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# Antimicrobial activity of *Croton zambesicus, Acacia ehrenbergiana,* and *Fagonia cretica*

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**Abstract**
A total of six plant extracts were screened for antimicrobial activity against standard bacterial and fungal species. These were carried out by the cup plate agar diffusion on Mueller-Hinton agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. The Minimum Inhibitory Concentrations (MICs) of the most active extracts were determined by agar plate dilution method. Six extracts exhibited inhibitory activity against one or more of the six tested bacteria. Two extracts were inhibitory to the six tested organisms. Methanol extract of *Acacia ehrenbergiana* had a wide spectrum activity against all the bacteria tested with promising minimum inhibitory concentrations. However, the extracts were inactive against the fungal species tested.

**Keywords:** Traditional medicine, Antimicrobial activity, agar diffusion, Croton zambesicus Acacia ehrenbergiana, Fagonia cretica

## 1. Introduction
Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses [1]. It is widely used and of rapidly growing health system and economic importance [2]. World Health Organization (WHO) has estimated that perhaps 80% of more than 4000 million inhabitants of the world rely chiefly on traditional medicines for their primary health care needs [3]. In Africa, up to 80% of the population uses traditional medicine (TM) to meet their health care needs. In Asia and Latin America, populations continue to use TM as a result of historical circumstances and cultural beliefs. In China, TM accounts for around 40% of all health care delivered [2]. Meanwhile, in many developed countries, complementary & alternative medicine (CAM) is becoming more popular. The global WHO Traditional Medicine Strategy indicates that rapidly increasing use of herbal medicines throughout the world has made regulation of herbal medicines an urgent issue [2]. Eighty five countries including Sudan reported that they have registration systems for herbal medicines [4].

Research on medicinal plants has attracted a lot of attention globally and there has been an explosion of interest regarding plants and their medicinal value [5, 6]. Herbal treatments are the most popular form of traditional medicine, highly lucrative in the international marketplace and have often maintained popularity for historical and cultural reasons [7]. The (WHO) estimated that herbs are medicines for about two thirds of the initial world's population or about 4 billion people [2]. *Croton zambesicus* Muell (Euphorbiaceae**)** is distributed in Sudan, Guinea Savanna, Cameroon and tropical Africa. Leaves contain trachylobane, diterpenoid, crotonadiol, crotozambefurans A, B and C, betulinol, lupeol and sitosterol. The essential oil contains monoterpenoids, spathulenol and linalool. The leaf decoction is used as antimicrobial, antihypertensive and ant malarial [8]. *Acacia ehrenbergiana* Hayne (Mimosaceae) is distributed in north and east Africa and contains Gallic acid, methyl gallate, rutin, myricetin, quercetin, myricetin 3- *O* -(3 ''' - *O* -galloyl)-β-D-rutinoside and catechin were isolated [9]. It is used in medicine as emollient (10). *Fagonia cretica* (Zygophyllaceae) is widely spread and contains sapogenins and oleanolic acid, alkaloids (harman and harmine), triterpenoides, sterols, alcohols (ceryl alcohols and n-tricontanol, flavonol, glycosides and fatty acids have been isolated. The fumigant of the whole plant is used against muscular pain [11].

In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Antibiotics are sometimes associated with adverse effects on the host like hypersensitivity. Therefore; there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from other sources, such as plants. Natural products of higher plants may be a new source of antimicrobial agents possibly with novel mechanisms of action [12]. Therefore this study will be carried out to investigate the antimicrobial activity of *Croton zambesicus*, *Acacia ehrenbergiana*, and *Fagonia cretica* and to provide scientific evidence for their use.

## 2. Materials and methods
### 2.1Plant material
The plants used in this study were collected from different parts of Sudan by Herbalists from June 2012 to August 2012. *Acaia ehrenbergiana* collected from Marawi (Northern Sudan), *Fagonia cretica* from Dongola (Northern Sudan) and *Croton zambesicus* purchased from local markets (Khartoum). They were authenticated in Medicinal and Aromatic plants and Traditional Medicine Research Institute (MAPTMRI).

Voucher specimens were deposited at the herbarium of the institute.

