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The Antimicrobial Activity Of Crude Extract Coleus Blumei

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Abstract: The results indicated the higher effect concentration of Coleus leaf water extract was 3.5 and 4 mg.ml^{-1} which had an inhibitory effect against Gram positive bacteria S. aureus, Gram negative bacteria K. Pneumonia, in addition, candida albicans had an inhibitory diameter zone at 3.5 and 4 mg.ml^{-1}. While, the ethanolic leaves extracts exhibited the largest inhibition zones of S. aureus, K. Pneumonia and candida albicans at the concentration 4 mg.ml^{-1}

1. INTRODUCTION

Many developing countries, traditional medicine is one of the primary health care systems (Chandrappa *et al.,* 2010). The natural activity of plants is due to the presence of secondary metabolites that it would be produced from the plant cell in small amount, in specific parts of plant and in the specific period of plant growth (Hartman, 1996).
Phytochemicals play a significant role in improvement of new pharmaceuticals using raw materials to produce synthetic ones (Khan *et al.,* 2012).
Plants are considered as good source for important compounds such as phenolic, which have anti-oxidant, anti-tumor, anti-mutagenic, and diuretic activities. Plant materials used in folk medicine are readily available in rural areas (Starry and Hans, 1998).
A significant increase in the use of therapeutically natural compounds extracted from plants, especially antimicrobial drugs prescribed by physicians (Hemphill and Cobiac, 2006).
Coleus species are used for ornamental purposes, but it is also used for medical purpose. In Malaises, it has been used for the treatment of high blood pressure, cuts and wounds to stop bleeding (Natasa *et al*., 2002).
Coleus plant extracts are rich in phenolic contents therefore; it can be used as free radical scavengers. Phenolic compounds are known to be synthesized by plants in response to environmental stresses (Baydar, 2004).

2. MATERIAL AND METHODS

Plant material: - *Coleus blumei*, (Lamiaceae) plants were purchased from local nurseries in pots. Plants were identified by Prof. Dr. Ali H. AL-Moosawi, Dept. of Biology, College of Science, University of Baghdad. Some plants were grown directly in the soil for continuous supply as a source for vegetative parts for intact plant studies (fig. 1).

Fig. (1): *Coleus blumei*

Preparation of water and ethanol extracts of *Coleus blumei* leaves for antimicrobial activity: - Leaves of *C. blumei* were dried in shade and then grounded into fine powder by using grinding machine.

Water extract: - A quantity of 10 g of the dried leaves powder was extracted with 100 ml of distilled water. The mixture was left in a shaker incubator at 37 ^{0}C for 6 hrs, then filtered through a sieve with 2 mm. The filtrate was concentrated using rotary evaporator at 40 ^{0}C until dryness and the extract residue was weighted and then and then stored at 4 ^{0}C (Nair and Chanda, 2007).

Ethanol extract: - A quantity of 10 g of dried leaves powder was extracted with 100 ml of 70% ethanol and kept on a rotary shaker at 190-220 rpm for 24 h, then filtered through a sieve with 2 mm. The filtrate was collected and solvent was evaporated. The extract residue was weighted and stored at 4 ^{0}C in airtight bottles for further studies (Nair and Chanda, 2007).

Antimicrobial activity

Microorganisms: - Three Microorganisms *Staphylococcus aureus, Klebsiella pneumonia* and *Candida albicans* were used in this study. They were isolated from patients suffering from skin infections, it was obtained from the Dept. of Biotechnology, AL-Nahrain University.

Preparation of microorganism culture medium: - The Microorganisms *S. aureus*, *K. pneumoniae* and *C. albicans* were cultured on a nutrient agar by mixing 20 g of agar powder with 8 g of nutrient broth powder in a conical flask, then the volume was completed to 1 liter with Double distilled water (Tilton *et al.*,1992). Nutrient broth medium was prepared as recommended by manufacturing company. Culture media were sterilized by autoclaving at 121^{0}C under 15 Ib.in^2 pressure, for 15 min. (Cappuecino and Sherman, 1987).

