

The Effect of Alkaloid Extract of *Teucrium Polium L.* Against Some Pathogenic Bacteria of Urinary Tracts and on Pyelonephritis Induced in Rats

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Abstract

Objective: In view of the reported literature, the medicinal reputation of *T. polium* plant stills the researcher's attention. This study was conducted to evaluate the antibacterial effect of the alkaloid extract of aerial parts of *Teucrium polium L.* on some pathogenic bacteria and to evaluate the effect of the alkaloid extract in the treatment of induced pyelonephritis in rats.

Method: The aerial parts of *Teucrium polium* was powdered, extracted with ethanol, filtered, and dried. Antibacterial activity of alkaloid extract (250 mg/mL) was evaluated by agar diffusion method. 24 female rats became pyelonephritis by intraperitoneal injection of *Escherichia coli*. The animals were divided randomly into four groups. The experimental patients groups received this alkaloid extract and antibiotic gentamycin freely for 10 days but the control group received tap water. Biochemical parameters such as urea and creatinine (CRT) were measured by kinetic (Enzymatic) and colorimetric method

Results: Our results showed that antibacterial of alkaloid extract gave inhibitory efficacy against the studied bacteria. The results also showed that alkaloid extract can decrease serum urea and creatinine significantly ($P < 0.05$, $P < 0.001$ respectively).

Conclusion: Alkaloid extract of *T. Polium* have good antibacterial activity and it can be used in the treatment of pyelonephritis instead of antibiotic.

Keywords: *Teucrium polium L.*; alkaloid extract; pyelonephritis; rats.

Introduction

In recent years, due to alarming increase in the rate of infections with antibiotic resistant microorganisms and side effects of some synthetic antibiotics, there is increasing interest in medicinal plants as a natural alternate to synthetic drugs. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens and therefore can serve as antimicrobial substances^[1,2]. *Teucrium polium L.* (family *Lamiaceae*) is a medicinal plant growing in the western Mediterranean region. *T. polium* is a grass plant, durable, with 10-30 cm in height and callous white exterior that ordinarily have dispersal in rocky and sandy area of Europe zones, North of Africa and South West of Asia^[3]. *T. polium* has been used for different pathological conditions such as anti-diabetic, anti- spasmodic, anti-inflammatory, analgesic, and anti-oxidant effects^[4-10].

In view of the reported literature, the medicinal reputation of *T. polium* plant stills the researcher's attention. The present study was carried out to investigate the possibility of the drug in the treatment of urinary tract infections and pyelonephritis induced by *Escherichia coli* in rats.

Materials and Method

Extraction and Isolation: *Teucrium polium L.* was collected from Basrah, Iraq. The aerial parts were separated, dried for one week in the shade at room temperature and then ground to a fine powder, and stored at 7°C in glass containers until used. The powdered plant material (250 gm) was extracted with a suitable volume of ethanol and 10% acetic acid on continuous mixing under room temperature for 4 hours. The mixture was filtered by using filter paper and concentrated by a rotary

evaporator at 70°C. The sulfuric acid (2%) was added to the concentrated filtrate and the concentrated filtrate was treated with concentrated ammonia until the pH=9, then the solution was filtered and separated by addition 20 ml of chloroform. This procedure was repeated three times and then the organic layer was isolated and dried at room temperature^[11].

Bacterial Strains: Five bacterial species used in this study. The gram-positive species were *Staph scui* (*S. scui*) and Gram negative species bacteria were *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Proteus sp.*, and *Klebsilla pneumoniae* (*K.pneumoniae*). These species were originally isolated from patients and identified by standard biochemical reactions.

Antibacterial Susceptibility Testing: Agar well diffusion method was followed for the determination of antimicrobial activity. The assay was conducted as described by^[12]. Briefly, microorganisms from growth on nutrient agar incubated at 37°C for 18 hrs. were suspended in saline solution 0.85% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards (10^8 cfu/ml). The suspension was used to inoculate 90 mm diameter Petri plates with a sterile nontoxic cotton swab on a wooden applicator. Eight millimeters diameter wells were punched in the Muller Hinton agar (MHA) and filled with 100 µl of 250 mg/ml of alkaloids and other compounds isolated from *T.polium*. The dissolution of the compounds was aided by 1% (v/v) DMSO which did not affect microorganism's growth, according to our control experiments. Commercial antibiotics were used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters by millimeter.

MIC Determination: Minimal inhibitory concentration (MIC) of alkaloidal crude extract of *T.polium* was determined by agar Well diffusion method. Alkaloidal crude extract was dissolved in DMSO into different concentrations (1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 80, 160, 200 mg/ml). Standard inoculums of bacteria were spread on Muller Hinton Agar (MHA). Wells were made on the plates using yellow tip (8 mm diameter). Each sample of alkaloidic compounds (100 µl) was poured into the well and incubated for overnight

at 37°C^[13]. The lowest concentration at which zone of inhibition appeared was considered as MIC.

