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IN-VITRO, IN-VIVO ANTI MICROBIAL, ANTI INFLAMMATORY AND PERITONITIS ACTIVITIES OF *DURANTA ERECTA* SILVER NANO PARTICLES

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ABSTRACT

Duranta erecta, a widely distributed medicinal plant, has garnered attention for its diverse pharmacological properties. This study investigates the in vivo and in vitro activities of *Duranta erecta* silver nanoparticles (DE-AgNPs). The antimicrobial efficacy of (DE-AgNPs) was assessed through in vitro studies against spectrum of pathogenic microorganisms. Results demonstrated notable inhibitory effects on bacterial strains, indicating the promising antimicrobial activity of DE-AgNPs. In vivo investigations were conducted to evaluate the anti-inflammatory potential of DE-AgNPs using relevant animal models. The results exhibited significant reductions in inflammatory markers, suggesting the nanoparticles' ability to mitigate inflammatory responses. Furthermore, the study explored the efficacy of DE-AgNPs in a peritonitis model, revealing their potential in managing this life-threatening condition. The findings of this research highlight *Duranta erecta* silver nanoparticles as a multifaceted therapeutic agent with promising antimicrobial, anti-inflammatory, and peritonitis management properties. These results contribute valuable insights into the potential applications of nanotechnology-derived formulations from medicinal plants, paving the way for further exploration and development of novel therapeutic interventions.

Keywords: *Duranta erecta*, Silver nanoparticles, anti-inflammatory, peritonitis, marker.

1. INTRODUCTION

Intraperitoneal infection known as peritonitis is a major killer in the practice of clinical surgery and it is also one of the most frequent diagnoses in a surgical intensive care unit (ICU) leading to severe sepsis (Weiss *et al.*, 2007). Primary, secondary, and tertiary peritonitis (TP) are the three types. There is no obvious anatomical abnormality in primary peritonitis. It is also known as spontaneous bacterial peritonitis and has a minimal prevalence on surgical ICUs because it is managed solely by physicians without any surgical intervention. The most prevalent condition in critical surgical patients is secondary peritonitis, which is defined as an infection of the peritoneal cavity caused by a hollow viscous perforation, anastomotic leak, ischemic necrosis, or other gastrointestinal tract injuries (Calandra and Cohen, 2005). Primary peritonitis is a common monomicrobial infection of the peritoneal fluid that does not result in visceral perforation. The most common type of peritonitis is secondary peritonitis, which occurs when the integrity of a hollow

internal organ has been compromised. Tertiary peritonitis arises following secondary peritonitis treatment due to a failure of the host's inflammatory response or super infection. As a result, peritoneal cavity pollution can cause a variety of infections, sepsis, and multi-system organ failure, and is lethal if not treated promptly (Gupta *et al.*, 2006). Peritonitis describes the inflammatory process of the peritoneum, which is usually due to infection, but peritonitis can also be a sterile process (Simmen *et al.*, 1996). Drugs can be incorporated or dissolved in nanoparticles, as well as adsorbed or linked to their surfaces. Encapsulating medicines into NPs can improve drug solubility and pharmacokinetics, allowing for further clinical development of new chemical entities that have been halted due to poor pharmacokinetic features (Alexis *et al.*, 2008). Traditionally, the use of plants as a source for drugs is based on the knowledge and superstitions passed down orally from generation to generation (Puri A.V, 2018). Plants produce a variety of bioactive chemicals, many of which have medicinal properties, making them a rich source of pharmaceuticals. *Duranta erecta* Linn. a member of the Verbenaceae family, is a native of clear, open woodlands. Tropical countries utilize it as a decorative plant (Ganapathy *et al.*, 1997; Hiradate *et al.*, 1999). Traditionally the plant is used to for a variety of disease conditions. The silver nano particles prepared from the plant is used to treat inflammation, pain, wounds and skin ailments. It is also used to treat ulcers and malaria. Previous works investigating formulation and characterization of Silver nano particles obtained by the extract of *Duranta erecta* leaves. Therefore the present study was undertaken employing both in vitro and in vivo methods to explore possible anti microbial and anti-inflammatory peritonitis potential of the methanolic extract of *Duranta erecta* so as to establish a plausible mechanism of action of extract and to justify the traditional uses of this plant.

