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Antibacterial, anti-inflammatory and antioxidant activities of anthraquinones from *Ceratotheca triloba* (Bernh) Hook F

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9, 10-Anthracenedione and 1-hydroxy-4-methylanthraquinone showed antibacterial activity against *Staphylococcus aureus, Micrococcus luteus, Bacillus cereus* and *Escherichia coli*. Due to the synergistic effect of the individual compounds, the crude extract from leaves and roots of *Ceratotheca triloba* exhibited good potency (>500) against *S. aureus* and *M. luteus,* medium potency against *E. coli* and *S. typhimurium* (<100) and very low potency against *B. cereus* (<10). Although a similar trend was observed for 9, 10 anthracenedione and 1-hydroxy -4-methyl anthraquinone unlike the crude extract. A very low potency against *S. aureus* was observed for 9, 10 anthracenedione and a high potency for 1-hydroxy-4-methylanthraquinone. Thus 9, 10 anthracenedione is an effective drug against *E. coli* and *S. typhimurium* and 1-hydroxy-4-methylanthraquinone is effective against *S. aureus* and *M. luteus*. The crude root extract and 9, 10 anthacenedione, 1-hydroxy-4-methylanthraquinone and 5, 8-dimethoxy-2, 3, 10, 10a-tetrahydro-1H-phenanthrene-4, 9-dione showed a ± 50% reduction of the free radicals. No anti-inflammatory activity was observed. The purified extracts showed moderate toxicity against $HepG_2$ cells at high concentrations and no toxicity was observed against brine shrimp larvae. No mutagenicity was observed with the crude extracts using the Ames test. All purified and crude extracts showed potent inhibition of the human topoisomerase II enzyme.

Key words: Anthraquinone, 9, 10 anthracenedione, 1 hydroxy -4 methylanthraquinone, antioxidant activity.

INTRODUCTION

Despite the extensive traditional use of *C. triloba*, scientific data confirming its biological activity is lacking. In our previous research (Mohanlall et al., 2011) six secondary metabolites were isolated from *C. triloba* roots. Structures were confirmed with EI-LCMS. as; (i) 9, 10 anthracenedione; (ii)1-hydroxy-4-methylanthraquinone; (iii) 5,8-dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione; (iv) 1,2 benzenedicarboxylic acid, mono(2-ethylhexyl)ester; (v) Octadecanoic acid and (vi) androst-5-ene-3,17,19-triol.

Anthraquinones are widely applied in medicine, food and the dye industry. In the pharmaceutical industry, the natural and synthetic derivatives of 9, 10 anthraquinone are beneficial to mammals and humans as they can display antibacterial, antitrypanosomal and antineoplastic activities (Heyman et al., 2009; Tarus et al., 2002; Velez-Cruz and Osheroff, 2004). 9, 10 anthracenedione derivatives are known to exhibit a quite potent anticancer

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activity. It has also been reported that these compounds can be effectively employed in both antibacterial and antitrypanosomal therapy. Anthracenedione-based drugs are characterized by prominent anticancer properties and are widely used in clinical practice (Hortobagyi, 1997; Thomas and Archimbaud, 1997; Wiseman and Spencer, 1997; Arcamone, 1998). Their mechanism of action has been principally correlated to their ability to stimulate DNA cleavage mediated by the enzyme topoisomerase II (Malonne and Atassi, 1997). Nonetheless, recent studies have shown that the telomeric G-quadruplex structure, which interferes with the function of telomerase, can be efficiently targeted by anthraquinones (Perry et al., 1998, 1999). In addition, it is well known that the redox cycling of the anthracenedione ring system may generate reactive radical species that are believed to be responsible for a number of toxic effects, including cardiac toxicity (Frishman et al., 1997; Giantris et al., 1998). 1-hydroxy-4-methylanthraquinone is an excellent source of synthetic dyes for the textile industry. These compounds have long been known to be useful as dyes (Figure 1) but, in recent years, many of these compounds have found utility as dyes for synthetic fibers (French Pat. No. 2,002,124 and U.S. Pat. No. 2,533,178).

Excellent dyeing properties for synthetic fibers such as washing fastness and durable press have been discovered in new compounds of the generic structural formula (Figure 1) in which A represents hydrogen, chlorine, bromine or lower alkyl; R represents alkylene of from 2 to 4 carbon atoms, vinylene, 1,2-benzene, 1,2-cyclohexane, 1,8-napthalene or 1,2-cyclohex-4-ene, all of which may be substituted by chlorine, bromine or lower alkyl; R' is lower alkyl; and Y represents hydrogen, hydroxy, chlorine, bromine, lower alkyl or lower alkoxy. These compounds have substantially better sublimation fastness than the known, related compounds disclosed in French Pat. No. 2,002,124. 5,8-dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione belong to a group of compounds that modulate the activity of Janus kinases and are useful in the treatment of diseases related to activity of Janus kinases including, for example, immune-related diseases and cancer (Lamb et al., 1998). As there no reports on the biological and safety effects of *C. triloba*, this research deals with antimicrobial, anti-fungal, anti-oxidant and anti-inflammatory activities of the hexane crude extracts and purified compounds; 9.10 anthracenedione and 1 hydroxy-4-methyl anthraquinone and determination of their safety in *in-vitro* experiments.

