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The *in vitro* biological activity of selected South African *Commiphora* species

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ABSTRACT

Ten South African *Commiphora* (Burseraceae) species were investigated to validate their use in traditional healing rites. The leaf and stem extracts of each species were analysed for the anti-oxidant (ABTS and DPPH assays), antimicrobial (MIC and death kinetic assays), anti-inflammatory (5-LOX assay), anticancer (SRB assay) properties, as well as the cytotoxic effects (tetrazolium-based assay). The best anti-oxidant activity (ABTS assay) was observed for the stem extracts of *Commiphora tenuipetiolata* IC_{50} = 5.10 μg/ml), *Commiphora neglecta* (IC_{50} = 7.28 μg/ml) and *Commiphora mollis* (IC_{50} = 8.82 μg/ml). Extracts generally exhibited poor anti-oxidant activity in the DPPH assay, with the exception of *Commiphora schimperi* (stem), *Commiphora neglecta* (stem), *Commiphora tenuipetiolata* (stem and leaf), and *Commiphora edulis* (stem), with IC_{50} values ranging between 7.31 and 10.81 μg/ml. The stem extracts exhibited moderate to good 5-LOX inhibitory activity with *Commiphora pyracanthoides* (stem) displaying the greatest inhibitory effect (IC_{50} = 27.86 ± 4.45 μg/ml). For the antimicrobial (MIC) assay, a greater selectivity was exhibited by the extracts against the Gram-positive bacteria (0.01–8.00 mg/ml) and the yeasts (0.25–8.00 mg/ml) than against the Gram-negative bacteria (1.00–8.00 mg/ml). Using death kinetic studies (time–kill studies), the rate at which *Commiphora marlothii* (stem) kills *Staphylococcus aureus* over a 24 h period was determined. Mostly, a concentration-dependent antibacterial activity was observed beginning after ca. 30 min. All concentrations exhibited antibacterial activity, with complete bactericidal effect achieved by the 24th hour. The most active *Commiphora* species against the HT-29 cells (SRB anticancer assay) were *Commiphora glandulosa* (leaf and stem) and *Commiphora marlothii* (leaf). The MCF-7 cells (SRB anticancer assay) exhibited the highest sensitivity to indigenous *Commiphora* species, with *Commiphora edulis* (leaf and stem), *Commiphora glandulosa* (leaf and stem), *Commiphora marlothii* (leaf), *Commiphora pyracanthoides* (leaf and stem), *Commiphora schimperi* (stem), and *Commiphora viminea* (stem) all possessing a percentage inhibition greater than 80% at 100 μg/ml. *Commiphora glandulosa* (leaf and stem) and *Commiphora pyracanthoides* (leaf and stem) were the two most active species against the SF-268 cells (SRB anticancer assay), with IC_{50} values ranging between 68.55 ± 2.01 and 71.45 ± 1.24 μg/ml. The majority of the *Commiphora* extracts were largely non-cytotoxic against Graham human kidney epithelial cells when investigated in the MTT assay.

1. Introduction

The Burseraceae family consists of approximately 700 species from 18 genera of which *Commiphora* is one. The name *Commiphora* originates from the Greek words *kommi* (meaning 'gum') and *phero* (meaning 'to bear'). The majority of the species yield a fragrant oleo-gum-resin following damage to the bark (Steyn, 2003). Of the more than 200 species of *Commiphora* native to the seasonally dry tropics of Africa, Arabia and India, about 40 species occur in southern Africa. Traditionally, *Commiphora* species have been used in southern Africa for the treatment of colds (stem), fever (stem), malaria (stem), typhoid (fruit), wound healing (resinous exudates), as an antiseptic (resinous exudates), snake and scorpion bites, tumours (bark, resin, leaf), stomach aches (bark, resin, leaf), diseases of the gall bladder (bark), chest ailments (roots) and

Abbreviations: ABTS, 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid); ATCC, American type culture collection; CFU, colony forming units; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HPLC, high performance liquid chromatography; INT, *p*-iodonitrotetrazolium; 5-LOX, 5-lipoxygenase; MIC, minimum inhibitory concentration; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2*H*-tetrazolium bromide; NaCl, sodium chloride; NCTC, national culture type collection; NDGA, nordihydroguaiaretic acid; SRB, antiproliferative sulforhodamine B; TEAC, Trolox equivalent anti-oxidant capacity; TCA, cold trichloroacetic acid; TSA, tryptone soya agar.

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skin infections (resin) (Kokwaro, 1976; Lemenih et al., 2003; Steyn, 2003). *Commiphora* species have been a source of several novel and bio-active compounds. Previous studies on this genus include the isolation of four active compounds, namely mansumbinone, mansumbinoic acid, picropolygamain, lignan-1(methoxy-1,2,3,4-tetrahydropolygamain) from *Commiphora kua* var. *kua*. Also, the extract of stem bark yielded three labile C_{22} octanordammarene triterpenes. The dihydroflavonol glucoside, phellamurin has been isolated from *Commiphora africana* and seven dammarene triterpenes from the stem bark of *Commiphora dalzielli* have been isolated. Pentacyclic triterpene with anti-inflammatory activity was isolated from *Commiphora merkeri* (Hanuš et al., 2005).

The botanical diversity of this genus in South Africa warrants a study to provide scientific evidence for the traditional use of *Commiphora* species in African healing rites. Thus, 10 South African *Commiphora* species were investigated. In this study, the leaf and stem extracts of each species were analysed for their anti-inflammatory, anti-oxidant, antimicrobial, anticancer activities as well as the cytotoxic effects.

