

IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF TWO MEDICINAL PLANTS AGAINST SOME CLINICALLY IMPORTANT BACTERIA

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Abstract

The aim of the present study was to evaluate the antimicrobial potential of *Amaranthus viridis* (Chowlai) and *Cannabis sativa* (Bhang) against clinically important bacteria, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. The stem and leaf extracts of these plants were prepared in pure solvents, ethanol and methanol. Tetracycline was used as standard antibiotic for reference. Solvent extracts were screened for antimicrobial activities by disc diffusion method. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) method. A significantly high antimicrobial activity was observed in the ethanol leaf extracts of both plants with 13.8 to 21.33 mm zone of inhibition against *S. aureus* and *K. pneumoniae*. The study revealed that leaves of *A. viridis* and *C. sativa* possess broad spectrum antimicrobial activity and natural antioxidants that can be of considerable pharmaceutical importance.

Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immuno-compromised patients in developed countries (Al-Bari *et al.*, 2006). *Staphylococcus aureus*, Gram-positive bacteria, causes a diverse array of lethal infections including skin and soft-tissue infections and urinary tract infections (UTI) in sexually active young women. *S. aureus* was a center of concern a decade ago, however, clinical microbiologists believe that Gram-negative bacteria pose a greater risk to public health (Cornaglia, 2009). A variety of human infections are caused by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. *P. aeruginosa* is the leading cause of pathogenic infections in acquired immunodeficiency syndrome (AIDS) patients, whereas *K. pneumoniae* causes pneumoniae, necrosis, inflammation and hemorrhage in lung tissues (Okeke, 2010). *Escherichia coli* cause severe urinary tract infections and has been a source of transferring antibiotic-resistant genes from infected food animals to human (Nordstrom *et al.*, 2013).

There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross infections. The development of new antibiotics should be continued as these are of primary importance to maintain for the effectiveness of antimicrobial treatment (French, 2005). In developing countries, the World Health Organization (2002) estimates about three quarters of the population relies on plant based preparations used in their traditional medicines and the primary health care. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin (Subramani and Goraya, 2003).

Over 60% population in the rural areas of Pakistan depends on traditional medicines for the treatment of their ailments. Microorganisms possessed natural resistance to antimicrobial agents even prior to the introduction of antibiotics, however, this natural resistance was present only in antibiotics producing microorganisms. Medicinal plants are rich source of antimicrobial agents and the bacterial resistance to these agents has been studied extensively (Mahesh and Satish, 2008).

Amaranthus viridis L. belongs to the family *Amaranthaceae* and has vernacular name as Chowlai or Karund. It is an annual herb, which is native to Africa and is found in Asia, Africa and Latin America in cultivated and uncultivated fields and grasslands. *A. viridis* is a plant of considerable medicinal importance. Leaves used for scorpion stings, traditionally used for constipation, inflammation, eczema, bronchitis, anemia and leprosy. *A. viridis* has also been used commercially to make yellow and green dyes (Kumar *et al.*, 2009).

Similarly, *Cannabis sativa* was used in the twentieth century B.C in Egypt to treat sore eyes. In India prior to the tenth century B.C., it was used as an anesthetic and anti-phlegmatic agent. Therapeutic values of *Cannabis sativa* derivatives have also been highlighted against HIV/AIDS (Abrams *et al.*, 2007). Flowers, leaves and stalks of the mature female plant are commonly used as the source of medicinal properties of *Cannabis sativa*. Moreover, Cannabis is commercially grown and processed for many uses. In third world countries Hemp is

grown for nutritional purposes whereas, oil and stalks are burned for biofuels. Hemp fiber is used to make plastic and composite materials (Shahzad, 2012).

Medicinal plants are also recognized to have natural antioxidants that help in prevention of infectious diseases and to clear the reactive oxygen species (ROS) from the living organisms which are the major cause of aging and main source of DNA damage. The antioxidant effects in plants are mainly due to the presence of phenol compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes. Natural antioxidants are important for human health because of low risk of heart diseases and possessing anti-mutagenic/carcinogenic properties, and being safer compared to the synthetic antioxidants (Shaker, 2006). Based on these properties of medicinal plants, the present study was designed to examine and compare the antimicrobial and antioxidant potential of leaf and stem extracts of commonly occurring plants, *Amaranthus viridis* and *Cannabis sativa*, against bacteria strains of clinical importance.

