)	12.5 100	25 100	50 -	50 -
Aegle marmelos (L.) Correa [VS; NO:AVK015]	Rutaceae	F	Methanol Aqueous	Antidiarrheic, digestive, aromatic, cooling, laxative (Kirtikar and Basu, 1935; Asolkar et al.,1992; Khan, 2002)	12.5 200	- -	25 100	25 200
Aloe vera (L.) Burm. F. [VS; NO:AVK013]	Liliacaeae	L	Methanol Aqueous	Antiseptic, antipyretic, stomachache, anthemintic (Kirtikar and Basu, 1935; Asolkar et al., 1992; Khan, 2002)	25 100	25 100	- -	- -
Amaranthus spinosus L. [VS; NO:AVK025]	Amaranthaceae	L	Methanol Aqueous	Antiseptic,blood purifier,piles,diuretic, scurofulo (Kirtikar and Basu, 1935; Asolkar et al., 1992; Khan, 2002)	- -	- -	- -	- -
Argemone mexicana L. [VS; NO:AVK055]	Papaveraceae	L	Methanol Aqueous	Antiseptic, antipyretic, Jaundice, skin diseases, tumors (Kirtikar and Basu, 1935; Asolkar et al., 1992; Khan, 2002)	25 100	- -	- -	- -
**Bacopa monnieri* (L.) Wettst. [VS; NO:AVK027]	Scrophulariaceae	L	Methanol Aqueous	Asthma, brain tonic, epilepsy, insanity, diuretic (Khan, 2002)	50 200	50 -	- -	- -
Calotropis procera (Ait.) R. [VS; NO:AVK065]	Asclepiadaceae	L	Methanol Aqueous	Antiseptic, antipyretic (Kirtikar and Basu, 1935, Asolkar et al.,1992; Khan,2002)	- -	50 200	25 100	25 -
Chenopodium album L. [VS; NO:AVK018]	Chenopodiaceae	L	Methanol Aqueous	Digestive (Kirtikar and Basu, 1935, Asolkar et al.,1992; Khan, 2002)	- -	- -	- -	- -
Citrullus colocynthis (L.) Schrad. [VS; NO:AVK0008]	Cucurbitaceae	F	Methanol Aqueous	Anti-inflammatory and antibacterial(Kirtikar and Basu, 1935, Asolkar et al., 1992)	50 -	25 -	12.5 -	- -
****Clerodendrum inerme*** (L.) Gaertn. [VS; NO:AVK035]	Verbenaceae	L	Methanol Aqueous	Antiseptic, antipyretic, alternative, febrifuge, diuretic, deobstruent, resolvent. (Khan, 2002)	25 100	50 -	25 200	25 100
Cusuta reflexa Roxb. [VS; NO:AVK122]	Cuscutaceae	W	Methanol Aqueous	Antiseptic, antipyretic (Kirtikar and Basu, 1935, Asolkar et al.,1992; Khan,2002)	25 100	25 200	50 -	- -

Table 2. Contd.