MATERIALS AND METHODS

Preparation of Plant Material

C. triloba (Bernh.) E. Mey. ex. Hook.f. was collected in Durban, Kwazulu Natal, South Africa, and identified by using available floral keys. A voucher specimen (Baijnath sn.) was deposited in the Ward Herbarium, University of Kwazulu-Natal (Westville Campus). The plant portals were carefully examined and old, insect-damaged, fungus-infected roots were removed. Healthy roots, stems, leaves, flowers and seed pods were spread out and dried in the laboratory at room temperature for 5 to 8 days or until they broke easily by hand. Once completely dry, plant material was ground to a fine powder using a Wareing blender. Larger quantities were crushed to a fine powder of 1.0 mm diameter using a Retsch Mühle mill at the Department of Biotechnology and Food Technology (Durban University of Technology). Material was stored in a closed container at room temperature until required.

Determination of antimicrobial activity

The antimicrobial activity of methanolic and aqueous plant extracts were carried out on selected bacteria and fungi by evaluating the bactericidal and antifungal effect and the minimum inhibitory concentration on selected bacteria and fungi in a petri dish using the agar disk diffusion method. A standard set of cultures are prescribed in these tests by the National Committee for Clinical Laboratory Standards, USA (NCCLS, 1990).

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that kills all the organisms and is usually determined by dilution methods. It is the antimicrobial susceptibility testing method against which other methods, such as disk diffusion are calibrated. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates or in test tubes or microplate wells of broth containing dilutions of the microbial agent.

In determining the MIC values growth indicators are used and not turbidity because plant extracts are frequently turbid or causes precipitates when mixed with microbial growth media. In this project p-iodonitrotetrazolium violet was used (Masoko et al, 2005).

The p-iodonitrotetrazolium violet (INT) reaction is based on the transfer of electrons from NADH, a product of the threonine dehydrogenase (TDH) catalyzed reaction, to the tetrazolium dye (p-iodonitrotetrazolium violet). Threonine dehydrogenase (TDH) from bacteria/fungi catalyses the NAD-dependent oxidation of threonine to form 2- amino-3-ketobutyrate and NADH. During the active growth of bacteria/fungi, an electron is transferred from NADH to p-iodonitrotetrazolium violet resulting in a formazan dye, which is purple in colour (Figure 2). Therefore, the clear zone(s) on the microplate wells indicate areas of inhibition (zones where no active growth of bacteria has taken place).

Antimicrobial activity assay

The ten bacteria used as test organisms were as follows: *Bacillus cereus* (DBT*_F), *B. stearothermophilus* (DBT*_Q), *Escherichia coli* (DBT*_L), *Klebsiella oxytoca* (DBT*_AM), *Micrococcus* sp. (DBT*_AR), *Pseudomonas aeruginosa* (DBT*_D), *Proteus mirabilis* (DBT*_O), *Salmonella typhimurium* (DBT*_AF), *Staphylococcus aureus* (DBT*_E) and *S. epidermis* (DBT*_ S).

* DBT is a reference for the Durban University of Technology Culture Collection which is based at the Department of Biotechnology and Food Technology.

The cultures were verified by their Gram reactions and antibiotic sensitivity patterns. Stock cultures were prepared from the Culture Collection and stored in micro bank vials using 50% glycerol. When required the cultures were plated out on Tryptone Soya Agar (Biolab) plates and were subsequently grown in Tryptone Soya Broth (Biolab) for 24 h at 37°C. The absorbance of bacterial cells

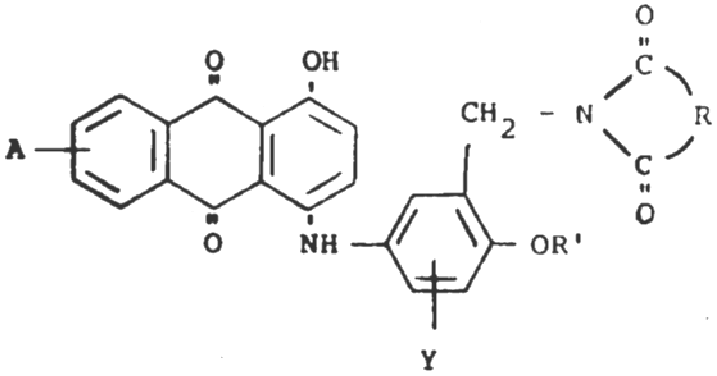

Figure 1. Generic structural formula for anthraquinone compounds with excellent dyeing properties.