Materials and Methods

Collection of plant material: The plants, *A. viridis* and *C. sativa* were collected from open fields of Lahore, Pakistan. Leaves and stem of the plants were separated and washed properly with distilled water to remove any impurities and dust settled on the surface of these plant parts. All Plant parts were dried under shade at room temperature for the prevention of loss of any active constituents. The dried leaves and stem of both plants were grounded separately to coarse powder using mechanical blenders followed by fine extraction using organic solvents.

Test organisms: The bacterial strains *Staphylococcus aureus* (Gram-positive), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Gram-negative) used in the present study were procured from Service Institute of Medical Sciences (SIMS), Lahore, and Post Graduate Institute of Medical Sciences (PGMI), Lahore, Pakistan.

Plant extraction: All plant extracts were prepared using the soxhlet extraction method. Ten grams of dry powder from leaves and stem of *A. viridis* and *C. sativa* were extracted with 100 mL of ethanol; and methanol, separately in soxhlet extractor until the final extraction was colorless. These ethanol and methanol extracts were then subjected to rotary evaporator at 78.4 °C and 64.7 °C, respectively. The crude extracts obtained were stored at 4 °C in airtight glass bottles for further use.

Antibacterial assay using Disc-Diffusion-Susceptibility method: Antibacterial properties of these extracts were evaluated using disc-diffusion-susceptibility method described by Bauer *et al.* (1966). The prepared Mueller-Hinton agar medium was poured into the pre-labeled sterilized petri plates. After solidification, freshly prepared culture in nutrient broth was swabbed with the help of sterilized cotton swab, carefully on the respective plates. Whatman filter paper discs (6mm diameter) impregnated with the test extracts were placed on the bacterial plates. Tetracycline (30 µg) and solvent (ethanol and methanol) discs were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 18-24 hours. All plates were used in triplicates and the average diameter of zone of inhibition on each plate was recorded.

Antioxidant activity [2,2-diphenyl-1-picryl-hydrazylhy (DPPH) assay]: The antioxidant activities of *A. viridis* and *C. sativa* extracts obtained by soxhlet extraction were determined by using DPPH free radical assay as described by Molyneux (2004). The working dilutions of plant extracts were prepared by dissolving 2,2-diphenyl-1-picryl-hydrazylhy (DPPH) in methanol and ethanol, separately. Ascorbic acid was used as positive control. One milliliter of plant extract was added to 3 mL of DPPH solution. All dilutions were used in triplicates. The absorbance was recorded at 517 nm by UV-Spectrophotometer against ascorbic acid.

Statistical analysis: Statistical analysis was carried out with SPSS (version 17.0) using ANOVA ($p < 0.05$ was used as significance level).

Results

Medicinal plants *A. viridis* and *C. sativa* were screened for their antimicrobial activity against *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* by disc diffusion method. The plant extracts showing positive and negative antibacterial activity against Gram-positive and Gram-negative strains are shown in Table 1. No inhibition zones were observed for negative controls. Maximum antibacterial activity was observed in ethanol (13.8 mm) and methanol (12.0 mm) leaf extracts of *A. viridis* against *S. aureus* (Figure 1) whereas, methanol stem extracts produced maximum antibacterial activity (10.9 mm) against *K. pneumoniae*. A statistically non-significant increase in the antibacterial activity of *A. viridis* extracts was observed against *P. aeruginosa*.

