

Antibacterial Activity of Hydroalcoholic Extract of *Callistemon citrinus* and *Albizia lebbbeck*

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Abstract: Problem statement: *Callistemon citrinus* and *Albizia lebbbeck* are two kind of native plants in Khoozestan province in Iran, according to the agreement that medicinal plant could be replaced the medicine we are trying to determine the antibacterial effect of the named plants. **Approach:** The antibacterial properties of ethanolic and methanolic extract of *Callistemon citrinus* leaf and *Albizia lebbbeck* leaf was studied against different pathogenic bacteria including *Streptococcus pyogenes*, *Bacillus cereus*, *Bacillus anthracis*, *Salmonella typhi*, *Kelebsiella pneumoniae*, *Streptococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* by disc diffusion method. The samples collected from young trees from Ahwaz city at may 2009. **Results:** The extract of *C. citrinus* showed significant activity against the majority of bacteria which is comparable with standard antibiotics. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for hydroalcoholic extract of *Callistemon* have been determined also for four bacteria; *S. pyogenesis*, *S. typhy*, *B. anthrasis*, *S. typhy*, *Albizia lebbbeck* extract despite previous reports didn't have any significant effect. **Conclusion:** According to the good effects of *Callistemon citrinus* on *Bacillus* species it has antiseptic effects and could be used as a therapeutic agent and therefore, it appears to be a potent antimicrobial agents that could be considered as a medicinal plant.

Key words: *Callistemon citrinus*, *Albizia lebbbeck*, antibacterial, MIC, MBC

INTRODUCTION

The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemistrie's found in plants for their therapeutic properties. All drugs from the plant are substances with a particular therapeutic action extracted from plants (Serrentino, 1991). The usage of herbal plants as traditional health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is reported to have minimal side effect (Doughari, 2006).

In this study 2 plant species have been selected; *Callistemon citrinus* and *Albizia lebbbeck*.

Callistemon citrinus (Family: Myrtaceae) the common name, "bottlebrush", perfectly describes this evergreen plant's bright red flower spikes. The flowers are followed by small; woody capsules that look like bead bracelets on the bark, and which last for years.

Albizia lebbbeck (native name: Borhan; Family: Leguminosae) is a deciduous tree with compound leaves, flat oblong fruits, round cream colored seeds,

grows wild and planted in almost all districts of Bangladesh (Ghani, 2003). This plant is found throughout India, Bangladesh, tropical and subtropical Asia and Africa (Kirtikar and Basu, 1980).

Many studies have been done on medical properties of different species of *C. citrinus*: Antibacterial, antifungal and antioxidant activities of methanolic extract obtained from *Callistemon linearis* DC. Leaf have been studied. Methanolic extract exhibited potential antimicrobial activity against both gram positive as well as gram negative bacteria and moderate activity against fungal species. This extract also shows good antioxidant activity (comparable with standard Ascorbic acid) which is concentration dependent (Anudwipa Das *et al.*, 2008).

There are also lots of data on therapeutic properties of *Albizia lebbbeck*: The Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties. The methanolic extract of the pod was investigated for antifertility effect (Gupta *et al.*, 2004; 2005). Although *A. lebbbeck* has traditionally been used in the treatment of many types of pain and inflammatory conditions. The analgesic and anti-

inflammatory effect of *A. lebbeck* have been reported (Achinto and Munirrudin, 2009). The *in vitro* antibacterial activities of 80% methanolic crude extracts prepared from the seeds of *Ablizia gummifera* was tested for inhibitory activity against the clinical isolates of six *S. pneumoniae* and twenty two *S. pyogenes* using agar dilution method (Abayneh, Unasho, 2005).

This study is an attempt to determine antimicrobial activity of *Calistemon citrinus* and *Albizia lebbeck* leaf ethanolic and methanolic extract on selected pathogenic bacteria isolated from patients.

