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Therapeutic Effect of *Portulaca oleracea* Against Diarrhea Induced by *Escherichia coli* in Male Rats

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A B S T R A C T

This study aimed to investigate the in vivo antidiarrheal efficacy of methanolic extract of *Portulaca oleracea* against diarrhea induced by *Escherichia coli* in male rats. The initial phase involved the extraction of *P. oleracea* using 99.8% absolute methanol through a Soxhlet extraction apparatus. Phytochemical analyses of the extract unveiled the presence of alkaloids, flavonoids, steroids, carbohydrates, tannins, and proteins. In the experimental phase, 20 Wistar albino male rats (*Rattus norvegicus*) were divided into four groups: the Negative Control (uninfected and untreated); the Positive Control (infected but untreated); POE group consisted of rats infected with *E. coli* (1×10^9 CFU/mL) and subsequently treated with 200 mg/kg BW of *P. oleracea* methanolic extract orally twice daily for seven days; and CIP group included rats infected and treated with 7.14 mg/kg BW of ciprofloxacin orally twice daily for seven days. Outcome measures encompassed clinical signs, frequency of watery stools, rectal bacterial count, and changes in BW. Remarkably, both POE and CIP groups demonstrated a statistically significant reduction in the frequency of watery stools ($P<0.05$) and a significant increase in BW ($P<0.05$) compared to Positive Control group. Notably, there was no significant difference in these parameters between POE and CIP groups, suggesting that *P. oleracea* methanolic extract performs comparably to ciprofloxacin in treating *E. coli*-induced diarrhea. The findings illuminate the potential of herbal medications such as *P. oleracea* as effective alternatives to antibiotics, thereby mitigating the overuse of antibiotics and the associated risk of bacterial resistance.

Keywords: *Portulaca oleracea*, *E. coli*, diarrhea, rat, ciprofloxacin

INTRODUCTION

Diarrhea serves as a prevalent disorder affecting the gastrointestinal system and is triggered by a diverse array of causative agents such as bacterial, viral, and parasitic pathogens. Manifesting primarily as frequent, loose, or watery stools, this condition typically arises following the consumption of tainted food or water. The clinical presentations can range from acute and hemorrhagic forms to chronic types, each posing unique health risks. Notably, the condition can lead to a cascade of complications including, but not limited to, electrolyte imbalances, severe dehydration, and in critical instances, can escalate to shock and fatality (1). Of particular concern is the high rate of mortality linked to diarrheal diseases among children under the age of five, which represented around 8% of all deaths within this demographic worldwide in 2017 (2). In the realm of veterinary medicine, diarrhea also imposes a significant economic toll, especially in bovine populations. For instance, both adult cattle and

neonatal calves experience heightened levels of morbidity and mortality due to diarrheal conditions, thereby incurring substantial economic losses (3).

Escherichia coli (*E. coli*) has emerged as a resilient pathogen, showing resistance to multiple antibiotics (4, 5). While ciprofloxacin has been somewhat effective in treating E. coli-induced diarrhea in newborn calves, resistance is reducing its efficacy (6). Ciprofloxacin's mechanism of action involves high-affinity binding to bacterial DNA gyrase, a feature making it more effective against bacterial DNA gyrase than its mammalian counterpart (7).

Given the escalating prevalence of antibiotic-resistant bacterial strains, there is an urgent need for alternative therapeutic interventions. Herbal medicine, when substantiated by scientific research, offers a promising avenue for treatment (8). Among plant-based alternatives, the Portulacaceae family, particularly *Portulaca* (*P.*) *oleracea* (commonly known as purslane), has gained attention for its versatile applications in both food and medicine (9). The phytochemical constituents of *P. oleracea*, such as alkaloids, coumarins, flavonoids, and saponins, have demonstrated potent antibacterial, anti-inflammatory, and analgesic activities (10-13).

In vitro studies have indicated that *P. oleracea* exerts bactericidal activity against *E. coli* by disrupting the bacterial cell membrane, impeding biofilm formation, and affecting cell morphology, all without adverse host effects (13,14).

