

Antimicrobial activity of different tea varieties available in Pakistan

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Abstract: In this antimicrobial study, various extracts of Green and Black tea (*Camellia sinensis*) and Lemon grass (*Cymbopogon citrates*) were evaluated for antimicrobial activities against six bacterial strains including both human pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*) and plant pathogenic bacteria (*Erwinia carotovora*, *Agrobacterium tumefaciens*) and one fungal strain *Candida albicans* by disc diffusion susceptibility method. Of human pathogens, *P. aeruginosa* was most susceptible to all three different tea varieties; though rest of the strains also demonstrated prominent sensitivity. In comparison, black tea extracts were less activities than green tea and lemon grass. However, all the three tea varieties illustrated profound activity against plant pathogenic bacteria. Similarly, when extracts of tea were tested against *C. albicans*, green tea and lemon grass exhibited significant activity while black tea was mostly inactive.

Keywords: Antimicrobial, green and black tea, lemon grass.

INTRODUCTION

Camellia sinensis L., the tea plant is an evergreen shrub belonging to Theaceae family. Tea is generally divided into three types, which depends on the process of fermentation. Fermented teas are black and red tea, oolong tea is partially fermented while Green tea is unfermented form. By an oxidation process, fermentation of black tea is carried out with the help of an enzyme polyphenols oxidase, while red or Pu-Erh tea is attained by fermentation using microorganisms (Cabrera *et al.*, 2006). Tea extract contains various properties such as antioxidant, antimutagenic, antitumour, antiviral, anticoagulant, against heart diseases, blood pressure, fighting against cancer and cholesterol lowering activity (Jarald and Jarald, 2006). Tea contains different constituents like xanthines, caffeine, tannins, theobromine, flavonoids, polyphenols, fats and vitamin C (Chevallier, 2000). Tea flavanols are called tea catechins. Major catechins are (-)-epicatechin (EC), (-)-epicatechingallate (ECG), (-)-epigallocatechingallate (EGCG) and (-)-epigallocatechin (EGC). These catechins are water-soluble compounds imparting astringency and bitterness to tea infusion. Other characteristics like taste, color and aroma are linked directly or indirectly to these catechins. Decrease in catechin content leads to enhanced aroma quality of tea (Wang *et al.*, 2000).

Cymbopogon citrates, which is commonly known as Lemongrass, a tall perennial grass belongs to the genus *Cymbopogon* of aromatic grasses and contains essential oil with fine lemon flavor. In Asia Lemongrass is widely

used as essential component for health. In India it is used as sedatives for the central nervous system (Brian and Ikhlas 2002). The *Cymbopogon* essential oils are characterised by monoterpene constituents like citral, citronellal, citronellol, elemol, linalool, 1,8-cineole, limonene, β -carophyllene, geraniol, methylheptenone, geranylformate and geranyl acetate. Citral is one of the important ingredients of the oil present in several species of *Cymbopogon* with large industrial uses such as raw material for confectionery, perfumery and vitamin A (Huynh *et al.*, 2008).

The aim of this present study was to evaluate the antimicrobial activity of above mentioned plants. Green and Black tea are obtained from the main single plant *C. sinensis*.

MATERIALS AND METHODS

Plant materials

C. sinensis (Green and Black tea) and *Cymbopogon citrates* were collected from local market Kohat and were identified by plant taxonomist Kohat University of Science and Technology, Kohat, Pakistan.

Test microorganisms

In this study, various extracts of *C. sinensis* and *Cymbopogon citrates* were evaluated for antimicrobial activities against gram positive, gram negative and fungi. The bacterial strains such as *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9721, *Staphylococcus aureus* ATCC 6538 and clinically isolated *Salmonella typhi*, *Erwinia carotovora*, *Agrobacterium tumefaciens* and one fungal strain *C. albicans* were tested by Disc Diffusion Susceptibility Method. Microorganisms

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were provided by PCSIR Laboratories Complex, Peshawar.

Extract preparation

Camellia sinensis (How much quantity was taken?) (Green tea and Black tea) and *Cymbopogon citrates* (Lemon grass) were soaked in methanol for 10 days and were extracted three times at room temperature in the same solvent. Then it was filtered through Whatman No. 1 filter paper. The filtrate was evaporated under reduced pressure to give extract, which was further suspended in water and partitioned successively with *n*-hexane, chloroform and ethyl acetate, to obtain *n*-hexane-soluble, chloroform-soluble and ethyl acetate-soluble fractions, respectively. The crude plant extracts and subsequent solvents soluble fractions were subjected to antimicrobial bioassay.