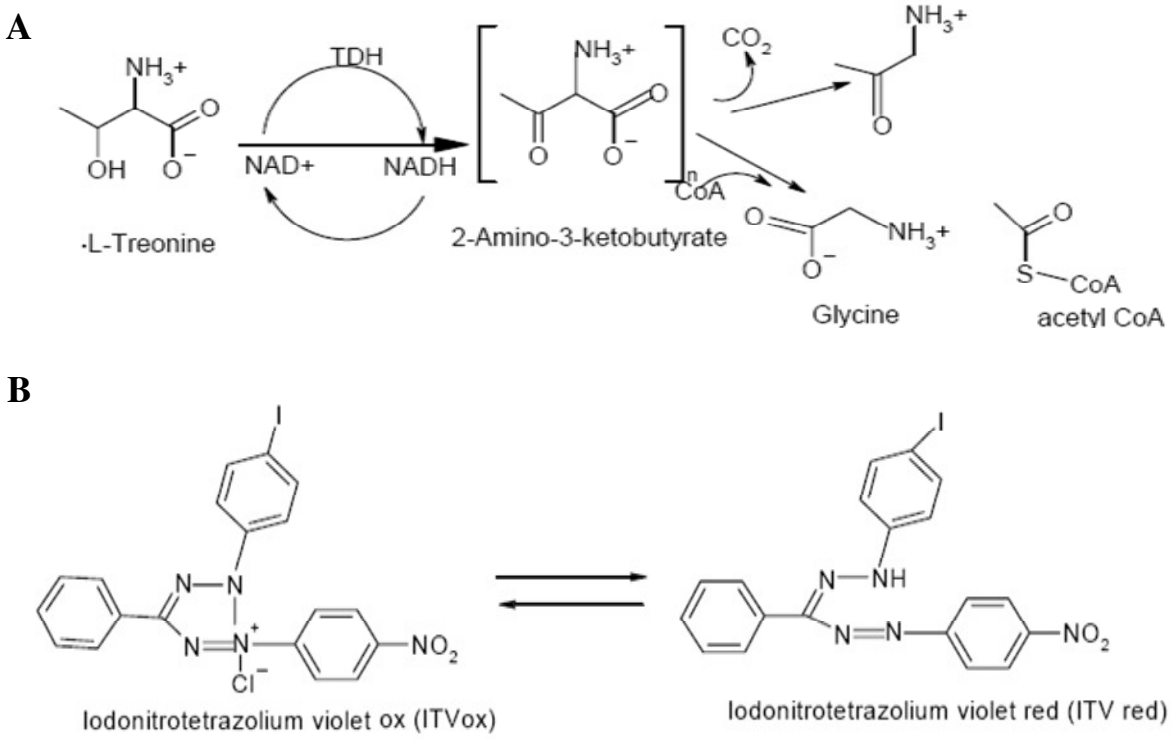

Figure 2. (A) Reaction pathways for the assay of threonine dehydrogenase, (B) INT, coupling reagent for the colorimetric assay.

was adjusted to MacFarland Standard of 0.5 which corresponded to 10^8 CFU/ml.

Molten (45°C) sterile tryptone agar (10 ml) in a flask was inoculated with a 0.1 ml of 10^8 cfu/ml of each of the respective bacterial strains. This was poured over the base plates containing 10 ml tryptone agar in sterile 9 cm Petri dishes. Fifty microliters of plant extracts at different concentrations were pipetted on 5 mm sterile filter paper disks (Whatman No. 1); and air dried in a biological safety cabinet laminar. Sample containing discs were placed on the surface of the inoculated bacterial plates and incubated at 37°C for 24 h. Dimethyl sulphoxide was used as the negative control and gentamycin was used a poitive control for the Gram Negative organisms and ampicillin was used a positive control for the Gram positive organism. All tests were carried out in triplicate.

Minimum inhibitory concentration (MIC) was determined by serial dilution of extracts beyond the level where no inhibition of growth of test organisms was observed (Eloff, 1998). This was performed in microplates by filling all wells, with 50 µL sterile Mueller Hilton broth. In row A, 50 µL (100%) of the extract was placed with a micropipette. From row A, 50 µL was transferred to row B after taking up and releasing three times to ensure adequate mixing. The

process was repeated until all the rows were completed and the additional 50 µL from row H was discarded. Two wells were used as a sterility and growth control respectively with the sterility control containing only Oxoid® Mueller Hilton broth (MHB), whilst the growth control containing both MHB as well as test organism.

After adding 50 µL of the bacterial suspension to each row (except for the sterility control), the microplate was sealed and incubated at 37°C at 100% relative humidity overnight. The following morning 50 µL of a 0.2 mg/ml solution of INT (p-iodo-nitrotetrazolium violet) was added to each row and the plate was returned to the incubator for at least half an hour to ensure adequate colour development. P-INT is a dehydrogenase activity detecting reagent, which is converted into corresponding intensely coloured formazan by metabolically active microorganisms (Navarro, 1998).

Inhibition of growth was indicated by a clear solution or a definite decrease in colour reaction. This value was taken as the minimum inhibitory concentration (MIC) of the extract. Extracts used for MIC determination were either dissolved in acetone or solubilized in DMSO (100 to 200 µl/mg) and made up as a stock solution (200 µg/ml) with distilled water. Well A had typically a final concentration of 100 µg/ml. Positive controls for test organisms were usually made up to a concentration of 1000 µg/ml and are listed in section.

Determination of total activity or potency

The mathematical model used to determine the total activity is expressed as:

$$\text{Total activity (ml)} = \frac{\text{Amount extracted from 1g of plant material (mg)}}{\text{MIC (mg/ml)}}$$

Determination of antifungal activity

For the antifungal activity two strains of fungi *Aspergillus flavus* (DBT*_AR) and *Fusarium verticilloides* (DBT*_FM) was utilized. These were previously isolated from corn samples and stored in the Durban University of Technologies culture collection. These were prepared by growing them on Sabourand Dextrose Agar at 28°C for 4 to 7 days until they sporulated. The spores were collected in 10 ml sterile distilled water, counted in a counting chamber (Neubauer) and the concentration adjusted to 10^6 spores/ml. Sterile distilled water containing the fungal spores (10^6 spores/ml) were poured over the Sabourand Dextrose Agar (SDA) base plates (Biolab). 50 µl of leaf and root extract was transferred onto each of three sterile 5 mm discs (Whatman No. 1). 50 µl of DMSO served as the negative control, and 5 µg/ml Amphotericin B (Fluka, Biochemika), was used as a positive control. Each plant extract and control was tested in triplicate. The plant extracts and ethanol impregnated discs were dried in sterile Petri dishes and incubated at 30°C. Antifungal activities were recorded as the width (mm). The minimum inhibitory concentration (MIC) was taken as the lowest concentration that inhibited growth.