In light of the aforementioned challenges posed by antibiotic resistance and the compelling evidence of *P. oleracea*'s therapeutic potential, this research aims to examine the efficacy of methanolic extracts of *P. oleracea* in treating *E. coli*-induced diarrhea in rats. This study seeks to contribute to the field of veterinary medicine by offering a scientifically-backed alternative to antibiotics for the management of infectious diarrhea.

MATERIALS AND METHODS

Ethical Approval

The procedures of the study were reviewed and approved by the local Research Ethics Committee, College of Veterinary Medicine, University of Baghdad (Approval Number 1109 dated 29th May 2023), ensuring that all procedures performed in this study were in line with ethical standards.

Plant Materials and Authentication

Mature specimens of wild *P. oleracea* (roots and stems) were manually collected from various regions in Baghdad province. The plant was authenticated officially by the Ministry of Agriculture, State Board for Seed Certification and Testing, located in Abu Ghraib, Baghdad, Iraq under certification number 3273, dated 6th November 2022.

Extraction Process and Yield Calculation

The harvested plant material was meticulously washed with tap water and then air-dried under shade at 25 °C. The dried plant was segmented into small pieces and pulverized to powder form. Amount of 100 g of the powdered plant material was subjected to Soxhlet extraction using absolute methanol (Fluka, Switzerland) as the solvent. The extraction was performed at 60 °C for a duration of 6 h. After extraction, the filtrate was passed through filter paper (Sigma Aldrich, USA) and concentrated using a rotary evaporator (Heidolph, Germany) at 45 °C. The concentrated extract was stored in a dark environment at 4 °C until further use (15).

The yield percentage of the extract was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract (g)}}{\text{Inintial weight of powder}} \times 100$$

Phytochemical Screening

Phytochemical analysis of the *P. oleracea* methanolic extract was carried out employing standard methodologies designed for the identification of secondary metabolites. The extract was partitioned into multiple aliquots, and each was subjected to specific indicating reagents to identify the presence of various metabolites (16).

Identification of *E. coli*

The *E. coli* strain used for inducing diarrhea in this study was isolated from Al-Fallujah Teaching Hospital laboratories, Al-Anbar province, Iraq. Detailed methods for isolation and identification, which include traditional biochemical tests, Vitek 2 test system (BioMérieux, France) with GN-ID cards, and Polymerase Chain Reaction (PCR), have been described in our earlier publication (Manthoor and Saliem, 2023, *in press*).

Antidiarrheal Activity of *P. oleracea* Methanolic Extract and Ciprofloxacin

Animals

A total of 20 males healthy Wistar albino rats (*Rattus norvegicus*), aged in average three months and weighing between 195.5 and 198.2 g, were obtained from and kept at the animal house (in a special room belongs to the Department of Physiology, Biochemistry and Pharmacology) of the College of Veterinary Medicine, University of Baghdad. The rats were accommodated in plastic cages with dimensions of 20×50×75 cm^3 and were allowed a two-week adaptation period prior to the commencement of the experiment. Standard rodent diet (commercial feed pellets) and tap water were freely available. Environmental conditions were maintained at 20 (±5) °C with a 14/10-h light/dark cycle. Ventilation vacuums were used to regularly replace air, and litter in the cages was changed weekly.

Experimental Design

The animals were randomly divided into four groups, each containing five rats. The first group, designated as NC (Negative Control), received only distilled water orally for seven days, serving as a baseline comparison. The second group, labeled PC (Positive Control), consisted of rats infected with *E. coli* but left untreated, to demonstrate the effects of the infection. The third group, referred to as POE (*P. oleracea* Extract), involved rats infected with *E. coli* and subsequently treated with a methanolic extract of *P. oleracea*. This treatment entailed an administration of 200 mg/kg BW, given orally twice daily for a period of seven days (14). The fourth group, termed CIP (Ciprofloxacin), also consisted of rats infected with *E. coli*, but these were treated with ciprofloxacin. The dosage for this group was set at 7.14 mg/kg BW, administered orally twice daily over a span of seven days (17). The commencement of all treatments occurred 24 h post-infection, allowing for a standardized treatment initiation across the study.

