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Antimicrobial activity of Crude *Calotropis procera* extract with special reference to Sheep *Salmonellosis*

Mosa M. Bahnass[1], Noura E. Attia[2], Elshaima M. Fawzi[1]

[1]*Department of Animal Medicine "Infectious Diseases", Faculty of Veterinary Medicine, Zagazig University, Egypt.*
[2]*Department of Animal Medicine "Internal Medicine", Faculty of Veterinary Medicine, Zagazig University, Egypt.*

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ABSTRACT

Antimicrobial resistance of bacteria is one of the biggest problems worldwide. *Calotropis procera* has been used in traditional and folk medicine due to presence of different active compounds. The present study was conducted to determine the antimicrobial activity of crude aqueous Calotropis *procera* extracts against field isolates of some gram negative and positive bacteria. The aqueous extract of the *Calotropis procera* was studied for its Antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Salmonella typhimurium, Samonella arizonae subsp.3A, Salmonella typhi and Salmonella pullorum*. In vitro antimicrobial activity was conducted by Kirby-Bauer disc diffusion technique on Mueller-Hinton agar. The extracts pronounced significant effect on the tested organisms. highest Zone of inhibition –mm (ZI) (35.4 ± 2.4) was in latex extract. There was a statistically significant ($P < 0.05$) difference between groups of bacteria in the antimicrobial effect of latex, leaves and flowers extracts. ZI of Latex, leaves and flowers extract for *Salmonella typhimurium* isolated from sheep was 7.667±.3, 3.583 ±.3 and 2.167±.2 respectively at Minimum Inhibitory Concentration (MIC) of 6.3 mg/ml. We concluded that the extracts of *Calotropis procera has* an antimicrobial activity against gram positive and negative bacterial isolates. The latex extract had more antimicrobial activity than leaves and flower. in addition, the leaves extract had more antimicrobial activity than flowers. (MIC) of *Calotropis procera* extracts was 6.3 mg/ml. It has been recommended to conduct further investigations in vivo to know the extent of the effect and control measure on salmonellosis and others bacterial diseases

1. INTRODUCTION

Latex of Calotropis procera has strong inhibitory effect on bacteria than the leaves and the plant used in traditionally medicines (Kareem et al., 2008). cysteine peptidases of Calotropis procera latex play a main role in control and treatment against fungus microbes (Freitas *et al.*, 2020). Aiton is synonyms of Calotropis procera or W.T. Aiton and it is related to Family Apocynaceae known apple of Sodom. It is worldwide distributed specially in semi aired and aired regions. It is local tree and plant of Middle East, north of Africa, Arabian part of Peninsula and south area of Asia. Furthermore it is grass or a weed in many parts of area as sides of road, waste sites, beside any water area, dunes of sand, land grass and pastures (Hassan *et al.*, 2015). Egyptian and Saudi Arabia Essential Oils of Calotropis procera were different in quantity and quality due to the effect of different environment and climates. The Essential Oils (EOs) of both plant species in both countries have significant important activity against microbes (antimicrobial and allelopathic effect). Also, it was found that essential Oils of Calotropis procera have strong phytotoxic activity to different species of weeds. Egyptian EOs had more effect against the weed of species Dactyloctenium aegyptium (Al-Rowaily *et al.*, 2020). Calotropis procera leaf extract was effective in vitro treatment of Bacillus cereus, Pseudomonas aeruginosa and Proteus mirabilis (Bilal *et al.*, 2020). Paenibacillus Polymyxa AALI Endophyte Isolated from Calotropis Procera has Antimicrobial activity and depended on 16S rRNA gene analysis. The free cell supernatant extract and crude Calotropis procera had antibacterial effect against Klebsiella pneumonia, Staphylococcus aureus and E. coli (El-Rahman *et al.*, 2020). Applied studies in vivo and vitro on sheep revealed that crude aqueous and crude methanolic extracts of Calotropis procera flowers had a strong antiparasitic activity against gastrointestinal Nematodes, but their activity were less than that showed by levamisole drug (Iqbal *et al.*, 2005).