Bioautography (Begue and Kline, 1972) was also used to verify above results. Developed chromatography plates (5 µl of 10 mg/ml = 50 µg) of extracts and fractions were dried overnight and sprayed with a suspension of growing cells of the bacteria listed above and incubated at 37°C in a chamber at 100% relative humidity for 18 h. After spraying with tetrazolium violet, clear zones on the chromatogram indicated inhibition of growth after incubating for 1 h at 37°C.

Determination of antioxidant activity

The antioxidative properties of the partially purified extracts (CTREH 01, CTREH 02 and CTREh 03) from *C. triloba* roots were tested using the DPPH (1.1-diphenyl-2-picrylhydrazyl radical) photometric assay described by (Choi et al., 2002).

The freeze dried aqueous leaf and root extract was diluted in methanol (1000 µg/ml). One millilitre of a 0.3 mM DPPH in ethanol was added to 2.5 ml of plant extract and kept at room temperature for 30 minutes. One millilitre ethanol plus plant extract solution (2.5 ml) was used as a blank, while DPPH solution and 2.5 ml ethanol was used as a negative control. The positive control was DPPH solution (1 ml) plus 2.5 ml 1 mM Quercitin 3- Rutinoside (Sigma). The radical scavenging activity was measured as the decolourization percentage of the test sample. All tests were carried out in triplicate. The absorbance values were measured in a Varian Cary 1E UV-visible spectrophotometer at 518 nm. The average absorbance values were converted into the percentage antioxidant activity, using the following equation:

$$\% \text{ Decolourization} = \frac{[\text{Av Controls} - (\text{Av sample}_{DPPH} - \text{Av sample}_{MeOH})] \text{ X100}}{\text{Av Controls}}$$

Where Av Controls=average absorbance of all DPPH control wells−average absorbance of all methanol control wells; Av sample $_{DPPH}$ average absorbance of sample wells with DPPH and Av sample $_{MEOH}$ = average absorbance of sample wells with methanol.

Determination of the 5-lipoxygenase activity

In normal biological systems 5-lipoxygenase is known to catalyze the oxidation of unsaturated fatty acids containing 1–4 pentadiene structures using arachidonic acid as the biological substrate coverting them into conjugated dienes which results in continuous increase in absorbance at 243 nm. 5-lipoxygenase activity of the plant extracts was determined using the method as published by Evans (1987), Baylac and Racine (2003) and (Alitonou et al., 2006) with linoleic acid as the substrate for the 5-lipoxygenase enzyme. Soybean lipoxygenase (EC. 1.13.11.12) was purchased from Fluka and Nordihydroguaiaretic acid (NDGA) [500.38.9] and linoleic acid sodium salt (822-17-3) were obtained from Sigma Chemical Co; potassium phosphate buffer 0.1 M, pH 9 was prepared with analytical grade reagents purchased from standard commercial sources. Deionized water was used for the preparation of all solutions.

Standard linoleic acid was enzymatically converted to conjugated dienes resulting in an increase in absorbance at 234 nm. Absorbance was plotted graphically against the different concentrations used. The slopes of the straight-line portions of the sample and the control curves were used to determine the percentage activity of the enzyme (Lourens et al., 2004). Nordihydroguaiaretic acid (NDGA), a known inhibitor of soybean lipoxygenase, was used as a reference drug.

5-Lipoxygenase assay

The reaction was initiated by the addition of aliquots (50 µl) of a soybean LOX solution (prepared daily in potassium phosphate buffer 0.1M pH 9.0) in a sufficient concentration to give an easily measurable initial rate of reaction to 2.0 ml of sodium linoleate (100 µM) in phosphate buffer; the enzymatic reactions were performed in

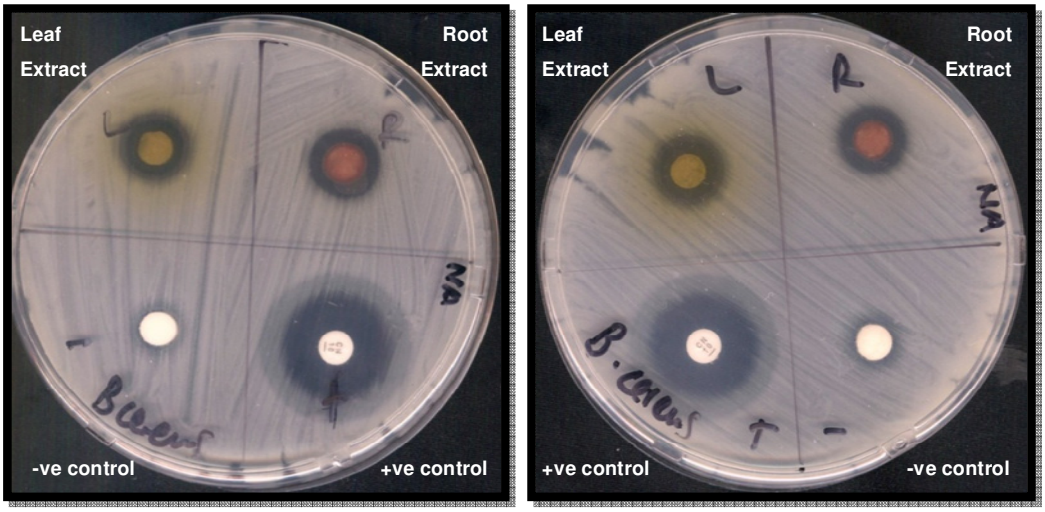

Figure 3. Antimicrobial screening showing zones of inhibition produced by crude extracts of leaves and roots from *C. triloba* against the gram positive bacteria, *Bacillus cereus.* Ampillicin and dimethylsulfoxide were used as positive control and negative control respectively.