Preparation of E. coli Inoculum and Induction of Diarrhea

A stock culture of *E. coli* was transferred to sterilized peptone water (HiMedia, India) and incubated at 37 °C for 18-24 h for activation. Following this initial incubation, a portion of the culture was transferred to a new flask containing Tryptic Soya Broth (HiMedia, India) for further activation. This flask was also incubated at 37 °C until the bacterial concentration reached the desired density. The bacterial cell concentration was quantitatively assessed using a spectrophotometer (at optical density 600 nm (OD_{600} nm), and adjustments were made to achieve a final activated cell concentration of 1×10^9 CFU/mL (18). The prepared *E. coli* inoculum was then administered orally using a sterile oral gavage needle.

Dose Preparation for P. oleracea Extract and Ciprofloxacin

The dose of *P. oleracea* methanolic extract was set at 200 mg/kg BW (14). To prepare this, 2 grams of the methanolic extract were dissolved in 20 mL of distilled water. For ciprofloxacin (Neopharma, UAE), a dose of 7.14 mg/kg BW was prepared by dissolving 42.84 mg in 12 mL of distilled water. The final oral dose volume for animals weighing approximately 100 g was 0.2 mL for either *P. oleracea* methanolic extract or ciprofloxacin.

Clinical Signs and Fecal Examination

Animal behavior and activity were continuously monitored throughout the seven-day experimental period. Each morning, the animals were placed in individual cages with filter paper flooring, which was replaced on an hourly basis. The frequency of wet stools was recorded on the first, third, and seventh days' post-treatment.

Body Weight Assessment

The weight of the animals was recorded prior to, during, and post-treatment to evaluate the potential impact of *E. coli* infection and the therapeutic effects of *P. oleracea* methanolic extract and ciprofloxacin.

Rectal Bacterial Count for E. coli

Sterile cotton swabs (Meheco, China) were used to collect fecal samples for bacterial enumeration. Fecal samples were collected before infection, 24 hours' post-infection, and on the third and seventh days' post-treatment. About 100 mg of each fecal sample was dissolved in 1 mL of a 0.1% peptone and 0.85% saline diluent, achieving a 1:99 ratios. The bacterial count was performed using the pour plate method, wherein serial dilutions of the initial nutrient broth (Oxoid, UK) were cultured on McConkey agar plates (HiMedia, India). Colonies were counted after 24 hours of incubation at 37 °C, and counts were adjusted based on dilution factors (19, 20).

$$\text{Number of Colonies (CFUs)} = \frac{\text{Number of Bacteria in mL}}{\text{Dilution X Amount Plated}}$$

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS, 2018). The Least Significant Difference test (Analysis of Variance-ANOVA) was employed to compare means and assess the impact of different factors on study parameters (21).

RESULTS AND DISCUSSION

Extraction Efficiency

The extraction of *P. oleracea* using absolute methanol yielded a dark blue, pasty extract with a 15% extraction ratio. The result of this study was in agreement with (22) who found that the percentage of Methanol extraction of 1.5 kg, purslane had produced 225.75 g (15.5% v/w) of water-soluble extract, which had been extracted by a Soxhlet. The similarity percentage that had been yielded may be due to the same solvent that was used in the extraction (28).

Phytochemical Analyses

The phytochemical analysis revealed the presence of various bioactive compounds such as alkaloids, flavonoids, steroids, carbohydrates, tannins, free amino acids, and proteins. Notably, terpenoids and quinones were absent (Table 1). These results corroborate the findings of (23, 24)، who also identified a similar phytochemical profile, saponins, glycosides, alkaloids, flavonoids, phenolics, steroids, di- and triterpenes, and tannins present in *P. oleracea* methanolic leaf extracts, but absent in ethanolic extracts. However, (25) reported an absence of alkaloids, flavonoids, and tannins in *P. oleracea* methanolic extracts, despite the presence of other constituents in the same solvent. This discrepancy could be attributed to different

plant origins, with using plants from Nigeria, while this study used Iraqi plants.