The aim of the present work was to explore Antimicrobial activity of *Calotropis procera* extract on isolated gram negative and positive Bacteria with special reference to sheep Salmonellosis.

2. MATERIAL AND METHODS

2.1 Geographical location of Present study:
Najran city present in south and west of Saudi Arabia country near Yemen the border. City of Najran represents Najran Province capital. Samples were collected from sheep 10 farms present in Arisa and Fisalia area near slaughterhouse.

2.2 Collection, isolation, and Processing of Calotropis procera Plant Samples:
The collected medicinal Plant was identified in Department of Herbs and Medicinal Plants, Faculty of Pharmacy, Najran University Then the plant was preserved in the laboratory. Fresh leaves, latex and flower of Calotropis procera medical plant were collected in clean sterile container and transported to the laboratory in Najran University. After perfect gently washing in distilled water, Leaves and flower were surface sterilized by ethyl alcohol 70 % immersion for 2 minutes, followed by Sodium hypochlorite (NaOCl) (active chlorine 2.5 %) for 5 minutes, after that in ethyl alcohol 70 % for about 30 seconds, and washed three times in sterile distilled water (Nascimento *et al.*, 2015).
Ten grams of grinded plant leaf and flower powder was soaked in hundred ml of distilled water in flask conical in shape and applied on shaker at a speed of 12 g for one day. Each mixture of the leaf and flower were filtered using filter paper ‘‘Whatman’’ NO. 1. Then filtrate was concentrated by using rotary evaporator or water bath at 100ºC followed by drying and lyophilization. The extracted powders of leaf and flower were dissolved separately in sterilized distilled water to make 1000 µg/ml solution. The mixture was used to conduct further antimicrobial assay (Kumar, Karthik and Bhaskara Rao, 2010).

* Corresponding author: Elshaima Mohamed Fawzi. Email: a.fawzy@zu.edu.eg

The latex of *Calotropis procera* was collected under aseptic condition. Then it was centrifuged at 200 g for 5 minutes. The supernatant fluid was discarded, and the Residual sediment was concentrated by using rotary evaporator or water bath at 100ºC followed by drying and lyophilization. Then extracted powders was dissolved in sterilized distilled water to make 1000 µg/ml solution for further antimicrobial assay (Kareem, Akpan and Ojo, 2008). Equal extract quantities of leaves, flower and latex dissolved in sterilized distilled water to make 1000 µg/ml solution for further antimicrobial assay.

2.3. Tested microorganism

The collected faecal samples were put into buffered peptone water and incubated at 37ºC overnight (16-20 hours). it was transferred to Selenite-F broth and incubated at 37°C for 18 hours. The inoculates were cultured in MacConkey agar media for 18 hours at 37°C. These were then subcultured into Deoxychocolate Citrate Agar (XLD). The suspected Salmonella spp. colonies according to TSI and selective media were followed by biochemical identification, the Microbact™ Gram-negative system (Oxoid, UK).

Eight bacterial microorganisms used in this study as test microorganisms including clinical isolates of four *Salmonella spp.* another bacterial isolates were obtained from the Microbiology Department of Najran king Khaled Hospital. This isolates were *E. coli , Staph. Aureus, pseudomonas spp. and Strept. Pyogens*

The isolated *Salmonella typhimurium* from the fecal samples which collected from diarrheic sheep (ewes) which subjected to thorough clinical examination including recording of body temperature, pulse, respiration, mucous membranes and ruminal movements investigation. The degree of dehydration has been estimated by capillary refill time and other clinical parameters according to Constable et al. (2016).

Another three salmonella isolates were from human (two isolates, first (S1) was *Samonella arizonae subsp.3A* and second isolate (S2) was *Salmonella typhi*) and Avian Salmonella pullorum isolate from table eggs (Bahnass, et al.,2015).

2.4 Negative and Positive control antibiotic discs

Ciprofloxacin disc (5 µg/disc) was used as positive control for *Salmonella species* and *E.coli*, Trimethoprim + Sulfamethoxazole disc (1.25 µg +23.75 µg/disc) for *S. aureus* , Ceftazidime disc (10 µg/disc) for *pseudomonas spp.,* Erythromycin disc (15 µg/disc) for *Strept. pyogens*. discs in Sterilized distilled water was used as negative control.