2. MATERIAL AND METHOD

2.1 Collection and authentication of plant material

The leaves of *Duranta erecta*, were collected from local region of Bhopal in the month of April, 2022. The plant was authenticated by Dr. Jagriti Tripathi, Department of Botany, Government College Khimlasi Sagar (MP). A voucher specimen (Ref No 2022076) has been allotted for authentication.

2.2 Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study was of analytical grade.

2.3 Preparation of Plant extract

2.3.1 Extraction of plant by soxhlet extraction method

Coarsely powered leaves of *Duranta erecta* was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 8-10 hrs at a temperature of 40-60°C using soxhlet apparatus (Alara *et al.*, 2019).

2.4 Synthesis of Silver Nanoparticles

The process for the synthesis of nanoparticle was followed as mentioned in the previous section of this paper published.

2.5 *In-vitro* Anti-inflammatory Activity (Protein denaturation Method)

a) Preparation of sample/ standard

Freshly prepared 1 mg/ml solution of Diclofenac sodium will be used as standard by dissolving with distilled water. 1 mg of test sample (extract and nanoparticle)/standard will be taken with methanol/DMSO and distilled water for standard to make 1mg/ml stock solution. Different concentration of sample (50-250 µg/ml)/standard (10-30µg/ml)) will be taken from stock solution in a set of test tubes and methanol/DMSO will be added to make the volume to 1 ml.

b) Protocol

The reaction mixture (5 ml) included 2.8 ml of phosphate buffered saline (PBS, pH 6.4), 0.2 ml of egg albumin (from hen's egg), and 2 ml of various plant extract concentrations, yielding final concentrations of 50, 100, 150, 200, and 250 µg/ml. A comparable volume of saline buffered with phosphate served as the control. The mixes were then heated to 70°C for 5 minutes after being incubated for 15 minutes at (25±2) oC in a Universal incubator. Following cooling, their absorbance was measured using a vehicle as a blank at 660 nm (Shimadzu 1700). For the purpose of determining absorbance, diclofenac sodium at final concentrations of 10, 15, 20, 25, and 30µg/ml was utilized as a reference medication and handled similarly. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The 50% inhibition (IC₅₀) of the extract (sample/drug, diclofenac sodium) was calculated by graphing the percentage inhibition relative to the control against the concentration of the treatment (**Chandra et al., 2012**).

2.6 Antimicrobial Activity (Well Diffusion Assay)

a) Preparation of Dilutions of the Samples

The samples were diluted to concentrations of 100µg/ml, 200µg/ml, 300µg/ml, and 400µg/ml, respectively. Distilled water was then used to make up the volume of the sample to 1 ml.

b) Preparation of Nutrient Agar Media

One liter of purified water included 28 grams of Nutrient Media dissolved in it. Prior to sterilization, the media's pH was measured. The media underwent a 15-minute autoclaving process at 121°C and 15 pounds of pressure. Plates containing nutritional media were filled and exposed to laminar air flow until the agar solidified.

