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Evaluation of Antimicrobial activity of Leaves extracts of *Bauhinia rufescens Lam. (Fabaceae)* on *Salmonella Spp.* isolated from Chicken intestine

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ABSTRACT

This study has been conducted to evaluate the antimicrobial activity of water extracts from leaves of *Bauhinia rufescens* on *Salmonella* species. In vitro antimicrobial test was performed by disc diffusion method on muller Hinton agar. Serial dilution was followed and concentrations corresponding to (0.2-0.4-2-4-6-8-10mg/ml) were prepared and then diluted to (10- 10^2 -10^3). Minimum inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) test were measured. The values of MIC for different times of incubation (24hrs, 48hrs, and 72hrs) were registered as : 0.1, 0.1, 2 respectively. The results of the study were subjected to statistical analysis and significances were found between concentration of *Bauhinia* extract . The results revealed that all concentrations used were effective for the inhibition of *Salmonella* sp. growth.

Key words: ***Bauhinia,*** **leaves,** ***Salmonella,*** **Antimicrobial,invitro,MIC**

Introduction

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. Thus the need of alternative drugs to reduce their burden of purchasing the synthetic drugs especially after the problem of getting resistant to many clinical patients against some antibiotics (Garbi *et al*.,2017). In addition antibiotics are sometimes associated with adverse effects on hosts including hypersensitivity, immune-depression and allergic reaction (Ahmad *et al*, 2004). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*, 2005).

In recent years, multiple drugs resistance in both human and plants pathogenic microorganisms have developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Loper *et al*., 1991,Hamidu *et al*; 2009).

People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore ,it is useful to investigate the potential of local plants against these disabling diseases with a view to validating their traditional usages and also serve as starting material for the development of clinically useful chemotherapeutic agents (koya *et al*.,2014).

*Bauhinia rufescens Lam. (*Fabaceae), known in western part of Sudan "*Kukul*" is a tropical forage plant that grows up to 8 meters high (Balogun *et al*., 1998). It is used for the establishment of hedges, as well as an ornamental tree (Asiedu *et al.*, 2012). In folk medicine, the plant is used for the treatment of gout, gingivitis, diarrhea, dysentery, diabetes, leprosy and malaria; Jansen et al (Compaoré *et al.*, 2011); (Tapsoba and Deschamps,(2006) Inngjerdingen *et al*.,(2004) ; *Maillard et al*.,(1991).

Previous phytochemical and bioactivity studies in the genus *Bauhinia* have resulted in the isolation of anti-inflammatory compounds including triterpenes from *B. tarapotensis* (Sosa *et al*., 2002), dihydrodibenzo oxepins from *B. purpurea* (Boonphong *et al*., 2007), and kaempferol and a triterpene caffeate from *B. variegat*e (Rao *et al*,2008), (Compaoré *et al.*, 2011) reported the inhibition activities of the leaves and bark extracts of *B. rufescens* against bacteria, *Gairdia lamblia*.

The core aims of this work were to screen *Bauhinia rufescens Lam* water extract phytochemically and to investigate the *in vitro* antimicrobial efficacy of its extract with a view of finding the most active against pathogenic bacteria (*Salmonella)* and to determine the minimum inhibitory and bactericidal concentrations.

Materials and Methods:

Plant sample

Collection and identification

Bauhinia rufescens (leaves) were collected from western Sudan in September 2018 University of Nyala Musai Campus. The plant was identified and authenticated by the a plant taxonomists Associ.Prof. Abduelrahman Tahir Agricultural Research Center (ARC-Nyala). All plant parts were air-dried, under the shadow with good ventilation and then ground finely in a mill and kept until their uses for extracts preparation.

Crude extract preparation

Extraction was carried out for *Bauhinia rufescens*(leaves (by using overnight maceration techniques according to the method described by Harbone, 1984. About 50 g were macerated in 250 ml of water at room temperature with occasional shaking for 24 hours, the supernatant was decanted and clarify by filtration through a filter paper, after filtration, the solvent was then removed under reduced pressure by rotary evaporator at 55°C.

Collection and Isolation of *Salmonella Spp*.