MATERIALS AND METHODS

Collection of plant material: The *C. citrinus* (Bottle brush tree) and *A. lebbeck* were collected from Ahvaz in Khuzestan province of Iran at May, 2009. The taxonomic identification of these plants was confirmed by department of biology, Chamran University, Iran.

Extract preparation: The leaves of each considered plant were shade dried for 48 h and crushed into powder using blender, 6 g of powder were used for extraction. 10 mL of ethanol distilled water (8:2w/v) g⁻¹ were used to make ethanolic extract and 10 mL methanol distilled water (8:2w/v) g⁻¹ for methanolic extract, then centrifuged (3000 rpm) for 15 min and the supernatants were harvested. The process repeated three times. Finally the alcohol was removed by evaporation through incubating at room temperature. (Seyyednejad *et al.*, 2001; Moazedi *et al.*, 2007) the methanolic extract prepared following the method described above by Okemo *et al.* (2001).

Test microorganisms: A total of eight bacterial species were tested including *Streptococcus pyogenes*, *Bacillus cereus*, *Bacillus anthracis*, *Salmonella typhi*, *Kelebsiella pneumoniae*, *Streptococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* that were isolated from medicinal samples. They were identified using standard biochemical tests.

Antimicrobial assay: The antimicrobial activity of the ethanolic and methanolic extract was determined based on inhibition zones in disc diffusion method described by (Bauer *et al.*, 1996). Mueller-Hinton agar was the media which is selected for preparing test plates. In all cases, the inoculums were contained in the nutrient agar plates for bacteria and previously prepared ethanolic extract and methanolic extract impregnated disc (5 mm in diameter blotting paper disc) at concentration of 100 µg mL⁻¹ for bacteria and were placed aseptically on sensitivity plates with appropriate

control were used as standard antibacterial respectively. The plates were incubated for 24 h at 37°C for bacteria (Bauer *et al.*, 1996). Control discs were soaked with the same extraction solvents and treated as the sample discs. The positive results (sensitivity) were established by the presence of clear zone of inhibition around active extracts which were measured with a meter rule and diameters were recorded based on mm.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of the extracts was determined for the most sensitive bacterial species for three times. A 16 h culture was diluted with a sterile Physiologic Saline solution (PS; 0.85% (w/v) sodium chloride) with reference to the 0.5 McFarland turbidometry to achievement of inoculums approximately 10⁸ colony forming units (cfu) mL⁻¹ (Burt and Reinders, 2003) a serial dilution was carried out to give final concentrations between 0.5-0.0025 from crude extract. The tubes were inoculated with 30 micro liter of the bacterial suspension mL⁻¹ of Muller Hinton broth, homogenized and incubated at 37°C. The Minimum Inhibitory Concentration (MIC) value was determined as the lowest concentration of the crude extract in broth medium that inhibited the visible growth of the test microorganism (Motamedi, 2009).

Determination of Minimum Bactericidal Concentration (MBC): To determine the MBC, for each set of test tubes in the MIC determination, a loop full of broth was collected from those tubes which did not show any growth and inoculated on sterile Muller-Hinton agar by streaking. The plates were inoculated at 37°C for 18-24 h. The highest dilution that yielded no colony fraction on a solid medium was considered as MBC (Motamedi, 2009).

RESULTS

The antimicrobial activity of Ethanolic and Methanolic extract of *Callistemon citrinus* was resulted to a growth inhibition pattern against the tested microorganism. The results of the antimicrobial activity were given in the Table 1. These data revealed that the ethanolic and methanolic extract showed good antimicrobial activity against bacteria. It is noteworthy in particular effect against *Salmonella*, *B. cereus*, *S. epidermis*, *B. anthracis* which is comparable with antibiotics. The results of MIC and MBC of 4 bacteria species are shown in Table 2.

In this case the methanol and ethanol extract of *Albizia lebbeck* were also examined and didn't have any antibacterial effect.

