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## Research Article

# Antimicrobial and antioxidant activity of seed extracts of *Croton zambesicus* Muell. Arg.

**Manal A. Ibrahim, Amna M. Ali, Mona A. Maky, Nafesa N. Abas, Sulafa H. Hassan**
Department of Botany, Omdurman Islamic University, Faculty of Science and Technology, Khartoum, Sudan

***Corresponding author**
Manal A. Ibrahim
Email: manalabdalla071@gmail.com

**Abstract:** The seed extracts of *Croton zambesicus* Muell. Arg. were screened for antimicrobial activity against some typed and pure cultures of bacterial and fungal species. These were carried out by the cup plate agar diffusion method on melton nutrient agar for bacteria and sabouraud dextrose agar for the fungi. Ethyl acetate, ethanol and chloroform used for the extraction, four bacterial strains (*Bacillus susbtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) and two fungal species (*Aspergillus niger* and *Candida albicans*) were tested for antimicrobial activities of the extract. The antimicrobial activity was of the limited type. Ethyl acetate and ethanolic extracts represented positive results against the entire microorganism at a high concentration (100mg/ml).While chloroformic extract showed negative results. The antioxidant activity proved that the ethanolic extract was able to reduce the DPPH free radicals (89%).
**Keywords:** *Croton zambesicus* Muell. Arg.; antimicrobial activity; antioxidant activity and DPPH; bacterial strains and fungal species.

## INTRODUCTION

The plant *Croton zambesicus* Muell. Arg (Syn. Name: C. amabilis Muell. Arg.) (Family euphorbiaceae) It is a species of widely spread in tropical Africa. The root used for menstrual pain[1] and as aperients[2]. The root is also used in some regions of Nigeria as anti malarial and antidiabetic[3]. The leaf decoction is used in Benin as a wash for fevers, dysentery, convulsions, antihypertensive and as antimicrobial for urinary infections [4] and in parts of Nigeria as antidiabetic and malarial remedy[5,6]. The seed decoction is commonly used to treat cough, malaria and to relieve menstrual pain[7].

*C. zambesicus* which has wide application in African folkloric medicinal usage has some of the sugar attachments (aglycons) to be therapeutically effective on several microbes. This study therefore is aimed at investigating the in vitro effects of the plant extracts on some microbes with a view to getting a better natural therapeutic agent that could be a solution to cases of resistance by pathogens to some of the chemotherapeutic agents as has been reported by Shalit *et al*.,[8]. The roots of *C. zambesicus* showed activity against some microorganism[9]; however, there is insufficient information regarding the antimicrobial activities of the seeds . Hence, there is need to investigate the antimicrobial properties and antioxidant activity of seed extracts.

## MATERIALS AND METHODS

### Preparation of crude plant extracts

The plant material was purchased from local market, dried and pulverized 100g were extracted using solvents of increasing polarities (Chloroform, ethyl acetate and ethanol). The plant materials were socked and shaken overnight, then filtered and evaporated to dryness.

### Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organism were aseptically distributed into nutrient agar slopes and washed off with 100 ml sterile normal saline, to produce a suspension containing about $10^8$-$10^9$C.F .U/ml the suspension was stored in the refrigerator at 4c till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique[10]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette into the surface of dried nutrient agar plates, Then were allowed to stand for two hours at room temperature to dry and incubated at37Cº for 24 hours. After incubation, the number of developed

colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

**Preparation of fungal suspension**

The fungal were maintained on sabouraud dextrose agar, incubated at 25 $^{o}$C for 4 days. The fungal growth was washed with 100 ml sterile normal saline and then the suspensions were kept at 4 $^{o}$C until use.

**Assay for antimicrobial activity**

The cup-plat agar diffusion method[11] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension$10^{8}$ – $10^{9}$ C.F. U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar. 20ml aliquots of the incubated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (no.4) and agar discs were removed. The crude plant extracts were dissolved in ethanol and four concentrations were made. Then 0, 1 ml sample of each the concentrations was dropped into each wells; leaving one well to be filled with 0.1 ml ethanol as a control. Using automatic microliter pipette. And allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 C0 for 18 hours. After incubation the diameter of the resultant growth inhibition zones were measured.