**2.2 Preparation of Crude Extracts:**

Each of the coarsely powdered plant material was exhaustively extracted for 20 hours with chloroform in Soxhlet apparatus. The chloroform extract was filtered and evaporated under reduced pressure using Rota-vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The methanol extract was filtered and evaporated under reduced pressure using Rota-vap. Each residue was weighed and the yield percentage was determined. The chloroform residue (2 g) was dissolved or suspended in a mixture containing methanol: petroleum ether (2:1) to a final volume 20ml (con. 100 mg/ml). The methanol residue (2g) was dissolved in methanol 20 ml (con. 100mg/ml), and kept in refrigerator until used.

**2.3Test microorganisms:**

The extracts were tested against two Gram positive bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Four Gram negtaive organsims, *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae (*ATCC 53657), *Proteus mirabilis* **(**ATCC 14153), *Pseudomonas aeruginosa* (ATCC 27853 ) and two standerd fungi, *Aperugillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596). The tested organsims were obtained from the Department of Microbiology, MAP TMRI and National Health Labroatory, Khartoum, Sudan.

One hundred clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*,, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *and Staphylococcus aureus* were collected during the year 2012 from Khartoum Educational Hospital, Khartoum North Educational Hospital, Military and Educational Hospital and Alribat National Hospital.

**2.4 Preparation of the test organisms:**

**2.4.1 Preparation of bacterial suspensions:**

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37ºC for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 MC farland units [13].

**2.4.2 Preparation of fungal suspensions**

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25º C for 4 days. The fungal growth was harvested and washed off with 100 ml sterile normal saline, and the suspension was stored in the refrigerator at 4° C till used.

**2.5 *In vitro* testing of extracts for antimicrobial activity**

**2.5.1Testing for antibacterial Activity**

The cup-plate agar diffusion method [14]. Was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 150-$10^6$ C.F.U/ ml were thoroughly mixed with 100 ml of Muller Hinton agar which was maintained at 45 ºC. 20ml aliquots of the inoculated Muller Hinton 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) were cut using a sterile cork borer (No. 4) and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each extracts using automatic microtitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

**2.5.2 Testing for antifungal Activity:**

The same method as for bacteria was adopted. Instead of Muller Hinton agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 ºC for two days for *Candida albicans* and three days for Aspergillus niger.

**2.6 Identification of clinical isolates**

After overnight incubation, the obtained isolates colonies were tested morphologically, and then examined microscopically using Gram staining technique, Gram positive cocci were purified by subculture on manitol salt agar. The purified isolates were identified by microscopical examination, cultural characters and biochemical tests.

**2.7 Determination of minimum inhibitory concentration (MIC)**

The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 6 segments. The organisms tested were grown in broth over night to contain $10^8$ CFU/ml [15].

**3. Results and Discussion**

The chloroform and methanol extracts of the three Sudanese plants (*Croton zambesicus*, *Acacia ehrenbergia*, *Fagonia cretica*). were tested for their antimicrobial activity against two Gram positive standard bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), four Gram negative standard bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa*) and two standard fungi (*Aspergillus niger, Candida albicans*).The antimicrobial activity of the most active plant extract was compared with that of the commercial antibiotics as amoxicillin, gentamicin, and tetracycline. In the preliminary screening, the total number of extracts examined against the six tested bacteria was six. They exhibited inhibitory activity against one or more of the six tested bacteria; two were inhibitory to the six tested organisms. The number of extracts exhibiting inhibitory effects against two bacteria amounted to three. One extract was active against three bacteria. *Escherichia coli* was the most susceptible organism; being inhibited by all extracts (100 %), followed by *Bacillus subtilis*; being inhibited by 5 extracts (83.3%), while *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed the lowest susceptibility; being inhibited by two extracts (33.3 %).Three extracts (50%) exhibited inhibitory effects against *Klebsiella pneumoniae*, whereas four extracts (66.6%) showed inhibitory effects against *Proteus mirabilis*. Two extracts (33.3%) exhibited inhibitory effects against both Gram positive organisms (*Bacillus subtilis* and

*Staphylococcus aureus*), and Gram negative organisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*). All chloroform extracts exhibited inhibitory effects against one or more of the tested micro bacteria. Two extracts (66.7%) exhibited inhibitory effects against two of the tested bacteria. All methanol extracts exhibited inhibitory effects against one or more of the tested bacteria. Two extracts (66.6%) exhibited effects against six tested bacteria. One extract (33.3) was active against two tested bacteria. The chloroform extracts showed an intermediate level of inhibition against four of the tested bacteria, one extract (33.3%) was active against three of the tested organisms. None of the extracts was active against standard fungi (Table 1).