Stool specimens were collected from intestines of chickens for bacteriological study using sterile cotton swab. Each of the collected swabs was inoculated into freshly prepared blood agar medium. Then the plates were incubated at 37°C for 24 hours aerobically in a bacteriological incubator. The plates were examined for the growth of bacteria; the colonies were cultured into tubes containing buffer Peptone water broth medium (B.P.W). Then the growth was sub-cultured into tubes containing Rapp-Port vassliadis single component broth medium(R.V.S), tetra thionate broth medium(T.M) and Xylose lysine Deoxy Cholate medium(X.L.D) were preformed from the suspected tubes containing *Salmonella* to obtain pure culture(Cheesbreugh,2003). Then incubated aerobically at 37ºC for 24 hours.

Salmonella colonies were picked up from XLD plates with bacteriological loop smeared on a separate glass slide and fixed by gentle heating, and stained with Grams Method of staining and examined under microscope at 100 magnification using immersion oil (Cowan and Steels,2003).

Motility test was performed using hanging drope slide to confirm the Genus of salmonella (Merchant and Packer,1967). The motile bacteria with swinging movement were identified as *Salmonella.*

Standardization of Inoculums:

The inocula were prepared from the stock cultures, maintained on nutrient agar slant at 4ºC and subculture onto nutrient broth using a sterilized loop. The density of suspension inoculated onto the media (Muller –Hinton) for susceptibility test was determined by caparison with0.5 Mcfarand standard (Cheesbreugh, 2002).

Antimicrobial Assay:

Disc agar diffusion technique described by Bauer *et al;* (1966) and demonstrated by Cakir et al; (2004) was employed for antibacterial bioassay. For *Salmonella Spp*. Susceptibility testing, the extracts were incorporated into appropriate medium and incubated at37°C for 24 hours. After the incubation period was completed the inhibition zones formed on the media were measured to determine antibacterial effect of the different concentration of the extracts. The results were interpreted using the National Committee for Clinical Laboratory Standards (NCCLS) (Cheesbreugh, 2006).

Determination of Minimum Inhibitory Concentration (MIC)&Minimum bactericidal :

Minimum Inhibitory Concentration for isolated salmonella was carried out using tube dilution technique as described by Akinymei *et al*; (2005). Stock solutions of concentrations equal to 0.2, 0.4,2,4,6,8,10 g in 100ml of sterilized distilled water were serially diluted to obtain $10,10^2,10^3$ g/ml.

The inoculums of *Salmonella Sp*. Was spread uniformly in Muller Hinton agar plates and sterilized paper discs were dipped into different *Bauhinia rufescens* extracts, were placed in inoculated plates .The plates were incubated for 24hrs at 37C and size of clear zones developed surroundings in each disc was measured by scaling to nearest mm for determination of MIC and the lowest concentration that revealed no visible bacterial growth after sub- culturing was taken as MBC.

Results and discussion

The statistical analysis and comparison between the effect of concentrated stock solution of leaves extract of *B.rufescens* and its tenfold diluted on the growth of *Salmonella* isolated from chicken after 24hrs, showed that there was no significant difference between them at (P≤0 .05) as it's displayed in table (1) .This results could be attributed to the fact that the active ingredient in leaves extract of *B.rufescens* has a very high biological activity on *Salmonella*.

Table(1) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its tenfold diluted on the growth of *Salmonella* after 24hrs.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock & its 10fold diluted solution at 24 hrs	1.12857	1.29707	.49024	-.07101	2.32816	2.302	6	.061

The results on table. (2) Shows the effect of the basic concentrated stock solution from the leaves of *B.rufescens* and its hundred times diluted solution on *Salmonella* after 24 hrs of incubation, this results revealed that there was a significant different between the two concentrations at(P≤0 .05). The basic concentration is more effective than the diluted one and this is normal , dilution decreases the effect of extract and biological activity on bacteria.

Table(2) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10^2)fold diluted on the growth of *Salmonella* after 24hrs.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock & its 10^2fold diluted solution on the growth of *salmonella Spp*. at 24 hrs	1.25714	1.43162	.54110	-.06688	2.58117	2.323	6	.059

From the results in table (3) it's obvious that there is a noticeable difference between the effect of basic concentrated stock solution of leaves extract of *B.rufescens* and its thousand time diluted (10^3) solution incubate for 24hrs. From these results it could be deduced that acute dilution of *B.rufescens* leaves extract leads to negative results.