c) Well Diffusion Assay

Bacterial strains (*S. mutans* MTCC 389 and *E. coli* MTCC 42) were cultivated on nutrient agar medium (NAM). The bacterial suspension was maintained in the shaker and standardized to 10⁸ CFU/ml of bacteria. Next, using a micropipette, extract 100µl of the inoculum (containing 10⁸ CFU/ml) from the broth and transfer it to a fresh, sterile, solidified Agar Media Plate (**Mohammadi-Sichani et al., 2012**). The inoculum was applied to the whole sterile agar surface of the agar plate using a sterile spreader to inoculate it. Using a sterile cork-borer, four 6 mm wells were drilled into the inoculation material. A varied concentration of samples (100µg/ml, 200µg/ml, 300µg/ml, and 400µg/ml) was added to each well. After being incubated for 18 to 24 hours at 37 degrees Celsius, it was given a half-hour at room temperature to disperse. Following incubation, plates were checked to see if a clear zone formed around the well, indicating that the chemicals under test had antimicrobial activity. The zone of inhibition (ZOI) was measured and recorded in millimeters. Zones were measured to the nearest millimeter using a ruler that was held on the back of the inverted Petri plate. A few inches above a black, non-reflective background was where the Petri plate was held. The diameters of the well as well as the zone of total inhibition (as determined by unaided eye) were measured (**Manandhar et al., 2019**).

2.7 In-vivo Study

a) Preparation for Bacterial Inoculum

To put it briefly, strains of *E. coli* ATCC 25922 and *E. Faecalis* ATCC 29212 were cultivated on LB Medium Catalog No.: M1245-1KG. From a single colony, the strains were autoclaved at 121°C temperature and 15 lbs pressure for 30 minutes. The cultures were then incubated for 16–18 hours. Following a 10-minute centrifugation at 200 rpm at 4°C, the bacteria were separated into pellets and resuspended in PBS until an OD of 0.1 at 660 nm, or 2×10^4 CFU/ml, was achieved using a spectrophotometer.

b) Systemic Infection of Peritonitis

In this study, the animals were divided into four-four groups for both bacterial strain and *E. Faecalis*, each group containing six animals (n=6).

***E. coli* treated:**

Group-I Control (Normal Saline+ *E. coli* (i.p.) Group-II Standard Drug (Ofloxacin 5 mg/kg body weight (p.o.)+ *E. coli* (i.p.) Group-III *Duranta erecta* methanolic extract (200 mg/kg body weight (p.o.)+ *E. coli* (i.p.) Group-IV *Duranta erecta* methanolic extract (400 mg/kg body weight (p.o.)+ *E. coli* (i.p.)

***E. faecalis* treated:**

Group-I Control (Normal Saline+ *E. faecalis*(i.p.) Group-II Standard Drug (Ofloxacin 5 mg/kg body weight (p.o.)+ *E. faecalis*(i.p.) Group-III *Duranta erecta* methanolic extract (200 mg/kg body weight (p.o.)+ *E. faecalis*(i.p.) Group-IV *Duranta erecta* methanolic extract (400 mg/kg body weight (p.o.)+ *E. faecalis*(i.p.)

The experimental design was involved administration of each of the test samples (200mg and 400mg/kg body weight) of selected plant extract and Standard antibiotic Ofloxacin 5mg/Kg body weight by daily oral dose (p.o.) for a period of 7 days. The survival studies of 6 rats per treatment group were inoculated with *E. coli* and *E. faecalis* the mortality occurs primarily between 24 and 48 hrs after infection. The mortality was assessed every 2 h in this period, thereafter mortality was monitored every 6 hrs. The Survival was monitored for all experimental groups until 14 days.

After the monitoring of the mortality, the treated rats were sacrificed, and assess the *in vivo* peritonitis activity of *Duranta erecta* methanolic extract by determination of CFU/ml. All the four-four groups of treated rats were anesthetized by inhalation of diethyl ether and the peritoneal cavity was washed with 5ml of sterile PBS saline by using an 18-gauge needle and peritoneal lavage fluid was collected in sterile tubes for determination of CFU.

Fifty microliters (50µl) of peritoneal lavage fluid from all tested Groups I-IV of *Duranta erecta* methanolic extract on *E. coli* treated and Groups I-IV of *Duranta erecta* methanolic extract on *E. faecalis* treated were placed on ice and serially diluted with sterile saline. Twelve (12µl) microliters of each dilution were spread on sterile nutrient agar plates and incubated overnight at 37°C after which the number of colonies was counted.