Table 1. Chemical component of *Portulaca oleracea* methanolic extract

Constituent	Results
Alkaloids	+
Flavonoids	+
Steroids	+
Carbohydrates	+
Terpenoids	-
Tannins	+
Quinones	-
Free amino acids	+
Protein	+

Clinical Signs and Watery Stool

Diarrhea was observed in rats after 24 h of *E. coli* infection, with symptoms including anorexia, dehydration, weakness, slow movement, and weight loss. The most significant clinical sign was watery stool, which continued for various periods. The infected non-treated group (Positive Control) had more severe signs represented by dehydration, diarrhea, rough coat, emaciation, poor weight gain, and reduced skin elasticity, while the POE (*P. oleracea* Extract) and CIP (Ciprofloxacin) groups had mild to moderate signs. According to (26) rats infected with *E. coli* developed diarrhea that progressed over time. The diarrhea was characterized by thin and watery stools, fever, loss of appetite, dehydration (sunken eyes, dry mucus membranes, rough hair), and an inability to stand up straight. During diarrhea, the animals lost a significant amount of water and electrolytes. The quick onset of acidosis brought on by the dehydration that occurs during this infection may not give the lung enough time to adjust.

The results of watery stool after 24 h (Table 2) of induced infection, there was a significant (P<0.05) increase in the number of watery stools in all groups except Negative Control, after three and seven days of treatment, there was a significant (P<0.05) decrease in number of watery stool in both POE and CIP groups, when compared with the Positive Control. These findings align with (27), who identified distinct metabolites that were enriched in pathways relevant to amino acid metabolism, indicating that *P. oleracea* methanolic extract may cause intestinal amino acid remodeling. These metabolites are connected to intestinal disorders, diarrhea, bacterial infections, and inflammation, according to a functional study. Therefore, the *P. oleracea*'s anti-bacterial diarrheal actions may be regulated by amino acid metabolic pathways and gut microbiota profile and function. However, there might be another pathway for the anti-diarrheal action, the presence of alkaloids, coumarins, flavonoids, saponins, and tannins (14) as all as these constituents which present in the plant might induce such positive action on diarrhea.

Body Weight Changes

Significant variations in BW were observed post-infection and post-treatment (Table 3). After 24 h post-infection, a significant decrease in BW (P<0.05) was noted for the Positive Control, POE, and CIP groups, but not in the Negative Control group. After three and seven days of treatment, BW significantly increased (P<0.05) in the POE and CIP groups compared to the Positive Control group. These results agree with findings from (14), which reported a significant decrease (P<0.05) in BW in animals infected with *E. coli*. This weight loss was attributed to severe damage, shedding, and irregular arrangement of the duodenal villi in the diarrheal group. In contrast, the group treated with *P. oleracea* methanolic extract maintained the complete structure of the duodenal villi, preserving intestinal nutrient absorption and mitigating the body weight loss caused by the *E. coli* infection.

Table 2. Comparison of the Mean ± SEM of watery stools per 6 h across different groups at various time points post-infection and post-treatment

Groups	24 h After Infection	3 Days After Treatment	7 Days After Treatment
Negative Control	0.200 ± 0.20 ^{Ac}	0.00 ± 0.00 ^{Ac}	0.00 ± 0.00 ^{Ac}
Positive Control	6.60 ± 0.24 ^{Ba}	8.40 ± 0.24 ^{Aa}	5.40 ± 0.24 ^{Ca}
Portulaca oleracea Extract	4.40 ± 0.24 ^{Ab}	3.40 ± 0.24 ^{Bb}	2.40 ± 0.24 ^{Cb}
Ciprofloxacin	4.60 ± 0.24 ^{Ab}	3.60 ± 0.24 ^{Bb}	2.60 ± 0.24 ^{Cb}
LSD Value		0.733	