2.5 Antimicrobial susceptibility testing (Kirby-Bauer disc diffusion technique):

Three to five colonies well-isolated were transferred into a nutrient broth tube containing 5 ml of. Then it was incubated at 37°C until it achieves the turbidity of the 0.5 McFarland standards (usually within 6 hours). Plate of Mueller Hinton agar was inoculated by using a sterile swab. Discs of Sterile filter paper (7mm diameter) were immersed with the extracts in different concentrations and dried at 40ºC for thirty minutes. Prepared extracts discs (tested), Sterilized distilled water immersed discs (negative control) and antibiotic discs (positive control) were placed on the agar surface as. Then prepared plates were incubated at 37°C for 24 hours. Three replicated plates were conducted for each test organisms (Biemer, 1973). Antibacterial activity of the different extracts was evaluated by zone of inhibition measuring.

2.6. Minimum inhibitory concentration determination of (MIC):

It was used modified agar well diffusion method. Different extract of leaves, latex and flower stock solution were diluted double fold serially to make a concentration ranged from 0.1-100 mg/ml. Plates of Mueller Hinton agar were bacterial inoculated by using a sterile swab. Four wells were cut in diameter of 7 mm. 100 µl of each dilution was applied into wells. Then incubation at 37ºC for 24 hours. Antibacterial activity of the different extracts was evaluated by zone of inhibition measuring. Three replicated plates were conducted for each test organisms (Rios *et al.,* 1988).

2.7 Ethical consideration:

The ethical board of Najran University, Saudi Arabia has given a permit authenticated to work and carry out research study within the institutional research mandate as stipulated by the National Ethical Board.

2.8 Statistical analysis

For data analysis, Statistical Package for Social Sciences software, version 23.0 (SPSS Inc., Chicago, IL) was used. Quantitative Normality data was tested using Kolmogorov-Smirnov. Both descriptive and inferential statistics involving Pearson's correlation, one-way ANOVA, Duncan's Multiple Range Test and regression were used to present results. For each test, a p-value of less than 0.05 was considered statistically significant.

3. RESULTS

The results were expressed as "mean (SD)" (n=3) (Nagele, 2003; Tom, 2004). Leaves, flowers and latex Calotropis procera aqueous extract showed the antimicrobial activity against eight clinical isolates of bacteria. From the results shown in Table (1), the highest Zone of inhibition –mm (strong antimicrobial effect) were at latex and lowest in flowers extracts. The results also showed that the lowest Minimum Inhibitory Concentration (mg/ml) gave antimicrobial effect were 6.3 mg/ml (Table 1). As well as the results showed highest Zone of inhibition –mm (ZI) (35.4 ± 2.4) was in latex extract while in antibiotic was in Ciprofloxacin disc (5 µg/disc) (26.9 ± 2.1).

Clinical examination of diarrheic sheep revealed moderate to severe diarrhea which determined according to the consistency of the fecal matter, degree of dehydration, alertness of the animal, posture of the animal "in standing or in recumbent position" dullness, depression. Feces were semifluid to watery in consistency, greenish to yellowish in color. Feces contains mucous and sometimes blood. There was loss of appetite and moderate degree of dehydration. The perineum and tail were solid with feces. The boy temperature, pulse, respiration was within the reference range. There was a reduction in ruminal movement (0-1)/ 2 minutes.

ZI of Latex, leaves and flowers extract in sheep *Salmonella typhimurium* was 7.667±.3, 3.583 ±.3 and 2.167±.2 respectively at Minimum Inhibitory Concentration (MIC) of 6.3 mg/ml (Table 2 and Fig. 3).