3. RESULTS

3.1 *In-vitro* anti inflammatory activity

3.1.1 Protein denaturation Method

Table 1 Effect of Diclofenac sodium (Standard) and Methanolic extract of *Duranta erecta* on Protein Denaturation

SAMPLE	IC50 VALUE
Diclofenac sodium (Standard)	11.639
Methanolic extract of <i>Duranta erecta</i>	132.08
Nanoparticles of <i>Duranta erecta</i>	67.872

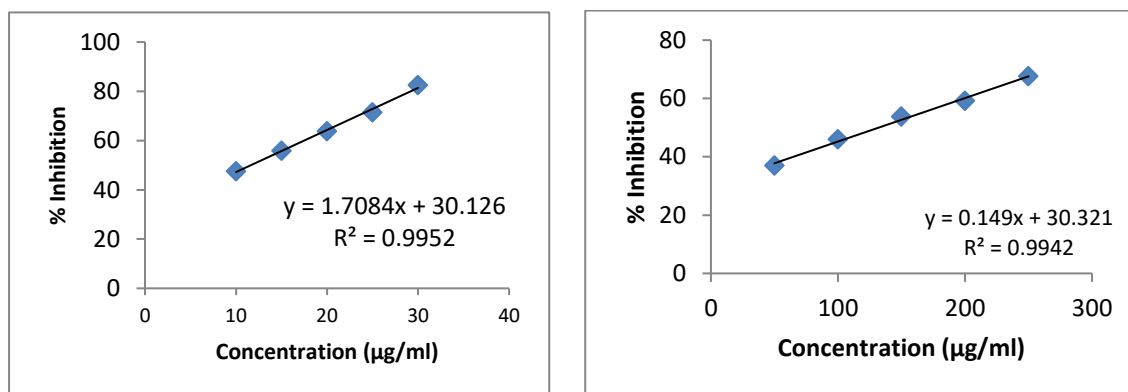


Figure 1: Bar Graph represents the Percentage Inhibition Vs Concentration of Diclofenac sodium (Standard) and Methanolic extract of *Duranta erecta*

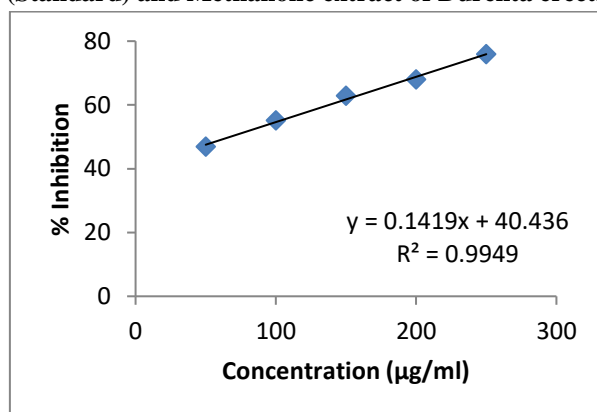


Figure 2: Bar Graph represents the Percentage Inhibition Vs Concentration of Nanoparticles of *Duranta erecta*

3.2 Antimicrobial Activity (Well Diffusion Assay)

The extract (sample/drug (diclofenac sodium) concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration (Chandra *et al.*, 2012).

Table 2: Antimicrobial activity of nanoparticle of *Durenta erecta* against *E.coli* and *E. faecalis*

Concentration	Mean±SD <i>E.coli</i>	Mean±SD <i>E. faecalis</i>
100	8±0	8.666±0.577
200	10.333±0.577	11.666±0.577
300	12.6667±1.154	15±1
400	15±1	18±1