absence or in presence of inhibitor and their kinetics were compared. The inhibitors were dissolved in DMSO in such a manner that an aliquot of each (30 µl) yielded a final concentration of maximum 100 µg/ml) in each assay. The initial reaction rate was determined from the slope of the straight line portion of the curve and the percentage inhibition of the enzyme activity was calculated by comparing with the control (using 30 µl of DMSO alone instead of 30 µl of the inhibitor solution). Each inhibitor concentration was tested in triplicate and the results averaged; the concentration (µg/ml) of the plant samples that gave 50% inhibition (IC_{50}) was calculated from the outline of the inhibition percentages as a function of the inhibitor concentration. The assay mixture without the plant extract was used as the negative control while one containing nordihydroguaretic acid (NDGA) was used as the positive control.

RESULTS AND DISCUSSION

The antibacterial activity of the root and leaf extracts of *C. triloba* against *B. cereus*, *S. aureus*, *M. luteus*, *P. aerouginosa, S. faecalis* and Gram-negative bacteria: *E. coli, S. typhimurium, E. aerogenes* and *K. pneumoniae* showed that the root extract showed inhibitory activity against *B. cereus* (Figure 3) and *M. luteus* (Figure 5) whereas the leaf extract showed activity against *B. cereus*, *E. aerogenes* (Figure 4) and *M. luteus*. The activity by the leaf and root extract was lower than that of the control.

Antibacterial assays – purified extracts

Root extracts of *C. triloba* were further purified by preparative thin layer chromatography and the extracts CTREh01, CTREh02 and CTREh03 were screened for antibacterial activity. The results of these screens are illustrated in Table 1. The purified extracts exhibited good activity against the Gram positive organisms *M. luteus, B. cereus* and *S. aureus.* Zones of inhibition ranging from 2 to 3 mm were identified for the Gram negative organisms, *E. coli* and *S. typhimurium*. DMSO and water did not inhibit growth of organisms. CTREh01, CTREh02 and CTREh03 were characterized and contained a combination of the six isolated compounds namely: 9,10 anthracenedione, 1-hydroxy-4-methylanthraquinone, 5,8-dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione, androst-5-ene-3,17,19-triol, 1,2 benzenedicarboxylic acid, mono (2-ethylhexyl) ester and octadecanoic acid. This combination of compounds or the synergy that exists between like molecules produced by the polyketide pathway could have resulted in the increased antibacterial activity. Test compounds exhibited similar activities against microorganisms, possibly due to similarities between structures and hence structure activity relationships. There appears to be a difference in inhibitory activity between Gram-positive and Gram-negative organisms, with the test compounds showing significant activity against Gram positive organisms (Figure 6B, C and E). No clear mechanisms have been identified that specifically indicate how these compounds target microbial invasion but is most probably not due to inhibition of cell wall synthesis. Some compounds appeared to be bactericidal as wells previously showing inhibition had not become infected after prolonged incubation periods. Data mining revealed

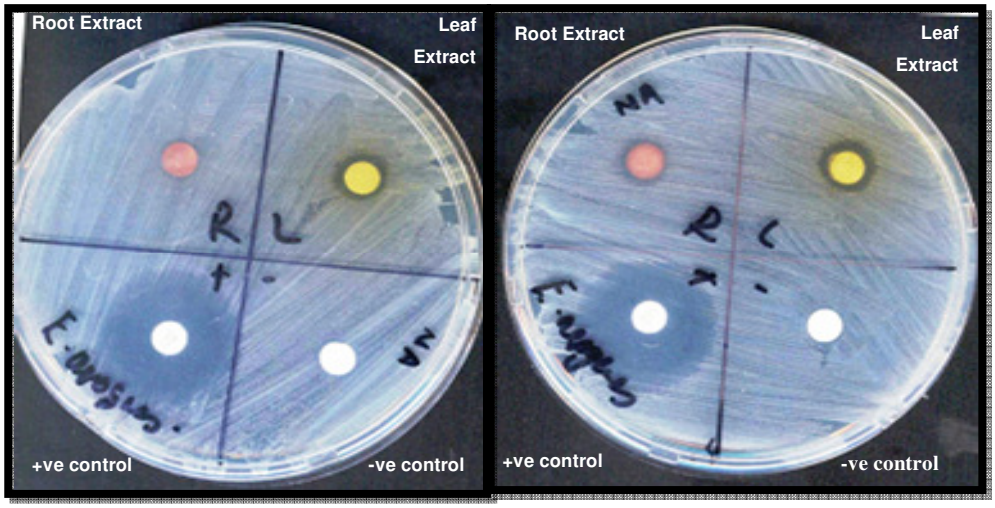

Figure 4. Antimicrobial screening showing zones of inhibition produced by crude extracts of leaves and roots from *C. triloba* against the gram negative bacteria, *Enterobacter aerogenes*.

no comparative work on *C. triloba*.