Antimicrobial activity of water and ethanol extracts (*in vitro*): - The hole-plate diffusion technique was used and the activities of extracts were determined against target microorganisms (bacterial and yeast isolates). The stock solutions were prepared by dissolved 0.1 g of plant extract residue with 10 ml sterile DDH$_2$O. The stock solutions were sterilized by filtration using Millipore filter 0.45 μm under aseptic conditions (Ibrahim, 2003). The extracts were prepared at concentrations 2, 2.5, 3, 3.5 and 4 mg.ml^{-1}, and the antimicrobial effect of each concentration was measured as a diameter of inhibition zone. The nutrient agar medium was mixed well and 20 ml was poured in petri-dishes. The medium was swabbed with 0.1 ml of a suspension containing 1.5x10^{5}cfu/ml of the pathogenic bacteria (*S. aureus* or *K.*

pneumoniae) or $1.5x10^3$ of the pathogenic yeast (*C. albicans*) using sterile cotton swab. Five plugs were removed from each agar plate using a sterile cork borer to make 5 mm-diameter hole. To each hole, 100 µl from different concentrations of each extract was added and allowed to diffuse at room temperature for 20 min (El-Astal *et al*., 2005). To identify the intrinsic extracts activity (water and ethanol extracts) of leaves, one control well was filled with 100 µl sterile DDH_2O. The plates were incubated at $37\ ^0C$ for 24 hrs. for bacteria and 48 hrs. for yeast. Each extract was tested against each microorganism in triplicate. The antimicrobial activity of the plant extracts was recorded as the mean diameter of the resulting inhibition zones of growth measured in millimeters (Santoyo *et al*., 2005).

Experimental design and statistical analysis: - In order to evaluate the variables in this study, a completely randomized design (CRD) was used with three replicates. Least significant differences (LSD) were calculated. Means were compared at probability of 0.05 ($p \leq 0.05$), using SPPS program (2010) (Rosner, 2010).

3. RESULT AND DISCUSSION

Antimicrobial activity for water and ethanol *C. blumei* leaves extract (*in vitro*) Effect of leaves water extract:- Results display in fig.(12) and table 16 indicate that high concentrations of *Coleus* leaf water extract 3.5 and 4.0 $mg.ml^{-1}$ have inhibitory effects against *S. aureus* resulted in 14.33 and 15.33 mm inhibition zone diameter respectively, while 12.000 mm was recorded at the concentrations 2.5 and 3 $mg.ml^{-1}$ and 11.00 mm at the concentration 2 $mg.ml^{-1}$.

K. pneumoniae was also affected by high concentrations of water exctract 3.5 or 4.0 $mg.ml^{-1}$ and showed a diameter of 13.33, 17.0 mm inhibition zones respectively, and 11.33, 11.33, 12.67 mm at the concentrations 2, 2.5 or 3 $mg.ml^{-1}$ respectively. *C. albicans* treated with high concentrations of water extract 3.5 or 4.0 $mg.ml^{-1}$ showed the diameter of 17.67 and 19.33 mm inhibition zone respectively and 14.67, 16.00 mm at the concentrations 2.5 and 3 $mg.ml^{-1}$ respectively, while 13.67 mm was recorded at the concentration 2 $mg.ml^{-1}$ (Fig.2).