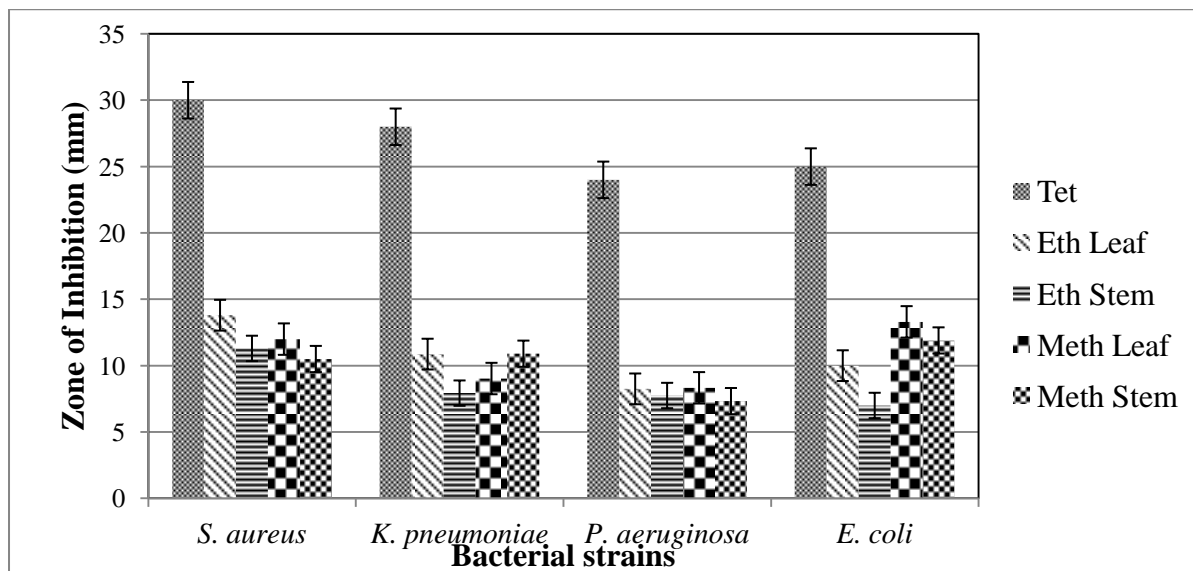


Fig. 1. Antibacterial activity of *A. viridis* extracts against bacterial strains. Tet; Tetracycline, Eth; Ethanol, Meth; Methanol.

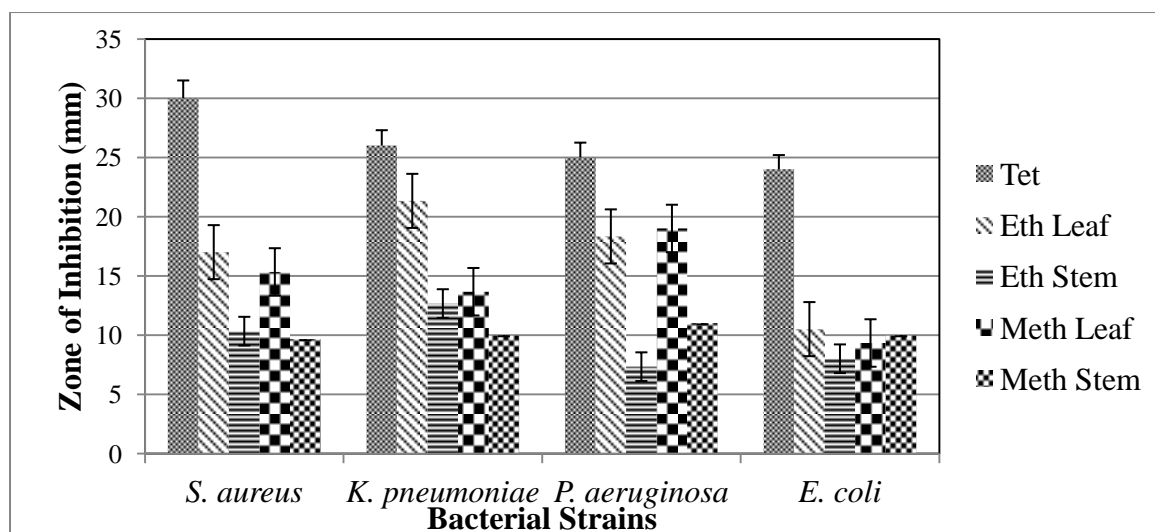


Fig. 2. Antibacterial activity of *C. sativa* extracts against bacterial strains. Tet; Tetracycline, Eth; Ethanol, Meth; Methanol.

Table 1. Antimicrobial activity of *A. viridis* and *C. sativa* extracts against bacterial strains.

Plant	Plant part	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	
		E	M	E	M	NC
<i>A. viridis</i>	Stem	+	+	+	-	-
	Leaf	+	+	+	-	-
<i>C. sativa</i>	Stem	+	+	-	+	-
	Leaf	+	+	-	+	-

Key: E: ethanol; M: methanol; NC: negative control (pure solvents); +: positive activity; - = not detected.