Animals: Adult female rats (150-200 g) was purchased from the animal house of the College of Veterinary Medicine/University of Basrah, Basrah, Iraq. The animal house temperature was maintained at 20± 2°C with a 12 h light/dark cycle. All animals were kept for one week prior to experimentation and were given free access to food and water.

Experimental Design: To induce experimental pyelonephritis animal, rats (n = 18) were injected with 0.5 mL of a freshly prepared *E. coli* solution into their left renal medullae ^[14]. Control rats (n =6) were administered 0.1 mL of distilled water. Pyelonephritic rats were divided into randomly and equally into 3 groups: first group considered as control, second group as treatment that received by intraperitoneal of 1 mL/rat (equivalent to 0.6 g/kg body weight) of alkaloid crude extract for 10 days, while third group were treated i.p. with 0.5 mL/rat of antibiotic gentamycin for 10 days. After collecting the blood samples, they were left for half an hour, to be coagulated. Then, blood serum was separated by centrifugation for 10 minutes at 3000 rpm. Biochemical parameters such as urea and creatinine (CRT) were measured by kinetic (Enzymatic) and colorimetric method.

Statistical Analysis: The data were analyzed as mean ± SD of variables in different groups using statistical program for social science (SPSS-18). P ≤ 0.05 was considered as the significant level.

Results and Discussion

The results of antibacterial activity of alkaloidal crude extract of *T.polium* against some pathogenic bacteria are shown in (Table 1). The results showed that antibacterial of alkaloid extract gave inhibitory efficacy against the studied bacteria relative to other active compounds isolated from *T. polium* where the diameter of the inhibition zone of alkaloid extract against *E.coli*, *S.scui*, *P.aeruginosa* and *K.pneumonia* was 25 mm while the alkaloidal extract showed the highest inhibitory diameter against *proteus sp.* where it was 30 mm. MIC results showed that MIC of alkaloidal crude extract against *E.coli* and *proteus sp.* was 40 mg/mL while MIC against *P.aeruginosa*, *K.pneumonia* and *S.scui* were 4, 5, 10 mg/mL respectively as shown in (Table 2).

Table 1: Antibacterial activities of alkaloid extract of *T.polium* against some urinary tracts bacteria

Bacterial species	Concentration of alkaloidal crude extract (mg/ml)	Antibiotic					
		ER	CAZ	TE	VA	CN	CIP
E.coli	25	25	15	R	R	R	R
S.scuiri	25	8	R	16	2	R	R
P.aeruginosa	25	21	14	18	27	R	23
K.pneumoniae	25	26	17	R	15	R	R
Proteus sp.	30	40	17	R	R	17	R

R: Resistant, ER: Erythromycin 15 µg; CAZ: Ceftazidime 30 µg; TE: Tetracycline 30 µg; VA: Vancomycin 30 µg; CN: Gentamycin 10 µg; CIP: Ciprofloxacin 5 µg

Table 2: The minimal inhibitory concentrations of alkaloid extract isolated from *T.polium* in mg/ml

Bacterial Species	MIC of alkaloidal crude extract (mg/ml)
E.coli	40
S.scuiri	10
P.aeruginosa	5
K.pneumonia	4
Proteus sp	40

Following the induction of pyelonephritis (10 days after administration of *E. coli*) the patients rats showed highly significant increased (27.217±2.6263 vs. 42.233±17.044 and 0.666± 0.2065 vs. 0.366± 0.1366, in urea and creatinine, respectively P<0.05, Table 3) as compared to the control group. However, 10 days after the initiation of treatments, alkaloid extract and gentamycin treated rats showed a significant decrease (0.243±0.1181 and 0.293±0.0265 in urea of treated rats with alkaloid extract and gentamycin, respectively vs. 0.666±0.2065 in untreated rats, P<0.001, Fig. 1), while alkaloid extract treated rats showed a significant decrease (29.100± 9.1945 vs. 42.233 17.044, P<0.05, Fig. 2) in level of urea as compared to the patients group.

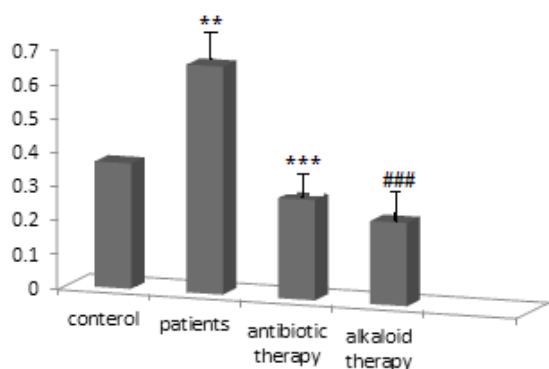


Fig. 1: effect of alkaloid extract of *T.polium* on the level of creatinine in treated pyelonephritis group as compared to untreated pyelonephritis rats.