Table 1 Antibacterial Properties of crude *Calotropis procera* Latex, Leaf anf flower Extracts with different concentration(mg/ml) using Paper Disc Method (Zone of inhibition - mm)(mean±sd) compared with antibiotics on tested Gram negative and postive bacetria :

Conc.	Plant part	Zone of inhibition (mm)	Conc.	Plant part	Zone of inhibition (mm)	Conc.	Plant part	Zone of inhibition (mm)
100 mg/ml	latex	35.4 ± 2.4	50 mg/ml	latex	27.8 ± 3.4	25 mg/ml	latex	24.2 ± 3.7
	Leaf	28.01 ± 3.9		Leaf	21.7 ± 4.5		Leaf	16 ± 3.4
	flower	21.1 ± 3.2		flower	14.6 ± 1.9		flower	7.4 ± 1
	mix	31.6 ± 2.4						
Conc.	Plant part	Zone of inhibition (mm)	Conc.	Plant part	Zone of inhibition (mm)	Conc.	Plant part	Zone of inhibition (mm)
12.5 mg/ml	latex	17.9 ± 3.6	6.3 mg/ml	latex	9.1 ± 1.8	3.1 mg/ml	latex	ND
	Leaf	8.1 ±1.7		Leaf	4 ± .8		Leaf	ND
	flower	3.7 ± .5		flower	1.8 ± .2		flower	ND
Conc.	Plant part	Zone of inhibition (mm)						
1.6 mg/ml	latex	ND*						
	Leaf	ND						
	flower	ND						
Antibiotics	Zone of inhibition (mm)							
Ciprofloxacin	26.9 ± 2.1							
Trimethoprim + Sulfamethoxazole	16.9 ±1.3							
Ceftazidime	19.9 ± 1.8							
Erythromycin	24.8 ± 1.9							

*ND= Not detected

Table 2 Minimum Inhibitory Concentration (mg/ml) of crude *Calotropis procera* Latex, Leaf and flower Extracts in relation to salmonella species compared with antibiotics:

Conc.		S. sheep	S. avian	S1. human	S2. human	F	*P value**
		Zone of inhibition (mm)					
100 mg/ml	latex	34.6 ± 1.2	32.6 ±1.3	34.6 ±1.4	36.5 ± 1.5	6.773	.001
	Leaf	27.7 ± 1.5	25.8± 1.1	20.9± 1.1	26.787 ± 1.2	20.480	<.001
	flower	19.9±1.3	19.9±1.6	19.9±1.5	20.9 ±1.7	12.765	<.001
	Mix	32.667±1.1	29.727±1.2	29.727±1.5	29.727±1.4	6.612	.001
50 mg/ml	latex	24.827±1.2	23.847±1	26.787±1	26.787±1.8	15.589	<.001
	Leaf	19.927±1.1	15.027±1.5	16.987±1.6	22.867±1.1	28.079	<.001
	flower	16.987±1.3	13.067±1	15.027±1.2	12.087±1.4	3.283	.023
25 mg/ml	latex	19.927±1.5	17.967±1.6	24.827±1.1	24.827±1.3	18.344	<.001
	Leaf	14.047±1.1	12.087±1.2	16.987±1.2	14.047±1.1	14.694	<.001
	flower	8.667 ±.8	6.667±.1	7.667±.6	6.167±.7	3.283	.023
12.5 mg/ml	latex	15.027±1.8	16.007±1.6	15.027±1	19.927±1.1	17.633	<.001
	Leaf	7.167± .7	6.167±.7	8.667±.7	7.167±.7	14.694	<.001
	flower	4.333±.1	3.333±.1	3.833±.2	3.083±.3	3.283	.023
6.3 mg/ml	latex	7.667±.3	8.167±.6	7.667±.7	10.167±.8	17.633	<.001
	Leaf	3.583 ±.3	3.083±.3	4.333±.3	3.583±.3	14.694	<.001
	flower	2.167±.2	1.667±.3	1.917±.1	1.542±.2	3.283	.023
Antibiotic discs		25.807±1	27.767±1	29.727±1.3	26.787±1.4	23.418	<.001

*Fisher exact test. **Significant correlation if *P- values*≤ 0.05 and non-significant if *P-value* ≥ 0.05