Minimum inhibitory concentration (MIC)

The MIC values were calculated using the well dilution method as described in materials and methods. For all organisms, a standard antibiotic, either gentamicin or ampicillin, was included as positive control. Since all compounds were solubilized in acetone and made up to final concentration of 5 mg/ml, the same quantity of acetone to water was included as a negative control (solvent). Gram positive organisms, *S. aureus,* and *M. luteus* were inhibited at low concentrations of the crude and purified compounds (40 µg/ml). Gram negative organisms, *S. typhimurium*, and *E. coli* (Figure 4) were inhibited at higher concentrations of the crude and purified compounds (312 to 620 µg/ml). The crude extracts showed the highest inhibitory effect against *S. aureus* and *M. luteus* (40 µg/ml) and the lowest activity was exhibited by *E. coli* and *S. typhimurium* (620 µg/ml) (Table 2). 9, 10 anthracenedione inhibited *S. aureus* (>1000 µg/ml), *M. luteus* (40 µg/ml), *S. typhimurium* (620 µg/ml), *E. coli* and *B. cereus* (>1000 µg/ml).

1-hydroxy-4-methylanthraquinone inhibited *S. aureus* (78 µg/ml), *M. luteus* (40 µg/ml), *S. typhimurium* (125 µg/ml) *E. coli* (150 µg/ml) and *B. cereus* (>1000 µg/ml). No antimicrobial data regarding this compound or 9,10 anthracenedione could be sourced in the literature.

The total activity of crude extracts showed a higher total activity than that of the individual compounds. The Gram positive organisms, *S. aureus* and *M. luteus* had the highest total activity of 530 g/mg/ml (Table 3). Gram negative *S. typhimurium* and *E. coli* showed moderate activity ranging from 34 to 67.9 g/mg/ml. The purified compounds also exhibited good total activity against Gram positive organisms (200 to 250 g/mg/ml). The total activity results as well as the minimum inhibitory concentration assays were in agreement with the disc diffusion assays and indicated 9,10 anthracenedione and 1-hydroxy-4-methylanthraquinone showed antibacterial activity against Gram positive organisms. No antifungal activity was observed for the two strains of fungi *A. flavus* and *F. verticilloides*.

Antioxidant activity

Quercetin-3-rutinoside was used as a positive control, which exhibited 100% scavenging activity of free radicals. The root extracts CTREh01, CTREh02 and CTREh03 (1000 µg/ml) showed a ± 50 % reduction of the free radicals. The experiments were carried out in triplicate. Figure 7 shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} of the standard compound quercetin was 0.01 mg/ml. The highest radical scavenging activity was showed by CTRE 02 with IC50= 1 mg/ml. The radical scavenging activity in the plant extracts decreased in the following order: CTRE 02 > CTRE 03 > CTRE 01. Most of the plants' extracts at different concentrations exhibited more than 50% scavenging activity (Figure 7).

Anti-inflammatory assay

In our study we used nordihydroguaiaretic acid **(**NDGA)

Figure 5. Antimicrobial screening showing zones of inhibition produced by crude extracts of leaves and roots from *C. triloba* against the gram positive bacteria, *Micrococcus luteus*.