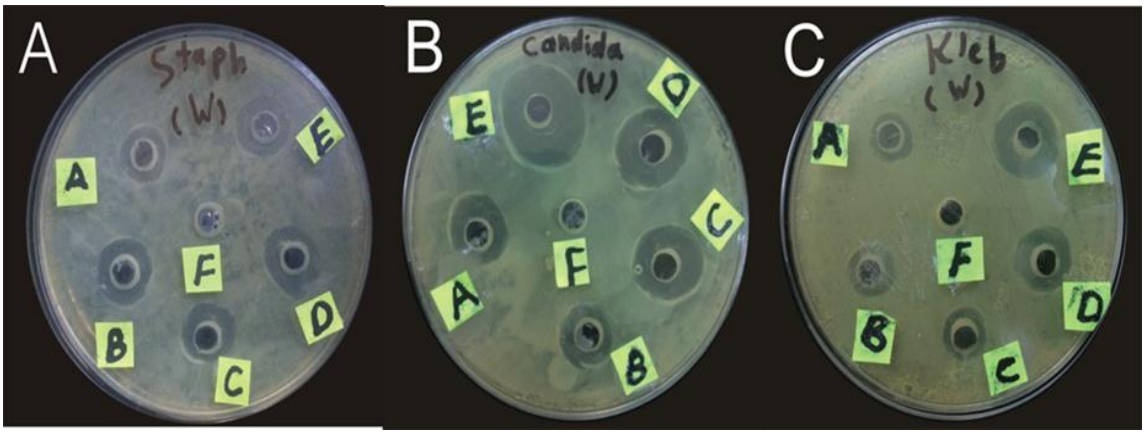

Fig. (2): Effect of *C.blumei* leaf water exctract on the growth of a- *K.pneumonia* b- *S.aureas*, c- *C.albicans*

A= 2 $mg.ml^{-1}$, B=2.5 $mg.ml^{-1}$, C= 3 $mg.ml^{-1}$, D=3.5 $mg.ml^{-1}$, E= 4 $mg.ml^{-1}$, F=control(DDH_2O).

Table (1) revealed the antibacterial, and antifungal investigations conducted on *Coleus blumei* by using water extract against *S. aureus*, *K. Pneumonia*, *C. albicans.* The water extract has

potent antimicrobial activity against Gram positive, Gram negative bacteria and the fungus indicating the presence of board spectrum of antimicrobial substances in the *Coleus* plant. This antimicrobial action of the water extract could be ascribed to the water soluble components in *Coleus*. Also water extract contains rosmarinic acid which exhibits antimicrobial activity as stated by Szabo *et al*. (1999).

The inhibitory ability of water extract was more pronounced against *Candida albicans* compared with other organisms. This result is in agreement with those of Kala (2014) who proved that *Coleus forskohlii* extracts which belongs to the family *Lamiaceae* and it is the most important species of the genus *Coleus* showed a significant activity against *Candida utilis*. Various workers have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria (Lin *et al*., 1999; Parekh and Chanda, 2006), while *Coleus* water extract showed high activity against gram negative bacteria and the yeast *Candida albicans* recording 23.33, 17.00mm inhibition zones for both *C. albicans* and *K. Pneumonia* respectively, compared with *Staph. aureus* which gave the diameter of 15.33 mm inhibition zone. Chandrappa *et al*. (2010) revealed similar finding about antibacterial activity of hot water leaf extract of *C. aromatics* using antibacterial test. Disc diffusion technique was used against pathogenic bacteria, and the water extract showed broad spectrum of inhibition by showing antibacterial effect for both Gram positive and Gram negative bacteria with inhibition zone ranged from 9 to 14 mm for hot water. Jawetz *et al*. (1998) described the mechanism thought to be responsible for phenolics toxicity against microorganisms to membrane disruption, binding or adhesion making a complex with cell wall, inactivation of enzymes, and binding to proteins.

Table(1) Diameter of inhibition zones (mm) caused by *C. blumei* leaves water extract at various concentrations against G +ve, G-ve bacteria and *Candida albicans* yeast.

water extract				
Concentration ($mg.ml^{-1}$)	***C.albicans***	***K.pneumonia***	***S.aureus***	**LSD P ≤ 0.05**
Control	0.00 Ad ± 0.00	0.00 Ac ± 0.00	0.00 Ac ± 0.000	
2	13.67 Ac ± 1.202	11.33 Ab ± 0.667	11.00 Ab ± 0.58	**2.978**
2.5	14.67 Abc ± 2.728	11.33 Ab ± 0.667	12.00 Ab ± 0.58	**5.729**
3	16.00 Aabc ± 0.577	12.67 Bb ± 0.333	12.00 Bb ± 1.00	**2.401**
3.5	17.67 Aab ±	13.33 Bb ±	14.33 Ba ±	**2.307**

	0.333	0.882	0.67	
4	19.33 Aa ± 0.33	17.00 ABa ± 1.15	15.33 Ba ± 0.33	**2.492**
LSD P ≤ 0.05	**3.87**	**2.22**	**1.88**	