Values are means±SEM. Means with different lowercase letters in the same column and uppercase letters in the same row are significantly different at $P \leq 0.05$. Negative Control (NC) - no infection, no treatment; Positive Control (PC) - infected with *E. coli*, no treatment; *Portulaca oleracea* (POE) - infected with *E. coli*, treated with *Portulaca oleracea* extract; Ciprofloxacin (CIP) - infected with *E. coli*, treated with ciprofloxacin

Table 3. Comparison of body weight changes (g) among different groups over various time periods

Groups	7 Days Before Infection	24 h After Treatment	3 Days After Treatment	7 Days After Treatment
Negative Control	196.30 ± 0.30 ^{Da}	204.30 ± 0.30 ^{Ca}	209.62 ± 0.18 ^{Ba}	219.98 ± 0.34 ^{Aa}
Positive Control	197.60 ± 0.22 ^{Aa}	190.80 ± 0.24 ^{Bc}	182.86 ± 0.22 ^{Cc}	176.16 ± 0.26 ^{Dd}
Portulaca oleracea Extract	196.92 ± 0.15 ^{Ba}	192.64 ± 0.18 ^{Db}	195.96 ± 0.15 ^{Cb}	200.90 ± 0.33 ^{Ab}
Ciprofloxacin	196.76 ± 0.15 ^{Ba}	191.72 ± 0.21 ^{Db}	194.90 ± 0.12 ^{Cb}	198.84 ± 0.13 ^{Ac}
LSD Value	1.792 *			

Values are means±SEM, n=5. Means with different lowercase letters in the same column and uppercase letters in the same row are significantly different at $P \leq 0.05$.

Rectal Bacterial Count for Different Periods

All four groups had a colony-forming unit/mL (CFU/mL) level of *E. coli* bacteria. There were significant ($P<0.05$) differences between the four groups seven days before infection. Except for Negative Control group, Positive Control, POE and CIP groups experienced a significant ($P<0.05$) rise in rectal bacterial count after 24 h of infection. These results concurred (28, 29), they demonstrated that giving rats 1.5×10^8 CFU/mL resulted in an efficient colonization of *E. coli* within 24 h after giving the rats pathogenic *E. coli* to produce an experimental infection. When compared to the Positive Control, after three and seven days of therapy, there was a significantly ($P<0.05$) decrease in number of bacteria in groups POE and CIP (**Table 4**). The results of rectal bacterial count gave good evidence about the use of *P. oleracea* methanolic extract, upon which the therapeutic value of this plant depends on their bioactive phytochemical components, which had specific physiological effects as defensive mechanisms against pathogens. The primary and secondary metabolites of *P. oleracea* include alkaloids, terpenes, coumarins, flavonoids, organic acids, and other ingredients (30), which have strong antibacterial, anti-inflammatory, immune enhancement effect (31, 12). The presence of these useful phytochemicals could be responsible for the observed antibacterial activities and can be seen as a potential source of antibiotic drugs. In general, the accumulation and concentration of secondary metabolites are responsible for the antibacterial activity of a plant (11).

Table 4. Comparison of rectal bacterial count (log_{10} CFU/mL) among different groups over various time periods post-infection and post-treatment

Groups	7 Days Before Infection	24 Hours After Infection	3 Days After Treatment	7 Days After Treatment
Negative Control	2.849 ± 0.03 ^{Ac}	2.838 ± 0.004 ^{Ac}	2.831 ± 0.002 ^{Ac}	2.818 ± 0.002 ^{Ac}
Positive Control	2.942 ± 0.03 ^{Da}	9.317 ± 0.007 ^{Aa}	6.872 ± 0.003 ^{Ba}	6.698 ± 0.006 ^{Ca}
Portulaca oleracea Extract	2.869 ± 0.02 ^{Cb}	9.259 ± 0.009 ^{Ab}	4.833 ± 0.002 ^{Bb}	2.778 ± 0.005 ^{Dd}
Ciprofloxacin	2.856 ± 0.02 ^{Db}	9.264 ± 0.009 ^{Ab}	4.842 ± 0.003 ^{Bb}	3.792 ± 0.002 ^{Cb}
LSD Value		0.0198		