The same method as for bacteria was used for fungi by using sabouraud dextrose agar instead of molten nutrient agar. The inoculated medium was incubated at 25C0 for two days for the *C. albicans* and three days for *A. niger*.

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of Shimada *et al*.,[12] with some modification. In 96-wells plant, the test sample were allowed to react with 2.2di (4-tert-octy1pheny1)-1-picry1-hydrazy1 stable free radical (DPPH) for half an hour at 37C0. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

**RESULTS AND DISCUSSION**

**Anti microbial activity**

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm their activity. Many efforts had been carried out to discover new antibacterial compounds from various kinds of plants[13]. The antibacterial effects exhibited by three solvent extracts varied with the bacterial strains used (Table1) .The ethyl acetate and ethanolic extracts reflected inhibitory effects against all bacterial strains at a concentration of 100mg/ml ,in addition to that *S. aureus* showed inhibitory effects at a concentrations of 50,25 and 12.5 mg/ml with ethyl acetate extract. The results clearly indicated that the seeds extracts had a limited antibacterial effect .The weak inhibitory effects may be attributed to low concentrations used. The antifungal activity exhibited by three solvent extracts (Table 2). Ethanol and ethyl acetate extracts showed weak inhibitory effects against the two fugal strains *A. niger* and *C. albicans*, while chloroform extracts reflected a negative results.

**Table (1): Mean inhibition zone diameter (mm) caused by the four concentrations (100, 50, 12.5, 6.25 mg/ml) of different extracts against the tested organisms.**

| Organism | Used extract | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ethanol | | | | Ethyl acetate | | | | Chloroform | | | |
| | Cons.mg/ml | | | | | | | | | | | |
| | 100 | 50 | 12.5 | 6.25 | 100 | 50 | 12.5 | 6.25 | 100 | 50 | 12.5 | 6.25 |
| *E. coli* | 12 | - | - | - | 11 | - | - | - | - | - | - | - |
| *P. arginoa* | 12 | - | - | - | 12 | - | - | - | - | - | - | - |
| *S. aureus* | 13 | - | - | - | 15 | 13 | 12 | 11 | - | - | - | - |
| *B. subtilis* | 11 | - | - | - | 15 | 11 | - | - | - | - | - | - |
| *C. albicans* | 12 | - | - | - | 13 | - | - | - | - | - | - | - |
| *A. niger* | 12 | - | - | - | 13 | - | - | - | - | - | - | - |

*S. aureus = Staphylococcus aureus*; *P. arginoa = Pseudomonas aeruginosa*; *B. subtilis =Bacillus subtilis*; *E. coli*; *Escherichia coli*; - = no inhibition

**Anti oxidant activity**

D PPH stable free radical method is an easy, rapid and sensitive way survey to the anti oxidant activity of specific compound or plant extracts[14]. Propyl galate was used as standard compound. The highest radical scavenging activity was showed by

ethanolic extract (89%) whereas chloroform and ethyl acetate extracts were showed a weak presence with a value of 16% and 29% respectively. The highest RSA resulted in ethanolic extract may be referred to the effect of the extractability was supported by Harborne [15] who had shown that more plant metabolites are isolated from plant materials when ethanol or water were used for extraction.

**Table 2: DPPH free radical scavenging activity EC50 values (µg/ml) of different extracts of plant**

| Extract used | EC50(µg/ml) | SD+ |
|---|---|---|
| Chloroform | 16 | .01 |
| Ethyl actate | 29 | .09 |
| Ethanol | 89 | .01 |
| Propyl galate | 83 | .01 |

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## CONCLUSION

In conclusion, ethyl acetate and ethanolic extracts represented positive results against the entire microorganism at a high concentration. While chloroformic extract showed negative results. Ethanolic extract had high antioxidant efficacy but other tow extracts showed weak results. Further studies of the biological and chemical properties of the active ingredients of the plant extracts may yield significant results.

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