Table 2. DPPH radical scavenging activity of *A. viridis* and *C. sativa* extracts against ascorbic acid Key: Antioxidant activity of ascorbic acid (positive control) = $53 \pm 0.1\%$. All values are mean \pm SD calculated from triplicates.

Plants	Plant Parts	DPPH scavenging activity (% age)	
		Ethanol	Methanol
<i>A. viridis</i>	Stem	17.6 ± 1.5	26.3 ± 0.1
	Leaf	22.4 ± 2.3	29.0 ± 0.2
<i>C. sativa</i>	Stem	29.4 ± 0.1	28.3 ± 0.1
	Leaf	33.27 ± 0.2	43.4 ± 0.1

C. sativa extracts produced significant antimicrobial activity against the tested bacterial strains while a non-significant antimicrobial activity was observed against *E. coli*. Ethanol leaf extract of *C. sativa* produced maximum antimicrobial activity against *K. pneumoniae* (21.3 mm) and *S. aureus* (17.0 mm). Methanol leaf extract also produced significant antimicrobial activity against *S. aureus*. Ethanol stem extract produced higher antimicrobial activity (12.7 mm) than methanol stem extract (Figure 2).

These plants were also assessed for their antioxidant activity by DPPH assay. The DPPH inhibition of leaf and stem extracts of *A. viridis* and *C. sativa* are recorded in Table 2. The free radical scavenging activity of positive control (ascorbic acid) was 53.5 ± 0.1 . Percentage scavenging activity of *A. viridis* extracts ranged from $17.6 \pm 1.5\%$ to $29 \pm 0.1\%$ and that of *C. sativa* extracts from $29.4 \pm 0.1\%$ to $43.5 \pm 0.1\%$.

Discussion

Medicinal plants are used as the source of active substances and drugs of pharmaceutical interests. Drugs of herbal origin are effective and have least side-effects in the treatment of infectious diseases (Leitao *et al.*, 2013). The present study was conducted using ethanol and methanol leaf and stem extracts of *A. viridis* and *C. sativa*. Significant increase in antibacterial and antioxidant activities was observed from these plant extracts. Iqbal *et al.* (2012) also reported the similar antimicrobial activities of leaf and seed extracts from *Amaranthus viridis*. Similarly, the potent antimicrobial activity of ethanol leaf extracts of *C. sativa* against *K. pneumoniae* is in accordance with the findings of other investigators who observed the same activity of *C. sativa* against multiple strains (Ali *et al.*, 2012; Wasim *et al.*, 1995). However, these findings were different from that of Borchardt *et al.* (2008) who found that stem and leaves extracts were only active against *Staphylococcus aureus*.

The study on antimicrobial potential of *A. viridis* and *C. sativa* proved ethanol as a better solvent for extraction of antimicrobial agents than methanol because of the ability of ethanol to solubilize and extract some active compounds such as flavonoids and other metabolites responsible for this activity. These antimicrobial substances have been an effective source of inhibition for various microorganisms (Rashid *et al.*, 2013). Moreover, ethanol formulations are relatively safe for human consumption as compared with other organic solvents such as acetone or methanol etc. This might be the reason that ethanol extracts showed better antimicrobial activity against test organisms (Wendakoon *et al.*, 2012).

Natural antioxidants present in the medicinal plants are responsible for inhibiting the deleterious consequences of oxidative stress. One such stable free radical DPPH has been accepted as a model compound for testing the free radical scavenging activity of natural products. The inhibition of DPPH radicals was observed by antioxidant-induced decrease in their absorbance at 517 nm. *Amaranthus viridis* extracts also possess the ability of acting as a free radical scavenger, has also been reported by Amin *et al.* (2006). Maximum percentage inhibition was observed in the methanol leaf extracts of *C. sativa* which possess the diverse nature of antioxidant compounds that inhibit DPPH radicals, has also been reported by Nadeem *et al.* (2012). The antioxidant activity might be due to the high phenol content and other constituents as flavones and hesperidin as also reported by Kaneria *et al.* (2009).

Conclusion

Leaf and stem extracts of *A. viridis* and *C. sativa* demonstrated a broad spectrum efficacy against Gram-positive and Gram-negative bacteria. These plants also exhibited good antioxidant activity.

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