Values are means±SEM. Means with different lowercase letters in the same column and uppercase letters in the same row are significantly different at $P \leq 0.05$

The study elaborates on the clinical use of herbal medication as an effective replacement for antibiotics, and to reduce the burden of exaggerated use leading to increased bacterial resistance, methanolic extract from *P. oleracea* effectively treated *E. coli*-induced diarrhea in rats, reducing clinical signs, and watery stools, while the body weight values increased in comparison to ciprofloxacin.

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N/A

Conflict of Interest

The authors declare no conflict of interest.

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التأثير العلاجي لمستخلص نبات الرجلة ضد الإسهال الناجم عن الإشريكيا القولونية في ذكور الجرذان

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فرع الفسلجة والكيمياء الحياتية والادوية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

تحرى البحث عن نشاط مستخلص الرجلة الميثانولي في الجسم الحي في الفئران التي اصيبت بالإسهال بواسطة الإشريكيا القولونية المعزولة من مختبرات مستشفى الفلوجة التعليمي وتم اثباتها بواسطة نظام اختبار نظام الفايتك 2. تضمنت الخطوة الأولى جمع واستخلاص الرجلة بالميثانول المطلق (٩٩,٨٪) في جهاز السكسوليت ثم أظهر التحليل الكيميائي النباتي للمستخلص قلويدات وفلافونويد ومنشطات وكربوهيدرات وعفص وبروتين. في الخطوة الثانية، كان عدد الحيوانات ٢٠ فأر ذكور، من أجل التأثير المضاد للإسهال، بدأ العلاج بعد ٢٤ ساعة من استحداث الإصابة ببكتيريا الاشريكيا القولونية 1.0 × 109 CFU / ml، والتي تم تضمينها: المجموعة (ا) هي المجموعة الضابطة السلبية (غير مصابة وغير معالجة)، المجموعة (ب) هي المجموعة الضابطة الإيجابية (المصابة وتركت دون علاج)، المجموعة (ج) التي أصيبت وعولجت بالمستخلص 200 مجم / كجم من وزن الجسم. فمويا لمدة ٧ أيام مرتين يوميا والمجموعة (د) مصابة وتعالجت بالسيبروفلوكساسين ٧,١٤ مجم / كجم من وزن الجسم. فمويا لمدة ٧ أيام مرتين يوميا. كانت المعايير المستخدمة في هذا البحث هي العلامات السريرية، وعدد البراز المائي، وعدد بكتيريا المستقيم، وتغيرات وزن الجسم. الحيوانات السليمة (المجموعة أ) لديها براز بني طبيعي، في حين أن المجموعة الضابطة الإيجابية (المجموعة ب) عانت من أعراض سريرية شديدة تتمثل في فقدان الشهية، والجفاف، والضعف، وبطء الحركة، والبراز الناعم أو الرطب أو السائل. قللت المجموعة (ج) والمجموعة (د) من عدد البراز المائي بشكل كبير ($P\leq0.05$) وزادت من وزن الجسم بشكل ملحوظ ($P\leq0.05$) بالمقارنة مع المجموعة ب. ومن المثير للاهتمام المجموعة (ج) التي عوملت بالمستخلص الميثانولي لنبات الرجلة ومقارنة مع الحيوانات المعالجة بالسيبروفلوكساسين (د) لم يكن هناك أي نتائج معنوية فيما بينها. تتناول الدراسة بالتفصيل الاستخدام السريري للأدوية العشبية كبديل فعال للمضادات الحيوية، ولتقليل عبء الاستخدام المفرط الذي يؤدي إلى زيادة المقاومة البكتيرية.

الكلمات المفتاحية: الرجلة، الاشريكيا القولونية، الاسهال، جرذان، سيبروفلوكساسين.