The minimum inhibitory concentration of the most active extract (methanol extract of *Acacia ehrenbergiana*) was determined against the standard bacterial organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas aeruginosa*). *Bacillus subtilis* and *Staphylococcus aureus* were the most susceptible organisms; Gram positive bacteria were more susceptible than Gram negative bacteria. The results were summarized in (Table 2) as mg/ml of crude extract.

The plant extract inhibited *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* higher than 40 µg /ml of amoxicillin, gentamicin and tetracycline and inhibited *Staphylococcus aureus* higher than 40 µg /ml of amoxicillin and gentamicin but less than the same concentration of tetracycline. It inhibited *Klebsiella pneumoniae* lower than 40 µg /ml of amoxicillin but higher than 40 µg /ml of gentamicin and 10 µg tetracycline, and inhibited *Proteus mirabilis* similarly to 40 µg /ml of tetracycline, higher than 40 µg /ml of amoxicillin and 5 µg /ml of gentamicin (Fig.1).

The methanol extract of *Acacia ehrenbergiana* was subjected to test against the clinical isolates of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris and Pseudomonas aeruginosa*. It was active against the 100 clinical isolates tested. The extract showed high activity against 16 of *P.aeruginosa*, 7 isolates of *S.aureus*, 5 of *E. coli*, 3 of *K. pneumoniae*, 2 of *P. mirabilis* and one of *P. vulgaris.* Moderate activity was shown against the rest of organisms (Photo.1, Fig. 2).

**Table 1:** Preliminary screening for antibacterial and antifungal activity of three Sudanese medicinal plant

| Family/Botanical name/Synonyms/ Vernacular name | Part used (extracted) | Solvent system | Yield % | Test organism used*/MDIZ mm** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ***B.s.*** | ***S.a*** | ***E.c.*** | ***K.p.*** | ***Pr.m.*** | ***Ps.a.*** |
| Euphorbiaceae<br>Croton zambesicus Muell. Arg.<br>Croton gratissmus Broun &Massey<br>Vern. Um gilaiglah | Fruits | $CHCL_3$<br>MeOH | 8.41<br>15.75 | -<br>28 | -<br>15 | 15<br>19 | 20<br>18 | 16<br>20 | -<br>23 |
| Memosaceae<br>Acacia ehrenbergiana<br>Acacia flava (Forssk.) Schweinf<br>Vern. Salam | Stem bark | $CHCL_3$<br>MeOH | 0.51<br>7.99 | 18<br>30 | -<br>25 | -<br>21 | -<br>20 | 14<br>18 | -<br>25 |
| Zygophyllaceae Fagonia creticaL.<br>Vern.Shok Elgomal, Um shwaika | Whole plant | $CHCL_3$<br>MeOH | 2.31<br>11.54 | 20<br>23 | -<br>- | 15<br>14 | -<br>- | -<br>- | -<br>- |

**Key**

**B.s. =Bacillus subtilis*, *S.a. =Staphylococcus aureus*, *E.c.=Escherichia coli*, *K.p.= Klebsiella pneumoniae*, *Pr.m.=Proteus mirabilis*, *Ps.a.= Pseudomonas aeruginosa*, *A.n.= Aspergillus niger, C.alb= Candida albicans*.

** M.D.I.Z. =Mean diameter of growth inhibition zones in mm. Average of two replicates (-): no inhibition. Cup diameter=10mm.Concentration used =200 mg/ml at 0.1 ml/ cup.

**Interpretation of results**

Active ≡ M.D.I.Z.>18 mm, moderately active ≡M.D.I.Z. =14-18 mm, Inactive ≡ M.D.I.Z. <14 mm