Table(3) comparison between the effect of concentrated stock solution of leaves extract of B. rufescens and its (10^3)fold diluted on the growth of *Salmonella* after 24hrs.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10^3fold diluted at 24 hrs	1.27143	1.46255	.55279	-.08120	2.62406	2.300	6	.061

Table (4) shows the comparison between the effect of the basic concentrated solution of *B.rufescens* leaves extract on *Salmonella* and its tenfold diluted solution after incubation for 48 hrs.Its clear that there is a significant difference between the two concentrations where it was found that the highest concentration was more effective. This study also proved that there is a correlation between the effect of the concentration and the time of incubation.

Table(4) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10)fold diluted on the growth of *Salmonella* after 48hrs.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10fold diluted at 48 hrs	-1.51429	1.60149	.60531	-2.99541	-.03316	-2.502	6	.046

There is significant difference (P≤0 .05). between the effect of the basic stock concentrated solution of *B.rufescens* leaves extract on *Salmonella* and its hundred fold diluted incubated for 48 hrs as shown on table (5) and this could be explained that increasing dilution decreases the amount of the active ingredient in the extract, hence its effect on *Salmonella*.

Table(5) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10^2)fold diluted on the growth of *Salmonella* after 48hrs.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10^2fold diluted at 48 hrs	1.15714	1.28304	.48494	-.02947	2.34376	2.386	6	.054

Table (6) shows the effect of 10^3 diluted solution of *B .rufescens* leaves extract on *Salmonella* and it's clear that it performed very weak effect.

Table(6) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10^3)fold diluted on the growth after *of Salmonella* 48hrs.

	Paired Differences					t	df	Sig. (2-tailed
	Mean	Std. Deviation	Std. Error Mean	Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10^3fold diluted at 48 hrs	1.24286	1.41758	.53579	-.06818	2.55390	2.320	6	.059

Results on tables (7,8,9) represent the significant differences between the effect of the basic stock solution and 10,100,1000 times diluted solution of *B .rufescens* leaves extract on *Salmonella* incubated for 72 hrs.

Table (7) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10)fold diluted on the growth *of Salmonella* after 72hrs

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10fold diluted at 72hrs	4.18571	4.37204	1.65248	.14225	8.22918	2.533	6	.044

Table(8) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10^2)fold diluted on the growth of *Salmonella* after 72hrs.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10^2fold diluted at 72 hrs	5.10000	5.35786	2.02508	.14481	10.05519	2.518	6	.045

Table(9) comparison between the effect of concentrated stock solution of leaves extract of *B. rufescens* and its (10^3)fold diluted on the growth of *Salmonella* after 72hrs

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Difference between the effect of the stock solution & its 10^3fold diluted at 72 hrs	5.45714	5.75322	2.17451	.13630	10.77798	2.510	6	.046

The values of inhibition zones of *Salmonella* growth affected by different concentrations of *B .rufescens* leaves extract. It could be seen that the greater the time of incubation the greater is the value of inhibition zones. These results is in agreement with Geetha and Padal(2014).In 2013, Amita *et al* found that *B. variegate* leaf exracts exhibited considerable antibacterial , antioxidant and anticancer activity.Emmanuel et al,2021 they found that *B .rufescens* extract of the whole plant have an antibacterial activity and used in traditional phytotherapuy. The values of MIC of different times of incubation (24hrs, 48hrs, and 72hrs) were recorded as follows: 0.1, 0.1,2 respectively.

Conclusion:

Bauhinia plant leaves extracts were used on *Salmonella spp*. to test growth inhibition of the bacteria .Minimum inhibitory concentration (MIC)and (MBC) for different times of incubation (24hrs,48hrs and 72hrs) were registered as 0.1,0.1,2 respectively .All of the different *Bauhinia* extract showed significances between them. So health professional should plan the strategies to use the extract for treatment with the aim to control the outbreak of typhoid fever in the community.

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