There was a statistically significant difference between groups of bacteria in the antimicrobial effect of latex 6.3 mg/ml (Minimum Inhibitory Concentration (mg/ml) as determined by one-way ANOVA (F = 17.633, p = <.001). Duncan's Multiple Range test revealed that latex extract (6.3 mg/ml) had statistically significantly highest antimicrobial effect against *Staph. aureus* with (Zone of inhibition –mm (11.667±.7), p = <0.001) compared to another bacterial group. (Table 2,3 and 5) (Figure 1&3)

While there was a statistically significant difference between groups of bacteria in the antimicrobial effect of leaves 6.3 mg/ml (Minimum Inhibitory Concentration (mg/ml) as determined by one-way ANOVA (F = 14.694, p = <0.001). Duncan's Multiple Range test revealed that leaves (6.3 mg/ml) had statistically significantly highest antimicrobial effect against *Strept. pyogens* with (Zone of inhibition –mm (5.833±.3), p = <0.001) compared to another bacterial group. (Table 2 and 3) (Figure 1&3)

Table 3 Minimum Inhibitory Concentration (mg/ml) of crude *Calotropis procera* Latex, Leaf and flower extracts in relation to bacterial speies compared with antibiotics:

Conc.		E. coli	Staph. aureus	Pseudomonas spp.	Strept. pyogens	F*	*P VALUE**
		Zone of inhibition (mm)					
100 mg/ml	latex	37.5 ± 1.1	33.647 ±1.2	34.6 ± 1.5	39.5 ±1.3	6.773	.001
	Leaf	29.7 ± 1.2	28.7± 1.1	29.7± 1.4	34.627 ± 1.7	20.480	<.001
	flower	19.9 ±1.4	19.9 ±1.4	19.9 ±1.4	28.7 ± 1.4	12.765	<.001
	Mix	34.627±1.7	32.667±1.4	29.727±1.1	34.627±1.4	6.612	.001
50 mg/ml	latex	29.727±1.2	26.787±1.5	29.727±1.6	34.627±1.1	15.589	<.001
	Leaf	27.767±1	19.927±1.4	24.827±1.3	26.787±1	28.079	<.001
	flower	16.007±1.3	15.027±1	14.047±1.4	15.027±1.6	3.283	.023
25 mg/ml	latex	24.827±1.5	24.827±1.4	26.787±1.9	29.727±1.8	18.344	<.001
	Leaf	17.967±1.7	15.027±1	15.027±1	22.867±1.4	14.694	<.001
	flower	8.167±.5	7.667±.6	7.167±.7	7.667±.8	3.283	.023
12.5 mg/ml	latex	13.067±1.2	22.867±1	21.887±1.3	19.927±1.7	17.633	<.001
	Leaf	9.167±.5	7.667±.8	7.667±.5	11.667±.7	14.694	<.001
	flower	4.083±.5	3.833±.4	3.583±.1	3.833±.3	3.283	.023
6.3 mg/ml	latex	6.667±.6	11.667±.7	11.167±.8	10.167±.9	17.633	<.001
	Leaf	4.583±.3	3.833±.3	3.833±.3	5.833±.3	14.694	<.001
	flower	2.042±.1	1.917±.2	1.792±.4	1.917±.1	3.283	.023
Antibiotic discs		24.827±1.3	16.987±1.4	19.927±1.2	24.827±1.1	23.418	<.001

*Fisher exact test, **Significant correlation if *P -values*≤ 0.05 and non-significant if *P-value* ≥ 0.05

Furthermore, there was a statistically significant difference between groups of bacteria in the antimicrobial effect of flowers 6.3 mg/ml (Minimum Inhibitory Concentration (mg/ml) as determined by one-way ANOVA (F = 3.283, p = 0.023). Duncan's Multiple Range test revealed that leaves (6.3 mg/ml) had statistically significantly highest antimicrobial effect against sheep salmonellosis with (Zone of inhibition –mm (2.167±.2), p = 0.023) compared to another bacterial group. (Table 2&3) (Figure 1&3)