***Cycas rumphii** Miq. [VS; NO:AVK039]	Cycadaceae	L	Methanol Aqueous	Anticancer, (Khan et al., 2011b)	50 -	50 -	50 -	50 -
***Dactyloctenium aegyptium** (L.)Wild. [VS; NO:AVK1171]	Poaceae	W	Methanol Aqueous	Antiseptic (Kirtikar and Basu, 1935, Asolkar et al.,1992; Khan, 2002)	50 -	50 -	- -	- -
***Eclipta alba** (L.) Hassk [VS; NO:AVK115]	Asteraceae	L	Methanol Aqueous	Antiseptic, antipyretic, hepatoprotective, hair Tonic. (Khan, 2002)	25 100	25 100	- 100	- 50
***Emblica officinalis** Webster [VS; NO:AVK150]	Euphorbiaceae	W	Methanol Aqueous	Antiseptic, hepatoprotective, hair tonic, cardio tonic (Kirtikar and Basu, 1935, Asolkar et al., 1992; Khan, 2002)	25 50	25 100	50 100	50 200
***Euphorbia hitra** L. [VS; NO:AVK085]	Euphorbiaceae	W	Methanol Aqueous	Antidiarrheic, digestive (Kirtikar and Basu, 1935, Asolkar et al., 1992; Khan, 2002)	50 -	100 -	50 -	50 -
***Euphorbia thymifolia** L. [VS; NO:AV066]	Euphorbiaceae	W	Methanol Aqueous	Antidiarrheic, digestive (Kirtikar and Basu, 1935; Asolkar et al., 1992; Khan, 2002)	25 200	50 -	- -	- -
***Malvastrum coromandelianum** (L.) Garcke [VS; NO:AVK075]	Malvaceae	L	Methanol Aqueous	General tonic (Kirtikar and Basu, 1935; Khan, 2002)	- -	100 -	- -	- -
***Melia azedarach** L. [VS; NO:AVK047]	Meliaceae	L	Methanol Aqueous	Antiseptic, antipyretic, digestive, skin diseases, hair loss.	12.5 100	25 100	25 -	- -
***Nerium indicum** Mill. [VS; NO:AVK088]	Apocynaceae	L	Methanol Aqueous	Antiseptic, antipyretic, Anticancer (Kirtikar and Basu, 1935; Khan, 2002)	25 - -	25	25 -	50 -
***Oxystelma esculentum** **(L.*f.*) Sm.** [VS; NO:AVK215]	Asclepiadaceae	L	Methanol Aqueous	Antiseptic (Pandya and Anand, 2011)	25 100	25 -	25 -	50 -
***Sida cordata** L. [VS; NO:AVK095]	Malvaceae	L	Methanol Aqueous	General tonic, burning mictrition (Kirtikar and Basu, 1935; Khan, 2002)	- -	- -	50 -	100 -
***Solanum nigrum** L. [VS; NO:AVK049]	Solanaceae	L	Methanol Aqueous	General tonic, hepatoprotective (Kirtikar and Basu, 1935; Khan, 2002)	50 200	50 -	- -	- -
***Trifolium alexandrinum** L. [VS; NO:AVK070]	Fabaceae	L	Methanol Aqueous	General tonic (Khan et al., 2012).	50 100	50 -	- -	- -

(VS; NO), Voucher specimen number; -, no antibacterial activity. Microorganisms: *Staphylococcus aureus* (*Sa*1), *Staphylococcus aureus* (*Sa*2), *Escherichia coli* (*E c*1) and *Escherichia coli Ec*2). Values are the mean of replication of three. {L= Leaf, F= fruit, W= whole plant}.

REFERENCES

Adnan M, Hussain J, Shah MT, Ullah F, Shinwari ZK, Bahadar A, Khan AL (2010) Proximate and nutrient Composition of Medicinal Plants of Humid and Sub-humid regions in Northwest Pakistan. J. Med. Plants Res. 4(4):339-345

Asolkar LV, Kakkar KK, Chakra OJ (1992). Second supplement to glossary of Indian medicinal plants with active principles: Pub. & Inf. Div (CSIR), New Delhi Part 1(A-K): XIVII, pp 414.

Bauer AW, Kirby WMM, Sherries T (1966). Antibiotic susceptibility testing by Biodiversity, National Academy Press: Washington pp. 83-97.

Boudreau MD, Beland FA (2006). An Evaluation of the Biological and Toxicological Properties of *Aloe Barbadensis* (Miller), *Aloe Vera*. J. Environ. Sci. Health Part C, 24:103-154.

Chu VH, Crosslin DR, Friedman JY, Reed SD, Cabell CH, Griffiths RI, Masselink LE, Kaye KS, Corey GR, Reller LB, Stryjewski ME, Schulman KA, Fowler VG Jr. (2005). Staphylococcus aureus bacteremia in patients with prosthetic devices: costs and outcomes. Am. J. Med. 118(12):1416.

Colle JA, Marr W (1989). Cultivation of Bacteria. In: Mackie & Mc Cartney: Practical Microbiology, Churchill Livingston: USA. 13th ed., pp. 121-140.

Cordell GA (2000). Biodiversity and drug discovery a symbiotic relationship, Phytochemistry 55:463-480.