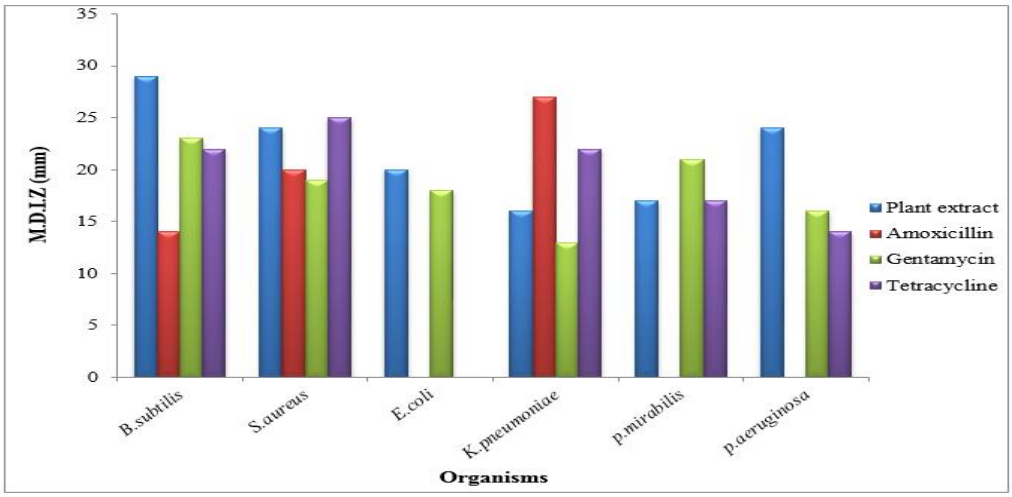


**Fig 1:** The antimicrobial activities of *Acaia ehrenbergiana* methanol extract (conc.: 200 mg/ml) and reference drugs (conc.: 40 µg /ml)

**Table 2:** Minimum inhibitory concentrations (MICs) of crude extract of *Acacia ehrenbergiana* against the standard organism

| Standard microorganisms | MIC of methanol extract |
|---|---|
| Bacillus subtilis | 3.125mg/ml |
| Staphylococcus aureus | 3.125mg/ml |
| Escherichia coli | 50 mg/ml |
| Klebsiella pneumoniae | 12.5 mg/ml |
| Proteus mirabilis | 12.5 mg/ml |
| Pseudomonas aeruginosa | 25 mg/ml |

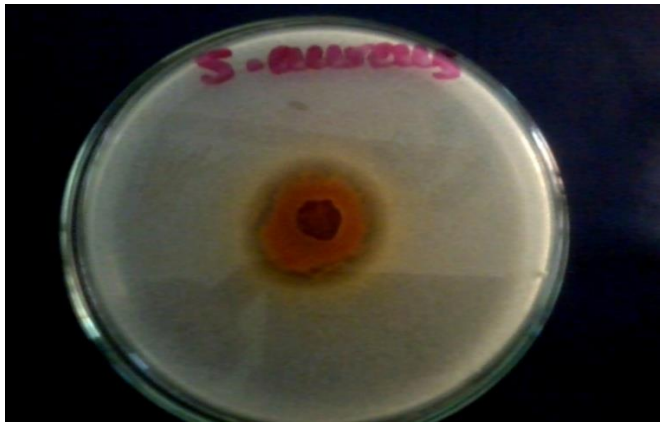


**Photo 1:** Antibacterial activity of methanol extract of Acacia ehrenbergiana stem bark against Staphylococcus aureus clinical isolate