A correlation and regression between Leaves, flowers and latex *Calotropis procera* aqueous extract (6.3 mg/ml) was also assessed. Significant positive a reasonable correlation (rho=0.481, p= .017) was observed between flower and leaves extract (6.3 mg/ml). Multiple linear regression analysis showed that leaves (6.3 mg/ml) extract (Zone of inhibition -mm) were significantly associated with Flower (6.3 mg/ml) (Zone of inhibition -mm) in term of 1 point increase on Flower corresponds to 1.729 points increase on leaves extract (P<0.01). (Table 4&5; Fig.2)

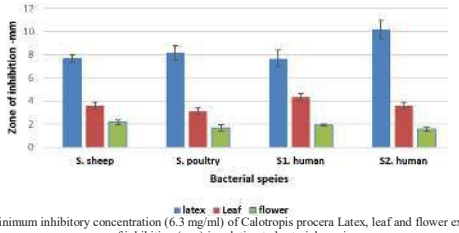

Fig. 1 Minimum inhibitory concentration (6.3 mg/ml) of Calotropis procera Latex, leaf and flower extracts zone of inhibition (mm) in relation to bacterial species.

Table 4 Correlation between latex 6.3, flower 6.3 and leaf 6.3 mg/ml

Variables	rho	*P*-value
latex 6.3 - flower 6.3 mg/ml	-.079	.712
latex 6.3 - leaf 6.3 mg/ml	.144	.501
flower 6.3 - leaf 6.3 mg/ml	.481*	.017

r = 0–0.2 : very low and probably meaningless.r = 0.2–0.4 : a low correlation that might warrant further investigation.r = 0.4–0.6 : a reasonable correlation. r = 0.6–0.8 : a high correlation. r = 0.8–1.0 : a very high correlation

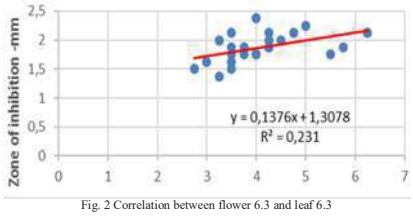

Fig. 2 Correlation between flower 6.3 and leaf 6.3

Table 5 Multiple linear regression of association between latex 6.3, flower 6.3 and leaf 6.3 mg/ml

variables		leaf 6.3 mg/ml			
		Unstandardized coefficient B*	95.0% Confidence Interval **for B	P value***	VIF****
Flower mg/ml	6.3	1.729	.366 - 3.093	.015	1.006
Latex mg/ml	6.3	.085	-.095 - .265	.339	1.006

* Unstandardized Coefficients. ** 95.0% Confidence Interval. *** statistically significant at p < 0.05. ****Variance inflation factor

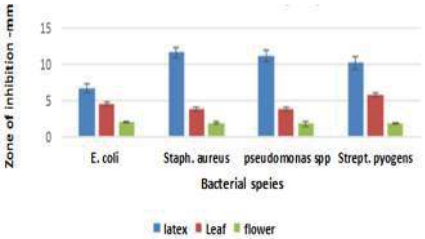

Fig. 3 Minimum inhibitory concentration (6.3 mg/ml) of Calotropis procera Latex, leaf and flower extracts zone of inhibition (mm) in relation to bacterial species (part 2).

4. DISCUSSION

Medicinal plants are considered an alternative source for obtaining active principals to treat many diseases and this depends on their physical and chemical properties. This is evident in their use in many traditional treatments throughout the ages in the present and the past. *Calotropis procera* plant is considered one of most important medicinal plants, as it is obtained abundantly in many agricultural and non-agricultural lands.