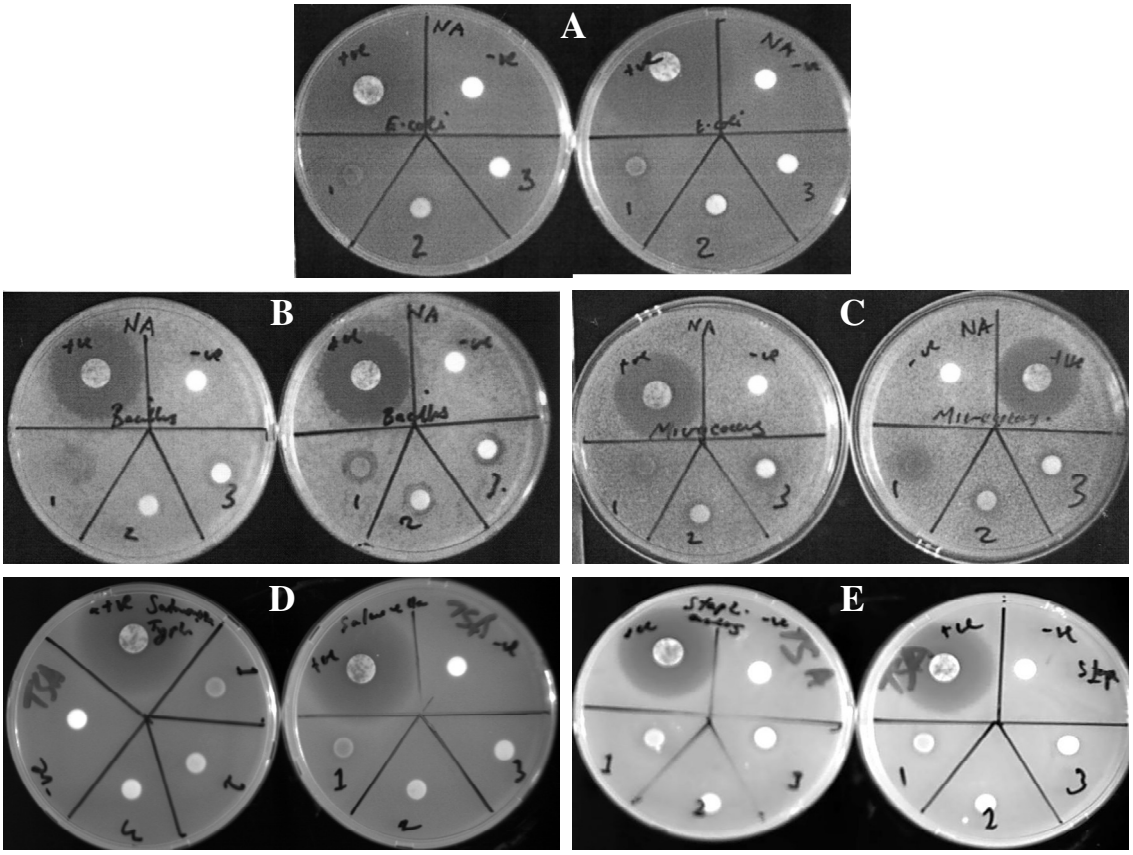

Figure 6. Antimicrobial screening showing zones of inhibition produced by purified extracts of roots from *C. triloba* against the Gram negative and Gram positive bacteria, A- *Escherichia coli, B-Bacillus cereus*, C- *Micrococcus luteus, D- Salmonella typhimurium and E- Staphylococcus aureus*. 1 - CTREh 01, 2 -CTREh 02 and 3 - CTREh 03

as a standard for the comparison of anti-inflammatory potential of the three famine plants. Reduced IC_{50} values

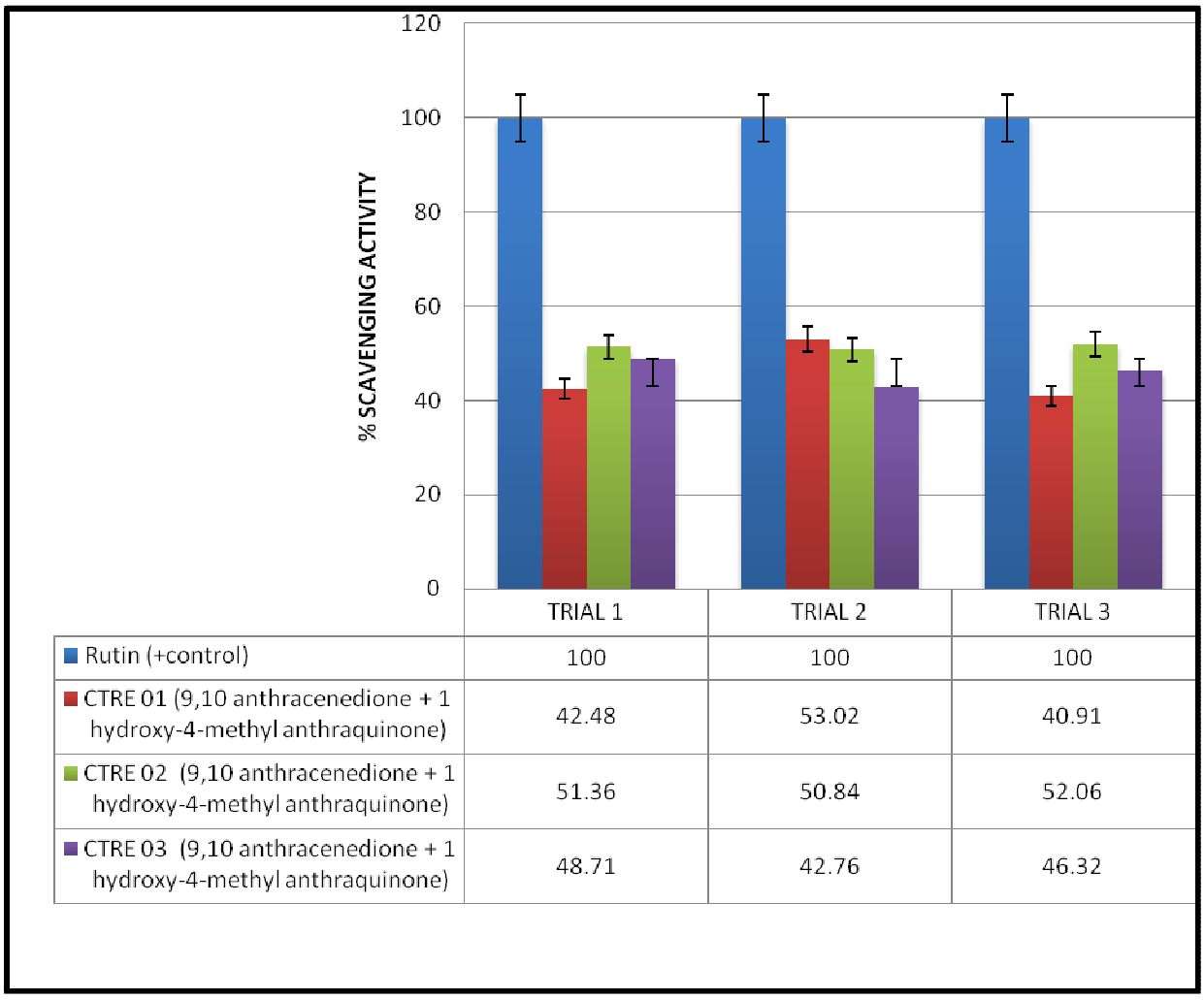

Figure 7. Antioxidant activity of the purified extracts (CTREh01, CTREh02 and CTREh03).