Antimicrobial assay

Disc diffusion method

The antimicrobial activity was done by agar disc diffusion assay by measuring the zone of inhibition against the test microorganisms by using the method of Kirby-Bauer method (Baur *et al.*, 1966). *In vitro* antimicrobial assay was screened by means of Nutrient Agar (NA). Few ml of molten NA agar media was poured into the sterilized petriplates. These plates were allowed to solidify, the standard 0.5 McFarland and 10⁶cfu/ml of 100µl of standardized microbial culture were spread on agar plates with the help of a glass spreader. Similar practice has been carried out for the fungi. Concentrations of various extracts (1 and 2mg/disc) in the form of 6 and 12µl were loaded on 6mm sterile individual discs and the plates were placed in incubator for 24 hours at 37°C. DMSO was used as negative control because all the extracts were dissolved in this solvent. Antibiotics (Azithromycin 50µg/6µl, Ciprofloxacin 30µg/6µl, Clotrimazole 50µg/6µl) were applied 6µl/disc on separate plate was used as positive control for gram-positive bacteria, gram-negative bacteria and *C. albicans* respectively. These studies were performed in duplicate to clear any uncertainty and the mean ratios of inhibitory zones were taken.

RESULTS

Effect of green tea in antimicrobial assay

Different extracts of green tea exhibited different zone of inhibitions (table 1). The ethyl acetate and aqueous extracts showed zone of inhibition in the range of 13-21 mm and 7-19mm, while ethyl acetate was inactive against *S. typhi*. The chloroform showed 10-21mm, while inactive against *E. coli* and *C. albicans* (table 4). Hexane showed 10-22mm and was ineffective against *S. aureus*, while methanol extract showed 9-14mm and was active against all the organisms. All the extracts showed highest zone of inhibitions against *P. aeruginosa*.

Effect of black tea in antimicrobial assay

Different extracts of black tea exhibited various zone of inhibitions (table 2). Aqueous and ethyl acetate fractions showed inhibitory zone in the range of 9-14mm and 7-14 mm, while not effective against *S. typhi*, *A. tumifaciens* and *C. albicans*. Chloroform showed 8-22mm. Hexane showed 7-15mm and was inactive against *S. typhi* and *C. albicans* (table 4), while methanol showed 12-20mm and was inactive against *S. typhi*, *E. carotovora*, *A. tumifaciens* and *C. albicans*. All the extracts showed highest zone of inhibitions against *P. aeruginosa* 9-22 mm, while it showed no activity against *S. typhi*.

Effect of lemon grass in antimicrobial assay

Similarly lemon grass also showed different inhibitory zone (table 3). Highest zone of inhibitions were shown against *P. aeruginosa* in between 9 and 26mm. The methanol extract showed zones between 11-26mm, while it showed no activity against *E. carotovora*. Hexane showed 8-15mm, chloroform 9-18mm, ethyl acetate 7-12mm and aqueous 7-15mm. Thus all the fractions were active against these organisms.

DISCUSSION

The antimicrobial activity of Pakistani medicinal is frequently available in literature (Khan *et al.*, 2012; Khan *et al.*, 2013). The current study deals with the strong antimicrobial activity of various extracts of different tea varieties available in Pakistan.

The green tea has good antimicrobial results than black tea. The reason for this distinction in activity could be due to different active constituents, which are present in green tea (Wang *et al.*, 1992). This suggested that many harmful bacteria such as Gram-positive *S. aureus* can be killed by the daily use of green tea. The tea consists of different antioxidant polyphenols, known as catechins, due to which it showed antimicrobial activity. It destroys bacterial cell membrane and inhibits its growth. Different catechins present in tea are epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC), gallic acid (GA), gallic acid-3-gallate (GCG) and epicatechin (EC) (Isogai *et al.*, 2001).

Additionally, tea extracts contains caffeine and other catechins, which could be helpful in the current antimicrobial show. As it has been observed in animals and plants cells, caffeine stops the division of cells (Aneja, 2001). In this way, it stopped spore germination and development. EGCG and ECG (Gallated Catechins) disengage proteins and breaks the bacterial lipid bilayer by shifting the membrane fluidity and morphology (Ikigai *et al.* 1993). In black tea caffeine present in large quantity as compared to green tea, while the natural polyphenols present in black tea are mostly oxidized. So as a result it showed less activity than green tea.