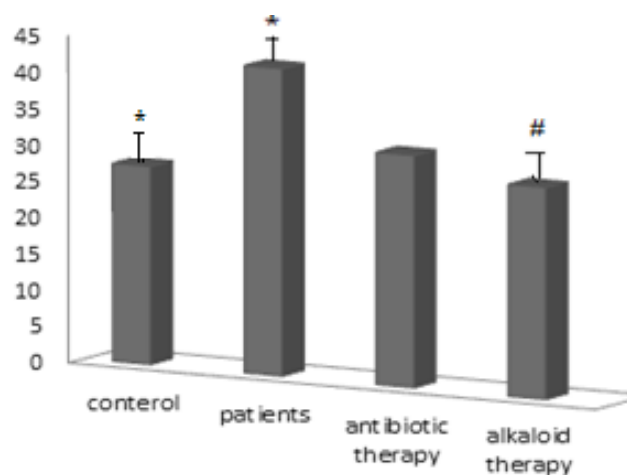


Fig. 2: The effect of alkaloid extract of *T.polium* on the level of urea in treated pyelonephritis group as compared to untreated pyelonephritis rat

In Figure 1, the effect of alkaloid extract of *T.polium* on the level of creatinine in treated pyelonephritis group as compared to untreated pyelonephritis rats. Data are presented as mean ± SD. ** indicates a significant difference (P<0.01) between patients and control group, *** indicates a significant difference (P<0.001) significance between antibiotic therapy and patients group, ### indicated a significant difference (P<0.001) between alkaloid therapy and patients group.

In Figure 2, the effect of alkaloid extract of *T.polium* on the level of urea in treated pyelonephritis group as compared to untreated pyelonephritis rats. There was also a significant difference in pyelonephritis rats as compared to healthy control group. Data are presented as mean ± SD. * indicates a significant difference (P<0.05) between patients and control groups, # indicates a significant difference (P<0.05) between alkaloid

Table 3: Comparison between the biochemical parameters in pyelonephritis and normal groups.

Group	Biochemical Parameters	
	Urea	Creatinine
Control	27.217±2.6263	0.366±0.1366
Patients	42.233±17.0448	0.666±0.2065**
Gentamycin therapy	31.916±7.6122	0.293±0.0265***
Alkaloid therapy	29.100±9.1945#	0.243±0.1181####

Data are presented as mean \pm SD. * indicates a significant difference ($P < 0.05$) between patients and control groups, # indicates a significant difference ($P < 0.05$) between alkaloid therapy and patients groups, ** indicates a significant difference ($P < 0.01$) between patients and control group, *** indicates a significant difference ($P < 0.001$) significance between antibiotic therapy and patients group, #### indicated a significant difference ($P < 0.001$) between alkaloid therapy and patients group.

Discussion

On the basis of this study, alkaloidal crude extract from the aerial parts of *T. polium* is effective against both gram-positive and gram-negative bacteria. Alkaloid crude extract at 250 mg/ml showed high antibacterial activity, so these findings support the previous report by Singh et al.^[15] and ^[16] who indicated that alkaloids have physiological effects and a great medical effect to treat many diseases, including urinary tract infections. Cowan ^[17] showed that the mechanism of action of alkaloids is the formation of chemical bonds between these active compounds and the nucleic acids (DNA, RNA) of these microorganisms and then inhibit metabolism. While ^[18] attributed the effectiveness of alkaloids to the interference with the respiratory chain enzymes containing the thiol group. Through substitution and replacement in the carbonyl group, the alkaloids are transformed into a more toxic compound. It also leads to the mutagenic proteins and interfere with the protein enzymes containing the thiol group and lead to the modification and sedimentation of cellular proteins and through the ability to link with the enzymes involved in the building of amino acids and enzymes involved in the construction of proteins, which leads to inhibition of work and the most important of these enzymes DNA ligase and RNA polymerase. The alkaloids act to break the covalent bonds of the nucleotides and thus destroy the DNA of the microorganism. The MIC for *K. pneumoniae*, *P. aeruginosa* and *S. scuri* was found at (4, 5, 10 mg/

ml) respectively, while for *E. coli* and *Proteus sp.* was 40 mg/ml. This result is in accordance with Darabpour et al.^[19] who confirmed that *Escherichia coli* and *Proteus sp.* were more resistant than others. This observed resistance probably could be due to the presence of outer membrane of peptidoglycan in *E. coli* and *proteus* may prevent the transport of compounds into the cell or due to other genetic factors. Our results also showed that alkaloid crude extract of *T. polium* has high activity against pyelonephritis induced in rats. A significant decrease in serum urea and creatinine was observed in rats with pyelonephritis after 10 days of treatment with alkaloid extract. These results confirm several previous studies that *T. polium* can be used as an anti-inflammatory ^[7].

In conclusion, the findings of the current study illustrate that intraperitoneal administered alkaloid extract of *T. polium* improved the renal function and good antibacterial alternative to antibiotic. These data suggest that alkaloid extract of *T. polium* might have a future role in the treatment of acute pyelonephritis.

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