Table 1. Summary of the antibacterial test using the purified extracts isolated from *C. triloba* roots. Inhibition zones (mm).

Bacterial culture	+ve control	-ve control	CTREh01 (mm)	CTREh02 (mm)	CTREh03 (mm)
Bacillus cereus	14	0	4	3	3
Esherischia coli	20	0	1	2	-
Micrococcus luteus	12	0	4	3	2
Staphylococcus aureus	20	0	4	1	1
Salmonella typhimurium	20	0	3	2	2
Pseudomonas aeroginosa	11	0	-	-	-

suggest better inhibitory action on 5 COX. The IC_{50} value of NDGA was 2.5 µg/ml and the extracts showed (Table 4) no anti-inflammatory activity. IC_{50} for the crude leaf and roots extracts of *C. triloba* was 300 µg/ml.

9, 10 anthracenedione and 1-hydroxy-4-methyl anthraquinone showed antibacterial activity against *S. aureus, M. luteus, B. cereus* and *E. coli*. Due to the synergistic effect of the individual compounds, the crude

Table 2. MIC results of crude and purified extracts from *C. triloba* (1mg/ml).

Minimum Inhibitory Concentration (MIC) µg/ml						
Bacterial culture	**Time (min)**	**Crude**	**9, 10 anthracenedione**	**1-hydroxy-4- methyl anthraquinone**	**Ampicillin (AMP)**	**Gentamycin (GEN)**
	30	0.620	0.620	1.250	0.620	
Salmonella typhimurium	60	0.620	0.620	1.250	0.620	
	90	0.620	0.620	0.620	0.620	
	30	0.040	1.250	0.078	NI*	
Staphylococcus aureus	60	0.040	2.500	0.078	NI	
	90	0.040	NI	0.040	NI	
	30	1.250	1.250	1.250		0.04
Bacillus cereus	60	2.500	NI	2.500		0.04
	90	2.500	2.500	2.500		0.04
	30	0.620	0.312	0.150		0.04
Escherichia coli	60	0.156	0.312	0.150		0.04
	90	0.312	0.312	0.312		0.04
	30	1.250	1.250	NI	0.620	
Micrococcus luteus	60	0.040	0.040	0.312	0.620	
	90	0.040	0.040	0.040	0.620	

*NI – no inhibition

Table 3 Total activity or Potency of purified 9, 10 anthracenedione and 1-hydroxy-4-methyl anthraquinone from *C. triloba*.

Total activity (g/mg/ml)	**Crude**	**9.10 anthracenedione**	**1 hydroxy-4 methyl anthraquinone**
Total quantity in mg extracted from 1g	**21.2**	**10**	**8**
Salmonella typhimurium	34	16	12.9
Staphylococcus aureus	530	4	200
Bacillus cereus	8.4	4	3.2
Escherichia coli	67.9	32.05	25.60
Micrococcus luteus	530	250	200

extract exhibited good potency (>500) against *S. aureus* and *M. luteus,* medium potency against *E. coli* and *S. typhimurium* (<100) and very low potency against *B. cereus* (<10). Although a similar trend was observed for 9, 10 anthracenedione and 1 hydroxy-4-methylanthraquinone unlike the crude extract. A very low potency against *S. aureus* was observed for 9, 10 anthracenedione and a high potency for 1-hydroxy-4-methyl anthraquinone. Thus 9, 10 anthracenedione is an effective drug against *E. coli* and *S. typhimurium* and 1-hydroxy-4-methyl anthraquinone is effective against *S. aureus* and *M. luteus*. The root extracts CTREh01, CTREh02 and CTREh03 (1000 µg/ml) showed a ± 50 % reduction of the free radicals. No anti-inflammatory activity was observed. All purified and crude extracts showed potent inhibition of the human topoisomerase II enzyme. The purified extracts showed moderate toxicity against $HepG_2$ cells at high concentrations and no toxicity was observed against brine shrimp larvae. No mutagenicity was observed with the crude extracts using the Ames test. 9, 10 anthracenedione and 1-hydroxy-4-methylanthraquinone can be used as antibacterial agents. Their antioxidative potential can be exploited for anti-cancer treatment as in many cancers reactive oxygen species are implicated in the aetiology of these cancers. Furthermore, this compound demonstrates potent anti-topoisomerase activity. Thus, the synergistic effect of 9, 10 anthracenedione and 1-hydroxy-4-methyl anthraquinone as anti-topoisomerases and anti-oxidative compounds can contribute to *C. triloba* extracts to fight cancer. However, the use of these compounds as anticancer agents needs further testing that can compare the activity of this compound in both normal and cancerous models.

Table 4 Inhibition of 5-lipoxygenase enzyme activity on linoleic acid by various concentrations (ppm) of leaf and root extracts of *C. triloba*

Concentration of plant extract (ppm)	% Inhibition[a] of 5-lipoxygenase activity		
	C. triloba (leaves)	*C. triloba* (roots)	NDGA
100	29.1	17.6	
200	41.6	31.8	
300	54.7	45.0	
400	61.3	52.1	
500	67.8	54.3	
600	67.5	54.1	
1.0			19.5
2.0			41.0
3.0			58.2
4.0			76.9
5.0			89.6
6.0			89.1
IC_{50} (ppm)[b]	**257.3**	**323.8**	**2.5**

a – Mean values obtained from experiments performed in triplicate. b - Mean value determined graphically.

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