***Values= are means of 3 sample readings± SE.**
***Means with capital and small letters indicate to comparison between columns and rows respectively, similar letters are non-significantly different.**

Effect of leaves ethanolic extract :- Results displayed in fig 13 and table (17) showed that *Coleus* ethanolic leaves extract had antibacterial action against *S.aureus* at all concentrations of the extract. The largest inhibition zones were observed against *K. pneumunia* recording 16.67 mm in diameter at the concentration of 4 mg.ml^{-1} whereas, reached 15.67 mm at the concentration of 3.5 mg.ml^{-1}.
At low concentrations of the extract 2, 2.5 or 3 mg.ml^{-1}, *K.pneumonia*e showed an inhibition zone reached 10.33, 12.33 and 13.67 mm respectively. This followed by *C. albicans* which showed 15.33 mm in diameter at 4 mg.ml^{-1} whereas, decreased to 14.00 mm at the concentration 3.5 mg.ml^{-1} The lower concentrations of the exctract 2, 2.5 or 3 mg.ml^{-1}. *C. albicans* showed 8.33, 10.67 and 12.33 mm respectively.
S. aureus growth was inhibited at the extract concentrations 2.5 , 3, 3.5 or 4 mg.ml^{-1}. It showed 8.00, 10.67, 12.33, 14.00 and 14.33 inhibition zones diameter respectively and slight inhibition at the concentration of 2mg.ml^{-1} (Fig. 3).

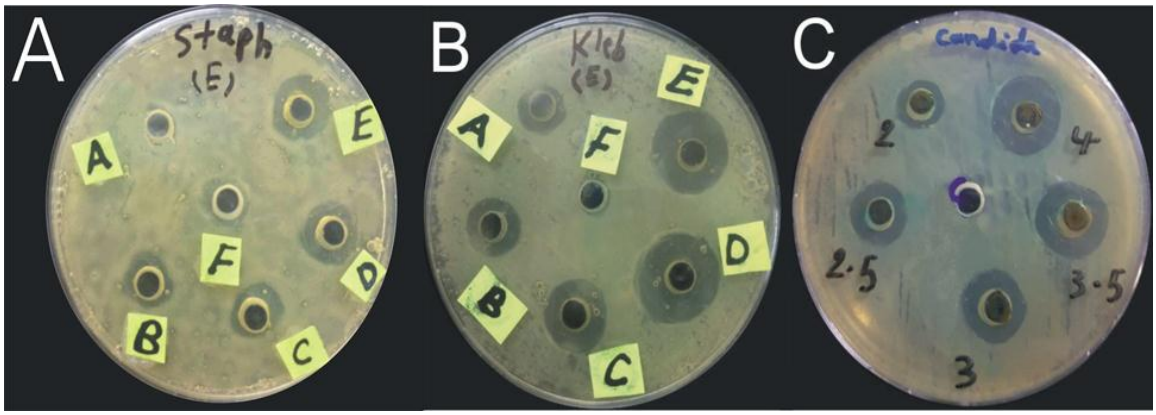

Fig. (3): Effect of C. *blumei* leaves ethanol exctract on the growth of a-*S.aureas* b-*K.pneumonia* , c- *C.albicans*. A=2 mg.ml^{-1}, B=2.5 mg.ml^{-1}, C=3 mg.ml^{-1}, D=3.5 mg.ml^{-1}, E=4mg.ml^{-1}, F=control(DDH$_2$O).

Results displayed in table (2) showed that *Coleus* ethanol leaves extract had antibacterial action against Gram positive, Gram negative bacteria and *Candida albicans*, the zone of inhibition ranged from 8-16 mm for ethanol *Coleus* extract. Ethanol *Coleus* extract showed potent of antibacterial effect. This may be because of alcohol extract is rich in polyphenol and other bioactive components. Similar observations have been reported that grape ethanol seed extracts are rich in polyphenols which exhibit antibacterial and antioxidant activity it's reported that active compounds responsible for the inhibition of Gram negative bacteria (Chandrappa *et al*., 2010). Cowan (1999) mentioned that most of the antibiotic compounds already identified