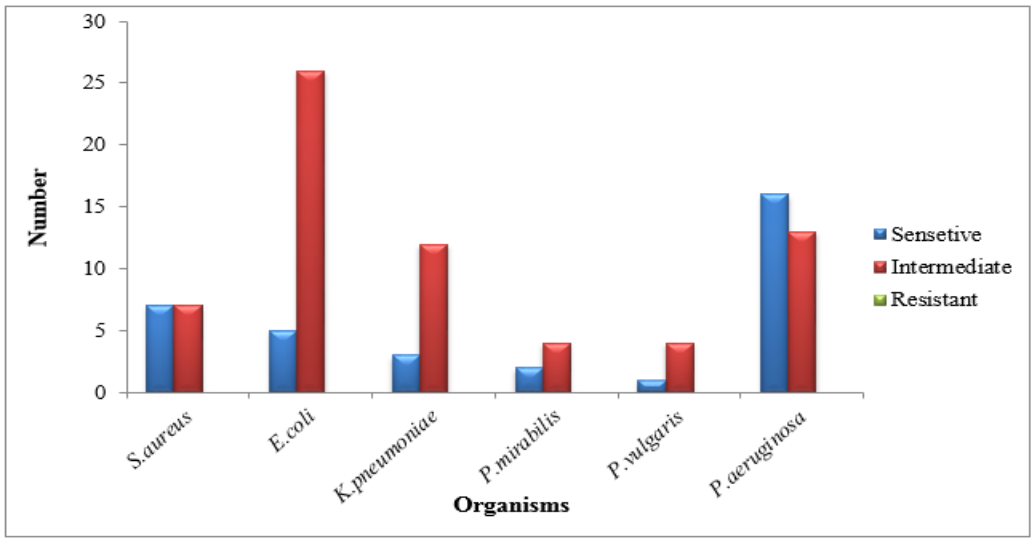


**Fig. 2:** Activity of *Acacia ehrenbergiana* stem bark against the clinical isolates

The extracts used showed antimicrobial activity against at least one of the tested standard organisms. Methanol extracts were found to be more active compared to the chloroform and aqueous extracts and this could be due to presence of polar secondary metabolites. This was in agreement with the findings of Kelmanson *et al.* when they used different methods of extraction and found that the methanol extract was the most active extract [16]. The percentage of inactive extracts was higher in the case of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (77.8%), *Klebsiella pneumoniae* (66.7%) and *Proteus mirabilis* (55.6%) when compared to those inactive against *Bacillus subtilis* (11.1%) and *Escherichia coli* (0%). Therefore the Gram negative organisms were more susceptible to extracts of *Croton zambesicus* than the Gram positive organisms and the two fungi tested. Abo *et al* [17]. screened the extract of the stem bark of *Croton zambesicus* which exhibited wide spectrum antibacterial effects comparable to those of Ampicillin and gentamicin. The methanol extract of stem bark of *Croton zambesicus* was screened by Reuben *et al.* [18]. for its antibacterial activity. The screening revealed the extracts to have broad spectrum activity on Gram positive, Gram negative organisms and fungal strain respectively. The highest activity was shown in *Staphylococcus aureus, Escherichia coli.* These results were similar to the results of current study, but they differed when the methanol extract of stem bark of *Croton zambesicus* showed antifungal activity against *Aspergillus Niger* and *Candida albicans*, this might be due to using different plant part from different habitat. Ajayi, Akintola [19] screened the antibacterial activity of the leave extract of *Croton zambesicus in vitro* against *Escherichia coli* and *Salmonella typhimurium* using the agar disc diffusion method. Extract exhibited high activity against *S. typhimurium* and *E. coli*. Okokon, Nwafor screened the root extract and fractions

of *Croton zambesicus* for their antimicrobial activity against some typed and pure cultures of bacterial and fungal species. The crude extract as well as chloroform and n-hexane fractions had activity against *Bacillus subtilis* only. While ethyl acetate fraction had a wide spectrum activity against all the bacterial organisms tested with promising minimum inhibitory concentrations. The result obtained in this study against *Bacillus subtilis* was contrary to those of Okokon, Nwafor [20]. and that might be due to using of different plant part and different solvents but there is an agreement about the fungal activity. The methanol extract of *A. ehrenbergiana* is more active than its corresponding chloroform and aqueous extracts and the Gram positive organisms are more active than the Gram negative organisms and the two fungi tested. Same result of methanol extract in current study was obtained when Gaara *et al*. evaluated the activity of some isolated compounds of *A. ehrenbergiana*. Potent antimicrobial activity was observed against tested Gram positive bacteria including *B.subtilis* and *S. aureus*. Gram negative organisms revealed weaker susceptibility than Gram positive for most tested compounds. Weak response was exhibited by *C.albicans* and this contrary to the present study; this due to that Gaara *et al* [9]. regarded diameter of inhibition zone (11 mm) was slightly active while in the present study this diameter was considered inactive. Ghulam *etal.* [21]. investigated the antibacterial activity of the methanol and n-hexane extracts of *F. cretica.* The study reported that the methanol extract of *F. cretica* showed highest antibacterial activity against *S. aureus*. Meanwhile, *F. cretica* showed no antibacterial activity against *S. aureus* in the present study. This low activity might be due to the negligible amount of active principles that were present in the plant. Amadi *et al.,* [22] reported that the bio efficacy of plant extracts is affected by the extraction method and concentration. Therefore the Gram positive bacterium (*B.subtilis*) is more active than the Gram negative bacterium (*E.coli*) and against the rest of organisms tested.

## 4. Conclusions

The methanol of *A. ehrenbergiana* was found to be active than that of *C. zambesicus* and *F. cretica*. The results obtained from the study showed that the methanl extract of *A. ehrenbergiana* inhibited the growth of all microorganisms to varying degrees and this implies that the stem bark of *A. ehrenbergiana* possess some active phytochemicals that can inhibit the growth of microorganisms.

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