Plate 1

Plate 2

Plate 3

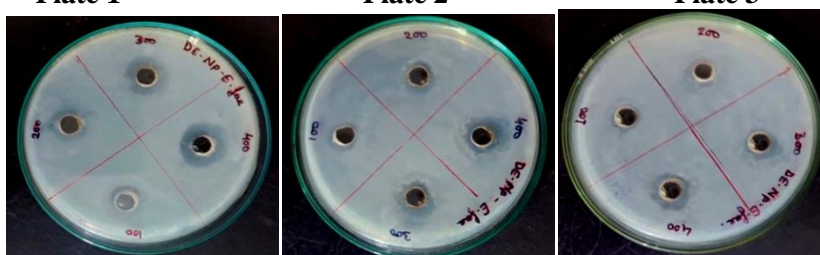


Plate 1

Plate 2

Plate 3

Figure 3: Antimicrobial activity of nanoparticle of *Durenta erecta* against *E.coli* and *E. faecalis*

Table 3 Antimicrobial activity of Methanolic extract of *Durenta erecta* against *E. coli* and *E. faecalis*

Concentration	Mean±SD <i>E.coli</i>	Mean±SD <i>E. faecalis</i>
100	7.333±0.577	7±0
200	9.333±1.154	8.333±0.577
300	11.333±0.577	11±1
400	14±0	14.333±1.527



Plate 1

Plate 2

Plate 3

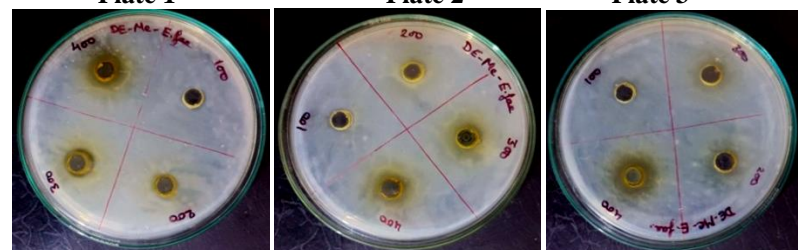


Plate 1

Plate 2

Plate 3

Figure 41: Antimicrobial activity of methanolic extract of *Durenta erecta* against *E. coli* and *E. faecalis*

Table 4: Comparative table of Antimicrobial activity of *Duranta erecta* at 400 (µg/ml) concentrations

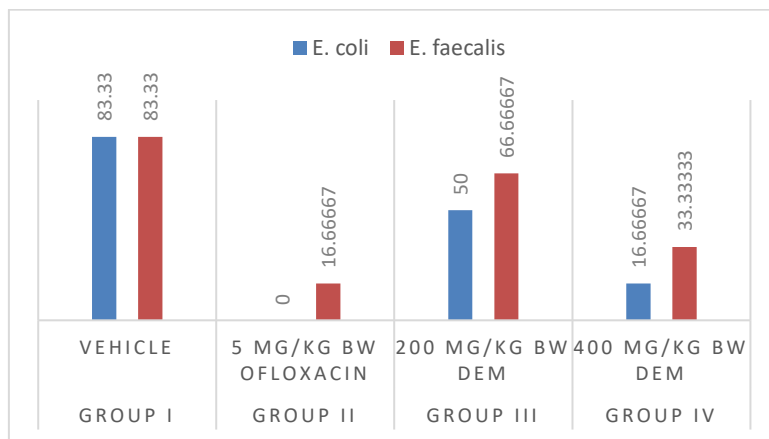
Sample name	Zone of Inhibition against <i>E. coli</i> (in mm)	Zone of Inhibition against <i>E. faecalis</i> (in mm)
Nanoparticle of <i>Duranta erecta</i>	15±1	18±1

Table 8: Mortality chart in *E. faecalis* induced groups treated with DEM in days

Gro ups	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 12	Day 14
I	0.67± 0.516	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408	0.83± 0.408
II	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00
III	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.17± 0.408	0.33± 0.516	0.50± 0.548	0.50± 0.548	0.67± 0.516	0.67± 0.516	0.67± 0.516
IV	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.33± 0.516	0.50± 0.548	0.50± 0.548