in plants are reportedly aromatic of saturated organic molecules which can easily solubilized in organic solvents. Similar results showing that the alcohol extract having the best antimicrobial activity as reported by Preethi *et al*. (2010) in *Leucas aspera*. It has been hypothesized that the microorganisms inhibition involves phenolic compounds, because these compounds are effective on phospholipids bilayer of the microbial cytoplasm membrane causing increased permeability, unavailability of vital intracellular constituents and /or impairment of bacterial enzymes system (Kim *et al*., 1995; Moreno *et al*., 2006). Essawi and Srour (2000) reported that the hole plate diffusion method is preferred over dick diffusion one. Therefore, this method was used for this purpose. The antimicrobial effectiveness of the alcohol extract was proven by Chandrappa *et al*. (2010); Kala (2014); Jayachitra and Chitra, (2015) against Gram positive, Gram negative bacteria and *C. albicans*. Generally, it is not surprising that there are differences in the antibacterial activities of the different extracts tested. This could be due to the difference in the phytochemical composition among plants (Dikbass *et al*., 2009).

Use of the water and ethanol extracts of the *Coleus* leaves at concentrations ranging between 2-4 $mg.ml^{-1}$ and comparing these concentrations with other plants in many studies, indicate the higher antimicrobial activity of *Coleus* extracts compared with others, such as *Rosmarinus officinalis* water extract at concentrations ranging from 20-80 $mg.ml^{-1}$ (Al-Mudhaffar, 2009). While the methanol extract of *Tagets patulal* flowers was used at the concentrations between 12.5-75 $mg.ml^{-1}$ against Gram positive bacteria, Gram negative bacteria and *Candida*. *albicans* (Al-saadi, 2009). Hameed, (2008) used flower extracts of *Chamomilla recutita* against eye infectious bacteria in Rabbits at concentrations ranging from 20-80 $mg.ml^{-1}$.

Table (2) Diameter of inhibition zones (mm) caused by *C. blumei* leaves ethanol extract at various concentrations against G +ve, G-ve bacteria and *Candida albicans* yeast.

Ethanol extract				
Concentrations ($mg.ml^{-1}$)	***C.albicans***	***K.pneumonia***	***S.aureus***	**LSD P ≤ 0.05**
Control	0.000 Ae ± 0.00	0.000 Ad ± 0.00	0.000 Ad ± 0.00	
2	8.33 Bd ± 0.33	10.33 Ac ± 0.33	Slight inhibition	**0.94**
2.5	10.67 Bc ± 0.33	12.33 Ab ± 0.33	8.00 Cc ± 0.58	**1.49**
3	12.33 ABb ± 0.33	13.67 Ab ± 0.33	10.67 Bb ± 0.88	**2.00**
	14.00 ABa	15.67 Aa	12.33 Bb	

3.5	± 0.58	± 0.67	± 0.33	**1.88**
4	15.33 Aa ± 0.88	16.67 Aa ± 0.67	14.33 Aa ± 0.88	**2.83**
LSD P ≤ 0.05	**1.51**	**1.39**	**1.78**	

***Values= are means of 3 sample readings± SE. Means with capital and small letters indicate to comparison between columns and rows respectively, similar letters are non-significantly different.**