2. Materials and methods

2.1. Collection of plant material

Aerial parts (leaves and stems) of 10 *Commiphora* species were identified and collected at random from natural populations within a selected geographical region in the Limpopo Province. The leaves and stems were air-dried and ground separately. Voucher specimens were prepared and deposited at the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

2.2. Preparation of extracts

Extracts were prepared over a period of 6 h (three extractions of 2 h each). The leaves and stems (10 g of each) underwent extraction in a conical flask using chloroform:methanol (1:1) in a water bath at 40 °C.

2.3. Determination of anti-oxidant activity

Two assays, viz. the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) were employed for the determination of potential anti-oxidant activity. The ABTS anti-oxidant assay is also known as the Trolox equivalent anti-oxidant capacity (TEAC) assay. The DPPH assay, as described by Shimada et al. (1992), was employed to determine the radical scavenging activity of the plant extracts. Aliquots of plant extract dissolved in dimethyl sulfoxide (DMSO, Saarchem) were plated out in triplicate in a 96-well microtiter plate. The DPPH solution (Fluka) was added to alternating columns of the test samples and high performance liquid chromatography (HPLC) grade methanol (Ultrafine Limited) for control of test samples, in the remaining columns. The plate was shaken for 2 min and incubated for 30 min in the dark. The percentage decolourisation was obtained spectrophotometrically at 550 nm using the Labsystems Multiskan RC microtiter plate reader, linked to a computer equipped with GENESIS® software. Percentage decolourisation was plotted against the concentration of the sample and the IC_{50} values were determined using Enzfitter® version 1.05 software. Vitamin C (L-ascorbic acid) and Trolox™ (6-hydroxy-2,5,7,8-tetramethylchromon-2-carboxylic acid) were used as positive controls. At least three independent tests were performed for each sample.

The quenching of the ABTS radical cation results in the evaluation of the radical scavenging activity. This assay was first reported by Miller et al. (1993) and Rice-Evans (1994). Stock solutions and serial dilutions of the plant extracts were prepared in DMSO. The total scavenging capacity of the extracts was quantified through the addition of $ABTS^+$ (Sigma–Aldrich) to the plant extract. The solutions were heated to 37 °C for 4 min and the absorbance read at 734 nm on a spectrophotometer (Milton Roy Spectronic GENESYS 5). All assays were done in triplicate. The percentage decolourisation was calculated relative to the control. Trolox™ was used as the positive control and ethanol as the negative control. The extent of inhibition of the absorbance of the $ABTS^+$ was plotted as a function of the concentration to determine the Trolox™ equivalent anti-oxidant capacity. The IC_{50} values and standard deviations were determined.

2.4. Determination of antimicrobial activity

2.4.1. Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) values were determined and modified from the microtiter plate dilution method (Eloff, 1998) on two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6358 and *Bacillus cereus* ATCC 11778), two Gram-negative bacteria (*Klebsiella pneumoniae* NCTC 9633 and *Pseudomonas aeruginosa* ATCC 9027) and two yeasts (*Candida albicans* ATCC 10231 and *Cryptococcus neoformans* ATCC 90112). The reference cultures (with the exception of *Candida albicans*, obtained from the South African Bureau of Standards) were obtained from the National Health Laboratory Services, Johannesburg. Stock solutions of the respective plant extracts were prepared by dissolving dry plant extract in DMSO, as a result of the insolubility of certain extracts in acetone. Aliquots of the stock solution were transferred aseptically into a microtiter plate, and serial dilutions (1:1) of the plant extract with sterile water were carried out several times. An equal volume of microbial culture at a concentration of 1×10^8 colony forming units (CFU)/ml, grown in tryptone soya broth (Oxiod) was added to each well prior to being incubated at 37 °C. Overnight incubation and 48 h incubation followed for bacteria for the yeasts, respectively.

After incubation, a 0.4 mg/ml solution of *p*-iodonitrotetrazolium (INT, Sigma–Aldrich) was added to each well (40 μl) as an indicator of microbial growth. The plates were incubated at 25 °C and the MIC values visually determined after 6 h (bacteria) and 24 h (yeasts). The concentration that inhibited bacterial/yeast growth completely (the first clear well) was taken as the MIC value. MIC values were determined at least in duplicate and repeated to confirm activity. Ciprofloxacin (Sigma–Aldrich) and amphotericin B (Sigma–Aldrich) were used as the positive controls for the bacterial and yeast strains, respectively. Negative controls contained only DMSO.

2.4.2. Death kinetic assay

Based on the preliminary promising results obtained from the MIC determination, *Commiphora marlothii* (stem) was identified as a suitable candidate for the inactivation broth death kinetic assay, as described by Lattaoui and Tantaoui-Elaraki (1994) and Christoph et al. (2001). *Staphylococcus aureus* (ATCC 6538) was cultured overnight on tryptone soya agar (TSA) at 37 °C, after which the resulting colonies were removed from the agar and used to inoculate a sterile 0.9% sodium chloride (NaCl, Labchem) solution. From this cell suspension, serial dilutions were prepared in 0.9% NaCl in order to obtain a suspension with an appropriate colony count of 1×10^6 CFU/ml. This was obtained by performing dilutions and counting viable colonies in order to extrapolate the bacterial cell concentration in the inoculum suspension. Solutions were prepared (extract dissolved in DMSO, broth and bacterial suspension) to yield final plant extract concentrations of 0.125, 0.25, 0.5, 0.75 and 1.0% (w/v), and were stabilised at 37 °C in a shaking water bath, after which bacterial inoculum was added. A sample of each incu-