Table 9: Survival in Rats infected with *E. coli* and *E. faecalis* treated with DEM at 200 and 400 mg/kg body weight

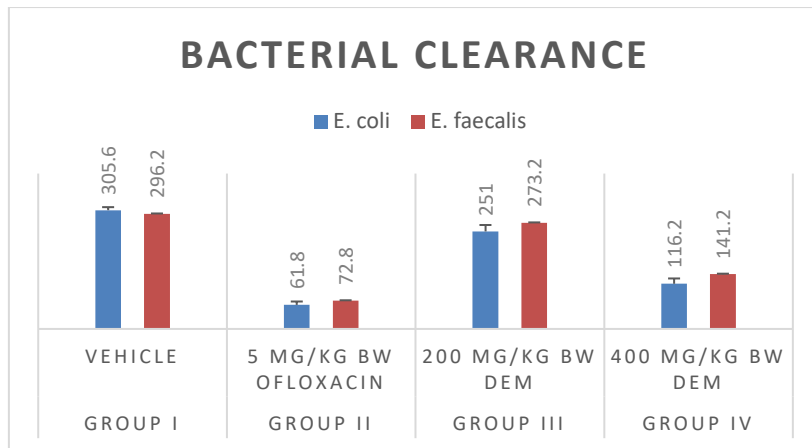
Groups	<i>E. coli</i>	<i>E. faecalis</i>
Group I	83.33	83.33
Group II	0	16.66667
Group III	50	66.66667
Group IV	16.66667	33.33333



Graph 2: representation of bacterial ratio

Table 10 : Bacterial clearance represented as CFU/ml in blood sample collected from rats infected with *E. coli* and *E. faecalis* treated with DEM (200 and 400 mg/kg), ofloxacin (5 mg/kg bw)

Groups	<i>E. coli</i>	<i>E. faecalis</i>
Group I	305.60±7.925	296.20±10.710
Group II	61.80 ±8.815	72.80±4.087
Group III	251.00±16.538	273.20±9.602
Group IV	116.20±13.737	141.20±10.183

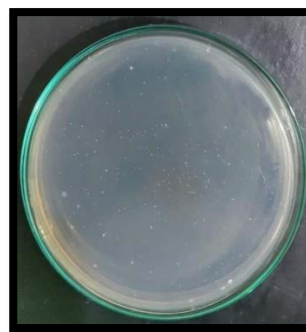


Graph 3: representation of bacterial clearance ratio

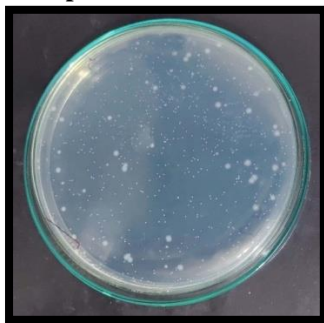
Figure 5 : CFU count of Groups I-IV of *Duranta erecta* methanolic extract on *E. coli*



Group I Vehicle treated



Group II 5 mg/kg bw Ofloxacin



Group III 200 mg/kg bw DEM

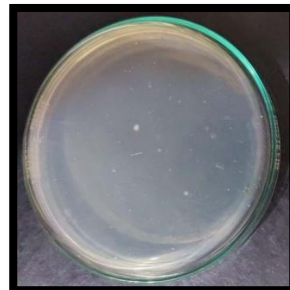


Group IV 400 mg/kg bw DEM

Figure 6: CFU count of Groups I-IV of *Duranta erecta* methanolic extract on *E. faecalis*