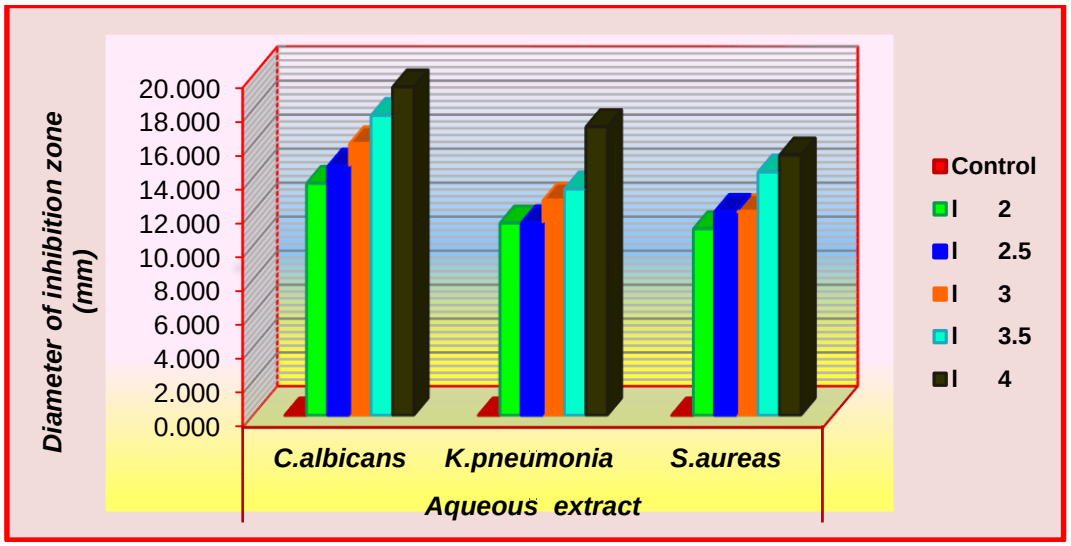

Fig.4 Diameter of inhibition zones (mm) caused by *C.blumei* leaf water exctract at various concentrations against two types of bacteria and C. albicans G +ve, G-ve bacteria and Yeast

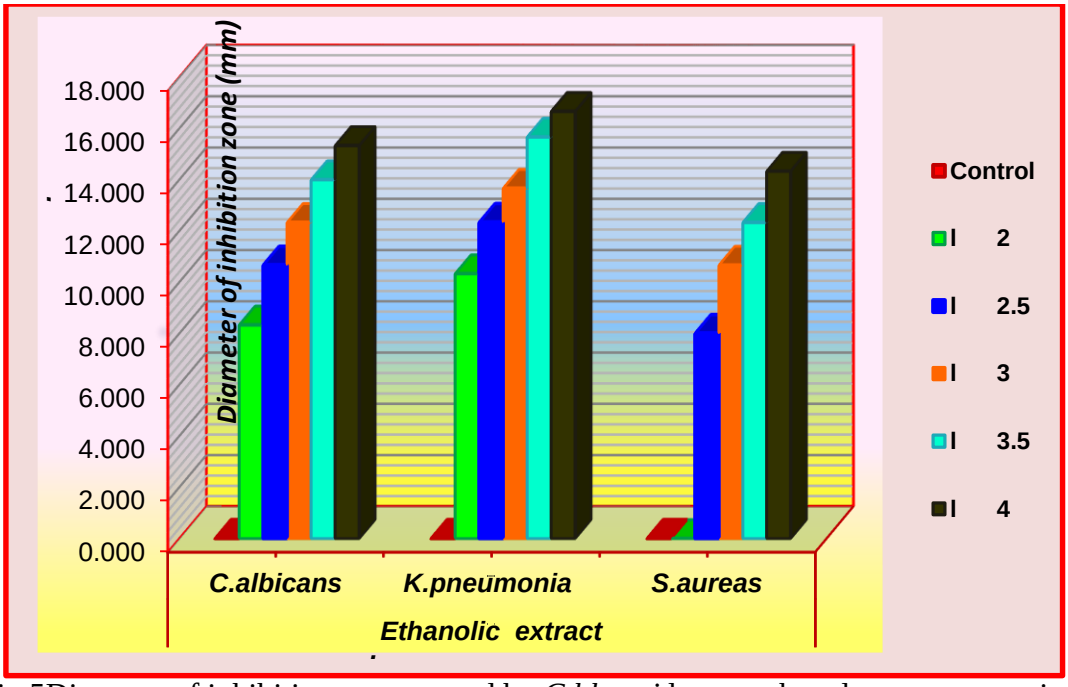

Fig.5Diameter of inhibition zone caused by *C.blumei* leaves ethanol exctract at various concentration against two types of bacteria and *C. albicans*

4. CONCLUSION

This research revealed high antimicrobial activity of both water and ethanol extracts for leaves of Coleus plant against Gram positive bacteria, Gram negative bacteria and the yeast *Candida albicans* was very clear in the current study.

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