Table 1: Results of antibacterial activity of ethanolic and methanolic extract of *Callistemon citrinus* leaf

Bacteria species	Ethanolic				Methanolic				Antibiotic disc*			
	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	NF	CB	NB	DX
Gram positive												
<i>S. aureus</i>	10	10	9	R	-	-	-	-	R	13	31	15
<i>B. anthracis</i>	13	11	10	R	22	19	19	20	R	28	20	32
<i>B. cereus</i>	17	17	15	14	17	16	16	15	R	7	18	18
<i>S. pyogenes</i>	24	23	22	25	25	25	41	30	R	36	39	21
<i>L. monocytogenes</i>	-	-	-	-	16	16	15	14	25	19	12	20
Gram negative												
<i>S. typhi</i>	22	21	17	15	15	12	12	11	R	27	34	30
<i>Kelebsiella pneumoniae</i>	R	R	R	R	11	9	11	9	R	R	11	R
<i>E coli</i>	R	R	R	R	R	R	R	R	R	R	17	11
<i>P. aeruginosa</i>	-	-	-	-	16	18	15	14	R	R	16	R

*: (6 mm) diameter disc; R: Resistant; -: Not used

Table 2: Antibacterial activity (MIC and MBC in mg mL⁻¹) of the ethanolic and methanolic extract of *Callistemon citrinus* on come tested bacteria

Bacteria species	Extract			
	Ethanolic		Methanolic	
	<i>S. pyogenes</i>	<i>S. typhi</i>	<i>B. anthracis</i>	<i>S. typhi</i>
MIC	5.0	40	10	20
MBC	2.5	80	20	40

DISCUSSION

The antimicrobial activities of various plants have been reported by many Researchers (Cowan, 1999; Dewanjee, 2008). As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism.

In the present study a variety of gram positive and gram negative strains were selected for screening antimicrobial effects of ethanolic and methanolic extract of *Callistemon citrinus* and *Albizia lebbek* leaf. The result of this study showed that the ethanolic and methanolic extract of *Callistemon citrinus* exhibited varied range of antimicrobial activity against the tested organism including gram positive and gram negative bacteria, which is comparable to standard antibiotic effect.

The *Callistemon* extracts exhibited the greatest antimicrobial activities (as determined by the diameters of the inhibition zones towards the most susceptible bacteria like *Bacillus* species, *S. pyogenes*, *S. Typhi* and *P. aeruginosa*.

Escherichia coli was resistant to methanolic and ethanolic extract that probably could be due to cell membrane permeability or due to other genetic factors

this result is supported by Nazif *et al.* (2002) and Motamedi *et al.* (2009).

Impact of methanolic and ethanolic extract of *C. citrinus* on *S. pyogenes* in lower dilution of extract increased. It could be related to reduce dilution of some inhibitory factors in the extract. Good effect of extract of this plant on *P. aeruginosa* that is a resistant bacteria is also noticeable. finally impact of the extract on gram positive bacteria are more notify than gram negatives, it is because of structure of membrane that the G⁺ bacteria are more simple than G⁻ ones. In some cases methanolic extract showed stronger effect than ethanolic extract, it could be because of the difference between extract compounds in this two extract.

Despite previous records, *A. lebbek* extract didn't show any inhibitory effect. It could because of ecological reasons. According to pharmacognosy science it is clear today that if the plant cultivated in different lands different types and concentrations of materials it will have. The other points that could be considered as the reason are plant growing location, height, climate, humidity and dryness, temperature, amount of sun levels, sex, soil, rainfall, time of collection, how to keep dry, age of plant and kind of organs used as therapeutic. Another problem due to medicinal effects of plants is geographical origin. The native place that the best and the most effective plants are grown up named geographical origin. A plant that is grows in different places in or out of geographical origin doesn't have the same substances and effects; it is because the weather condition is very decisive in producing officinal substances (Salehi Sormaghi, 2008).

CONCLUSION

Based on the result of this study it can be said that *C. citrinus* is an effective antimicrobial plant that can be used for folk medicine and will be a good source for

finding new antimicrobial agents in order to treat and control infections.

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