Group I Vehicle treated



Group II 5 mg/kg bw Ofloxacin



Group III 200 mg/kg bw DEM



Group IV 400 mg/kg bw DEM

4. DISCUSSION

Antimicrobial agents, found in foods, can inhibit microbial growth or cause death, with recent interest in naturally occurring antimicrobials like herbs, spices, and plants (Burt, 2004). The ability to compare the results for antimicrobial plant compounds or extracts from different studies is limited because of differences in the methodologies used. The study investigated the antimicrobial properties of *Duranta erecta* plant extracts and nanoparticles, aiming to develop a standardized procedure for assessing their potential in vivo applications. The results presented demonstrated that silver nanoparticles synthesized from *Duranta erecta* extract exhibited a notable zone of inhibition against all tested organisms. The maximum inhibitory effect was observed against *E. faecalis* (18 mm) and *Escherichia coli* (15 mm). *Duranta erecta* extract effectively inhibits bacterial growth, with silver nanoparticles showing significant inhibitory effects against both gram-positive and gram-negative strains. *Duranta erecta*, also known as "golden dew drop," is a shrub grown in Ghana for hedges and ornamental purposes, with phytoconstituents including coumarinolignoids and lamiide. (Hiradate *et al.*, 1999; Ijaz *et al.*, 2010). A number of bioactive compounds such as β -sitosterol, naringenin, acteoside, lamiide, sucrose, and raffinose have been identified and isolated from *Duranta repens* (Abou-Setta *et al.*, 2007). It has numerous natural uses including antifungal and insecticidal properties (Ekenma *et al.*, 2018). Peritonitis is a disease characterized by persistent abscesses and high mortality rates. Treatment focuses on controlling sepsis and eliminating contamination sources. Peritoneal infections can occur due to various factors, including translocation of microorganisms, surgical contamination, and bacterial translocation through the intestines. Anaerobic bacteria, such as *Bacteroides* species and facultative anaerobic and aerobic Gram-negative bacilli, contribute to 37% of peritoneal infections. Further research is needed to develop effective treatments (Baron *et al.*, 1990; Koneman *et al.*, 1997). The aim of the present study was to develop such a medicine, which allows the study of mechanism involved and prevention of, intra-abdominal abscess formation after surgical treatment of generalized peritonitis. In this infection model, the

peritoneal cavity saw rapid bacterial development due to a high inoculum in group II. DEM were able to limit the growth of the bacteria and eliminate them within 24 hours. The mortality rate among rats with infections caused by *E. faecalis* was similar to that among patients infected with *E. coli*. Survival percentage of rodents was calculated by inducing bacterial infection through *E. coli* and *E. faecalis*. The activity of the extract was calculated by the aggregate percentage of mortality chart count of the microorganisms induced groups study. Four groups containing vehicle, standard (5mg/kg), extract (200 mg/kg) and extract (400 mg/kg) was considered. The results obtained indicated the highest percentage survival rate in group I compared to others groups. About 40,000 deaths in 2001 were linked to *E. coli* sepsis, which accounts for 17% of all sepsis cases. Peritonitis continues to be an important problem in the health care system. Bacterial clearance study represented as CFU/ml in peritoneal lavage collected from rats infected with *E. coli* and *E. faecalis* treated with DEM (200 and 400 mg/kg), ofloxacin (5 mg/kg bw). The results obtained indicated a dose dependent antibacterial activity of the extracts; the extracts given at a concentration of 400 mg/kg had better activity than the one administered at 200 mg/kg. While the concentration presented an 116.20 ± 13.737 CFU/ml count for *E. coli* count and having a count of 141.20 ± 10.183 CFU/ml for *E. faecalis*. This shows the increased affinity of drug to treat the *E. coli* induced condition precisely followed by *E. faecalis*. Further, studies need to be done to verify the effect of phenolic compound explore the mechanisms and promote the clinical use.

5. CONCLUSION

The study on *Duranta erecta* silver nanoparticles provides insights into their potential therapeutic applications. In-vitro experiments showed robust antimicrobial properties, while in-vivo studies showed significant anti-inflammatory effects. Peritonitis activities suggest potential combating peritoneal infections. These findings highlight the multifaceted nature of *Duranta erecta* silver nanoparticles, making them a promising candidate for further research in nanomedicine. Further studies are needed to fully understand their safety and efficacy.

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