Table 1: Antimicrobial activities of *Camellia sinensis* (Green tea).

Test Microorganisms	Zone of inhibition (mm)										
	Crude		n-hexane		Chloroform		Ethyl acetate		Aqueous		Standard
Extract mg/ μ l	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	50 or 30 μ g/6 μ l
Human pathogenic bacteria											
<i>Escherichia coli</i>	12	13	11	12	-	-	16	20	11	15	28*
<i>Salmonella typhi</i>	11	14	13	15	11	14		-	10	11	30*
<i>Pseudomonas aeruginosa</i>	12	14	20	22	16	17	19	21	14	17	30*
<i>Staphylococcus aureus</i>	9	11	-	-	11	16	15	17	7	10	25**
Plants pathogenic bacteria											
<i>Erwinia carotovora</i>	10	11	10	14	10	12	15	19	9	11	24*
<i>Agrobacterium tumifaciens</i>	9	11	11	12	11	21	16	18	9	10	36*

Table 2: Antimicrobial activities of *Camellia sinensis* (Black tea)

Test Microorganisms	Zone of inhibition (mm)										
	Crude		n-hexane		Chloroform		Ethyl acetate		Aqueous		Standard
Extract mg/ μ l	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	50 or 30 μ g/ 6 μ l
Human pathogenic bacteria											
<i>Escherichia coli</i>	-	14	9	12	17	19	11	14	-	12	28*
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	-	-	-	30*
<i>Pseudomonas Aeruginosa</i>	17	20	9	12	16	22	12	14	11	14	30*
<i>Staphylococcus aureus</i>	12	14	13	15	8	9	7	8	-	-	25**
Plants pathogenic bacteria											
<i>Erwinia carotovora</i>	-	-	9	11	10	11	10	13	9	12	24*
<i>Agrobacterium tumifaciens</i>	-	-	7	9	8	10	11	14	-	-	36*

Table 3: Antimicrobial activity of *Cymbopogon citrates* (Lemon grass)

Test Microorganisms	Zone of inhibition (mm)										
	Crude		n-hexane		Chloroform		Ethyl acetate		Aqueous		Standard
Extract mg/ μ l	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	50 or 30 μ g/ 6 μ l
Human pathogenic bacteria											
<i>Escherichia coli</i>	15	23	12	14	13	17	9	12	12	14	28*
<i>Salmonella typhi</i>	14	17	11	12	9	10	11	11	9	10	30*
<i>Pseudomonas Aeruginosa</i>	22	26	10	15	14	18	9	11	12	15	30*
<i>Staphylococcus aureus</i>	17	18	8	11	9	9	7	9	8	8	25**
Plants pathogenic bacteria											
<i>Erwinia carotovora</i>	14	16	8	10	9	11	9	12	7	8	24*
<i>Agrobacterium tumifaciens</i>	11	16	8	11	9	11	7	11	7	8	36*

Table 4: Effect of different tea varieties commonly used in Pakistan on *Candida albicans*.

<i>Candida albicans</i>	Zone of inhibition (mm)										
	Crude		n-hexane		Chloroform		Ethyl acetate		Aqueous		Standard
Extract mg/ μ l	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	1/6	1/12	50 or 30 μ g/ 6 μ l
Greentea	11	12	10	13	-	-	13	18	10	11	25***
Black tea	-	-	-	-	9	11	-	-	-	-	
Lemon grass	11	16	8	11	9	11	7	11	7	8	

(-): No inhibition zone, Ciprofloxacin (*): 30 μ g/6 μ l, Azithromycin (**): 50 μ g/6 μ l.

Similarly, the essential oils of *C. citratus* showed the presence of citral, neral, limonene, geranial, citronellal, geraniol and myrcene (Schaneberg and Khan 2002). These classes of compounds could also contribute in the antimicrobial activity. The essential oil also contains terpenoids and phenols like eugenol, thymol (Didry *et al.*, 1993, Juneja and Friedman. 2007) are responsible for antimicrobial activity could also helpful in the antimicrobial effect of tea varieties.

CONCLUSION

Green and black tea and Lemon grass showed prominent antimicrobial activity at different concentrations against the tested microorganisms. Therefore, the present study validated the uses of these plants in folk medicine for the treatment of different infectious diseases and thus supported the uses of these beverages in limited.

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