Cox PA (1994). The ethnobotanical approach to drug discovery: strengths and limitations. In, Ethnobotany and the search for the new drugs, John Wiley & Sons: England, pp 25-36.

Eshun K, He Q (2004). *Aloe vera*: A Valuable Ingredient for the Food, Pharmaceutical and Cosmetic Industries-A Review. Critical Reviews in Food Science and Nutrition 44(2):91-96

Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B, Pappas PA, Anstrom KJ, Wray D, Fortes CQ, Anguera I, Athan E, Jones P, van der Meer JT, Elliott TS, Levine DP, Bayer AS. (2005). Staphylococcus aureus *endocarditis*: a consequence of medical progress. JAMA 293:3012-3021.

Gilani SA, Kikuchi A, Shinwari ZK, Khattak ZI, Watanabe WN (2007) Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta Decne*. Phytother. Res. 21:301-307

Gilani SA, Fujii Y, Shinwari ZK, Adnan M, Kikuchi A, Watanabe KN (2010). Phytotoxic studies of medicinal plant species of Pakistan. Pak. J. Bot. 42(2):987-996.

Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E (2006). Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet 368: 874-885

Haessler SD, Brown R (2009). Pneumonia caused by *Staphylococcus aureus*. Curr. Respiratory Med. Rev. 5:62-67.

Hussain JA, Khan L, Rehman N, Hamayun M, Shinwari ZK, Malik W, Lee IJ (2009). Assessment of herbal products and their composite medicinal plants through proximate and micronutrients analysis. J. Med. Plants Res. 3(12):1072-1077

European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2000). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. EUCAST Definitive Document E. Def 3.1. J. Clin. Microbiol. Infect. 6:509-515.

Farnsworth NR (1988). Screening plants for new medicines. Wilson EO (ed.) Biodiversity, National Academy Press: Washington, pp. 83-97.

Harbone JB (1988). Phytochemical Methods. A guide to modern technique of plant analysis, third edition. Kluwer Academic Publishers: United Kingdom, pp. 182-190.

Jain SK (1991). Dictionary of Indian Folk medicine and ethnobotany, Deep publication: New Delhi, 12:311.

Khan AV, Ahmad QU, Mir R, Khan AA, Shukla I (2011a) Antibacterial efficacy of the seed extracts of Melia azedarach against some hospital isolated pathogenic bacterial strains. Asian Pac. J. Trop. Biomed. 1:452-455.

Khan AV, Ahmad QU, Shukla I, Khan AA (2011b) Antibacterial activity of Cycas rumphii Miq. leaf extracts against some tropical human pathogenic bacteria. Res. J. Microbiol. 8(10):761-768

Khan AV, Ahmad QU, Shukla I, Khan AA (2012) Antibacterial activity of leave extract of *Trifolium alexandrinum* Linn. Against pathogenic bacteria causing tropical diseases. Asian Pacific J Tropical Biomed. 1:452-455.

Khan AV (2002). Ethnobotanical studies on plants with medicinal and anti-bacterial properties. (PhD Thesis). Aligarh Muslim University, Aligarh, India, pp. 1-293.

Kirtikar KR, Basu BD (1935). Indian Medicinal Plants. Lalit Mohan Publication: Allahabad, 3:1841

Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER,Fosheim GE, McDougal LK, Carey RB, Fridkin SK (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA, 298(15):1763-71.

Laupland KB, Church DL, Mucenski M, Sutherland LR, Davies HD (2003). Population-based study of the epidemiology of and the risk factors for invasive *Staphylococcus aureus* infections. J. Infect. Dis. 187:1452-1459.

Lescure FX, Biendo M, Douadi Y, Schmit JL, Eveillard M (2006). Changing epidemiology of methicillin-resistant Staphylococcus aureus and effects on cross-transmission in a teaching hospital. Eur. J. Clin. Microbiol. Infect. Dis. 25:205-207.

Leven M, Vanen BDA, Mertens F (1979). Medicinal plants and its importance in antimicrobial activity. J. Planta Med. 36:311-321.