In this study, the highest Zone of inhibition –mm (strong antimicrobial effect) of crude extract was with latex and lowest with flowers extracts. The obtained result was consistent with (Kareem *et al.,* 2008), who proved that The inhibitory antibacterial effect of *Calotropis procera* was more obvious in the latex extract than the leaf extract. From obtained results revealed that the effect of *Calotropis procera* crude extract had antibacterial on gram positive bacteria than gram negative. the findings come in accordance with (El-Rahman *et al.*, 2020), as it has been proven that *P. polymyxa* AALI crude extract pronounced antibacterial activity against both Gram-positive and Gram negative bacteria, however, it showed a big activity against S. aureus (Gram positive) compared with *K. pneumonia* and *E. coli*, (Gram negative). In general, negative bacteria are more resistant to antibiotics than positive bacteria, and this is due to the difference in the chemical composition of the cell wall. This confirms that in recent times, in the world, the phenomenon of multi-drug resistant strains has appeared on a large scale and has become of great interest to researchers (Asadi Karam, Habibi and Bouzari, 2019). Antimicrobial activity of *Calotropis procera* leaves extracts against Escherichia coli and Pseudomonas aeruginosa, *Staph. aureus*, *Strept. pyogen* evaluated by disc method revealed inhibitory effect on growth of bacterial isolates (Mako *et al.*, 2012) .The results of this study agree completely with the results of current research. Moreover, the highest inhibition zone of 24.80±20 was showed for aqueous extract of *Calotropis procera* with conc. 2.5 mg/ml against *E.coli*. Escherichia coli was most susceptible to extract than another bacteria in the study while *Salmonella typhi* had lower one (Abegunde Segun *et al.,* 2020). By comparing this study with our study, it was found that it agrees with *E. coli*, but it differs with *Salmonella typhi*, and this could be led to drug resistance to test organisms isolates of this study. The antibacterial activity of latex showed that E.coli was more effected by latex than *S. aureus*, while all isolates test organisms were controlled by latex extract in different degree (Al-terehi *et al.*, 2018).

The obtained result supported earlier report by (Farooq *et al.,* 2017) and *(Abegunde et al.,*2020) that the aqueous and ethanolic extracts of *Calotropis procera* leaves inhibit the development of *E.coli*, *Pseud.aeruginosa*, *Staph.aureus*, *Salmonella typhi* and *Strept. pyrogenes,* respectively at different concentrations. The results revealed that extracts of plants are potent against some bacteria. However, the performances increased with an increase in the concentration of each extract. Generally, the aqueous extract of *Calotropis procera* was the most effective one against all studied bacteria. The result of the present work justifies the use of an aqueous extract of *C. procera* in ethnomedicine for the treatment of infectious diseases caused by bacteria (Mainasara *et al.,* 2011).

5. CONCULOSIONS

We concluded that crude extracts of *Calotropis procera* has an antimicrobial activity effect on gram positive and negative bacterial isolates. The latex extract had more antimicrobial activity than leaves and flower. On the other hand, the leaves extract had more antimicrobial activity than flowers. Minimum Inhibitory Concentration (MIC) of *Calotropis procera* extracts was 6.3 mg/ml. It has been recommended to conduct further investigations in vivo to know the extent effect of *Calotropis procera* extracts in treatment and control of salmonellosis and others bacterial diseases.

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6. REFERENCES

1. Abegunde Segun M, Akinyele Simeon, A. and Ayodele-Oduola Roseline, O. 2020. Chemical analysis and antibacterial activities of Calotropis procera and Clusia rosea leaves extracts', GSC Biological and Pharmaceutical Sciences, 12(1): 025–030.
2. Al-Rowaily, S.L., Abd-ElGawad, A.M., Assaeed, A.M., Elgamal, A.M., Gendy, A. .-N., Mohamed, T.A.; Dar, B.A., Mohamed, T.K. and Elshamy, A.I. 2020. Essential Oil of Calotropis procera: Comparative Chemical Profiles, Antimicrobial Activity, and Allelopathic Potential on Weeds. Molecules (Basel, Switzerland), 25(21).
3. Al-terehi, M. Shershab, S., Alshukry, S. and Al-Saadi, A.H. 2018. Study of Antimicrobial Activity of Calotropis Procera Latex in Some Pathogenic Isolates', 18(2), 1989–1991.
4. Asadi Karam, M.R., Habibi, M. and Bouzari, S. 2019. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli', Molecular Immunology, 108 pp. 56–67.
5. Bahnass, M.M., Fathy, A.M., and Alamin, M.A., 2015. Identification of Human and Animal Salmonella spp . isolates in Najran region and control of it. Int. J. Adv. Res. 3(1): 1014–1022.
6. Biemer, J.J., 1973. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Ann. Clin. Lab. Sci., 3(2): 135–140
7. Bilal, H., Ali, I., Uddin, S., Khan, I., Said, A., Rahman, M.Ur., Khan, A.M., Shah, A.B. and Khan, A.A. 2020. Biological evaluation of antimicrobial activity of Calotropis procera against a range of bacteria. Journal of Pharmacognosy and Phytochemistry 9(1): 31-35.
8. Constable, P., Hinchcliff, W.K., Done, S. and Gruenberg, W. 2016. Veterinary Medicine 11th Edition A textbook of the diseases of cattle, horses, sheep, pigs and goats.
9. El- Rahman, T. M.A., El-Beih, A.A., Ali, A.M. and Salah, M.G. 2020. Antimicrobial Potential of Paenibacillus Polymyxa AALI Endophyte Isolated from Calotropis Procera. Int. J. Progress. Sci. Technol 20(2): 418–422
10. Farooq, U., Nisar, S., Merzaia, A.B. and Azeem, M.W. 2017. Isolation of Bioactive components from Calotropis procera Plant Latex-A Review. International Journal of Chemical and Biochemical Science, 11, 95-101.
11. Freitas, C.D.T., Silva, R.O., Ramos, M.V., Porfírio, C.T.M.N., Farias, D.F., Sousa, J.S., Oliveira, J.P.B., Souza, P.F.N., Dias, L.P. and Grangeiro, T.B. 2010. Identification, characterization, and antifungal activity of cysteine peptidases from Calotropis procera latex. Phytochemistry, 169 p. 112.
12. Hassan, L.M., Galal, T.M., Farahat, E.A. and El-Midany, M.M., 2015. The biology of Calotropis procera (Aiton) W.T.," Trees - Struct. Funct., 29(2): 311–320
13. Iqbal, Z. Lateef, M. Jabbar, A. Muhammad, G. and Khan, M.N. 2005. Anthelmintic activity of Calotropis procera (Ait.) Ait. F. flowers in sheep. J. Ethnopharmacol 102(2): 256–261.
14. Kareem, S.O., Akpan, I. and Ojo, O.P., 2008. Antimicrobial activities of Calotropis procera on selected pathogenic microorganisms. African J. Biomed. Res. 11(1): 105–110,
15. Kumar, G. Karthik, L. and Bhaskara Rao, K.V., 2010. Antibacterial activity of aqueous extract of Calotropis gigantea leaves - An in vitro study. Int. J. Pharm. Sci. Rev. Res.4(2): 141–144.
16. Mako, G.A. Memon, A. H., Mughal, U. R., Pirzado, A.J. and Bhatti, S.A. 2012. Antibacterial effects of leaves and root extract of Calotropis procera Linn. Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences, 28(2), 141–149.
17. Mainasara, M.M., Aliero, B.L., Aliero, A.A. and Dahiru, S.S. 2011. Phytochemical and Antibacterial Properties of Calotropis Procera (Ait) R. Br. (Sodom Apple) Fruit and Bark Extracts. International Journal of Modern Botany, 1(1), 8 – 11.
18. Nagele, P. 2003. Misuse of standard error of the mean (SEM) when reporting variability of a sample. A critical evaluation of four anaesthesia journals. Br J Anaesth. 90:514–6.
19. Nascimento, T.L. Oki, Y. Lima, D.M.M., Almeida-Cortez, J.S., Fernandes, G.W. and Souza-Motta, C.M., 2015. Biodiversity of endophytic fungi in different leaf ages of Calotropis procera and their antimicrobial activity. Fungal Ecol. 14: 79–86,
20. Rios, J.L., Recio, M.C. and Villar, A., 1988. Screening methods for natural products with antimicrobial activity: A review of the literature. J. Ethnopharmacol. 23(2–3): 127–149,
21. Tom, L. 2004. Twenty statistical error even YOU can find in p