Mainous A, Hueston W, Everett C, Diaz V (2006). Nasal carriage of Staphylococcus aureus and methicillin-resistant S. aureus in the United States, 2001-2002. Ann. Fam. Med. 4:132-137.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C (1999). Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

Mathur J, khatri P, Samanta KC, Sharma A, Mandal S (2010). Pharmacognostic and preliminary phytochemical investigations of *Amaranthus spinosus* (linn.) leaves, Int. J. Pharm. Pharm. Sci. 2(4): 221-224.

Mongkolrattanothai K, Jean CA, Peggy M, Barry MG (2009). Epidemiology of community-onset Staphylococcus aureus infections in pediatric patients: an experience at a Children's Hospital in central Illinois. BMC Infectious Diseases 9:112.

Nabera CK (2009). Staphylococcus aureus Bacteremia: Epidemiology, Pathophysiology, and Management Strategies. Clin. Infect. Dis. 48 (4): S231-S237.

Neill MA, Tarr PI, Clausen CR, Christie DL, Hickman RO (1987). *Escherichia coli* O157:H7 as the predominant pathogen associated with the hemolytic uremic syndrome: a prospective study in the Pacific Northwest. Pediatrics 80:37-40.

Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Jacobson C, Smulders M, Gemmen E, Bharmal M. (2007). National trends in Staphylococcus aureus infection rates: impact on economic burden and mortality over a 6-year period (1998-2003). Clin. Infect. Dis. 45:1132-1140.

Pandya DJ, Anand IS (2011). Anti-ulcer potential of Oxystelma esculentum. Int. J. Green Pharm. (5):65-68.

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL (2005). Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982-2002. Emerg. Infect. Dis. 11:603-609.

Shinwari ZK, Gilani S (2003). Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). J. Ethnophormacol. 84(2003):289-298

Shinwari ZK, Khan I, Naz S, Hussain A (2009). Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases. Afr. J. Biotechnol. 8(24):7082-7086

Shinwari ZK (2010) Medicinal Plants Research in Pakistan. Journ. Med. Pl. Res. 4(3): 161-176

Siegler RL, Pavia AT, Christofferson RD, Milligan MK (1994). A 20-year population-based study of post diarrheal hemolytic uremic syndrome in Utah. Pediatrics 94:35-40.

Stenhem M, Ortqvist A, Ringberg H, Larsson L, Olsson-Liljequist B, Haeggman S, Ekdahl K (2006). The Swedish Study Group on MRSA Epidemiology. Epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in Sweden 2000-2003, increasing incidence and regional differences. BMC Infectious Diseases 6:30

Sule A, Ahmed QA, Othman AS, Muhammad NO, Norazian MH, Laina ZMK, Ambar AY (2011). Bioassay guided isolation of antibacterial compounds from *Andrographis paniculata* (Burm.f.) Wall. ex Nees

(*Hempedeu bumi*). Am. J. Appl. Sci. 8:525-534.
Uddin Q, Malik A, Azam S, Hadi N, Azmi AS, Parveen N, Khan NU, Hadi SM (2004). The biflavonoids, amentoflavone degrades calf thymus DNA in the presence of copper ions. Toxicology in Vitro, 18:435-440.
Vanden BD, Vlietinck AJ (1991). Screening methods for antibacterial and antiviral agents from higher plants. In Dey P, Harbone JB, Hostettman K (eds.) Methods for plant biochemistry Assays for Bioactivity.6.San Diego, Academic Press, pp. 47-69.
Vogler BK, Ernst E (1999). Aloe vera: a systematic review of its clinical effectiveness. Br. J. Gen. Pract. 49 (447):823-828.
Walter C, Shinwari ZK, Afzal I, Malik RN (2011). Antibacterial Activity in Herbal Products Used in Pakistan. Pak. J. Bot. 43:155-162.
Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect .Dis. 39:309-317.
World Health Organization (WHO) (2004). The World Health Report, Changing History. Statistical annex. Death By cause, sex and mortality stratum in WHO regions, estimates for 2002, Geneva: Switzerland, WHO, pp. 120-121.
Yousaf Z, Shinwari ZK , Khan MA (2010). Phenetic Analysis of Medicinally Important Species of the genus *solanum* from Pakistan. Pak. J. Bot. 42(3):1827-1833.