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A phytochemical and pharmacological study of ten Commiphora species indigenous to South Africa

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ABSTRACT

Commiphora species (from which myrrh is obtained) has been a source of several novel and bio-active natural compounds. Traditionally, *Commiphora* (Burseraceae) is used in southern Africa for the treatment of ulcers, fevers, and as a remedy for snake and scorpion bites. In western Africa, the macerated stem is used in the treatment of rheumatic conditions. The resin of some *Commiphora* species is applied topically to aid in wound healing. Documented uses include antibacterial and antifungal properties, as well as cytotoxic, cytostatic and anti-oxidant activity. The botanical diversity of this genus in South Africa warrants a study of this plant group, to provide scientific evidence for the traditional use of *Commiphora* species in African healing rites.

Ten *Commiphora* species were investigated. Fresh plant material of the selected species were identified and collected from natural populations in the Limpopo Province. Active compounds, viz. kaempferol and dihydrokaempferol, in *C. glandulosa* (stem) were isolated using bioassay-guided fractionation and identified using nuclear magnetic resonance spectroscopy. The stem and leaf extracts of each species were analysed for *in vitro* anti-oxidant, antimicrobial, anti-inflammatory, anticancer activity, as well as cytotoxicity. The anti-oxidant activity of the extracts was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) assays. Extracts generally exhibited poor anti-oxidant activity in the DPPH assay, with the exception of *C. schimperi* (stem), *C. neglecta* (stem), *C. tenuipetiolata* (stem and leaf), and *C. edulis* (stem), which possessed IC_{50} values ranging between 7.31 μg/ml and 10.81 μg/ml. Isolated compounds were subjected to the DPPH assay to determine the anti-oxidant potential of each compound, separately and in combination to establish possible synergistic, antagonistic or additive effects. The flavonol, kaempferol (IC_{50} = 3.32 μg/ml) showed exceptional radical scavenging activity, in contrast to the low activity displayed by dihydrokaempferol (IC_{50} = 301.57 μg/ml), their combination being antagonistic. Greater anti-oxidant activity was observed for most species in the ABTS assay when compared to the results obtained in the DPPH assay. The best activity was observed for the stem extracts of *C. neglecta* (IC_{50} = 7.28 μg/ml) and *C. mollis* (IC_{50} = 8.82 μg/ml).

In vitro antimicrobial efficacy was determined against Gram-positive and Gram-negative bacteria as well as yeasts using the MIC microtiter plate assay. A greater selectivity was exhibited by the extracts against the Gram-positive bacteria and yeast than against the Gram-negative bacteria. Using death kinetics studies (time-kill studies), the rate at which the antimicrobial agent kills pathogens over a 24-hour period was determined. The antibacterial activity of *Commiphora marlothii* (stem) was observed to begin at ca. 30 min of the exposure of *S. aureus* to the different concentrations of plant extract. All concentrations exhibited antibacterial activity, with a complete bactericidal effect achieved by all test concentrations by the 24^{th} hour. *Commiphora pyracanthoides* (stem) displayed anti-inflammatory activity through good inhibition of the 5-LOX enzyme (IC_{50} = 27.86 μg/ml).

The ability of extracts and kaempferol to inhibit the *in vitro* growth of three human cancer cell lines, namely the colon adenocarcinoma (HT-29), breast adenocarcinoma (MCF-7), and the neuronal glioblastoma (SF-268), was evaluated using the sulforhodamine (SRB) antiproliferative assay. The most active *Commiphora* species against the HT-29 cells were *C. glandulosa* (leaf and stem) and *C. marlothii* (leaf). The MCF-7 cell line was the most sensitive to indigenous *Commiphora* species, with *C. edulis* (leaf and stem), *C. glandulosa* (leaf and stem), *C. marlothii* (leaf), *C. pyracanthoides* (leaf and stem), *C. schimperi* (stem), and *C. viminea* (stem) all possessing an inhibition greater than 80% at 100 μg/ml. *Commiphora glandulosa* (leaf and stem) and *C. pyracanthoides* (leaf and stem) were the two most active species against the SF-268 cells, with IC_{50} values ranging between 68.50 μg/ml and 71.45 μg/ml. The inhibition of the cancer cell proliferation by kaempferol in all three-cancer cell lines was determined, with IC_{50} values of 9.78 μg/ml in HT-29 cells, 20.21 μg/ml in MCF-7 cells and 43.83 μg/ml in SF-268 cells. The microculture tetrazolium cellular viability (MTT) assay was used to determine the cellular toxicity of the extracts against transformed human kidney epithelium (Graham) cells. *Commiphora glandulosa* (stem) proved to be most toxic (IC_{50} = 30.5 μg/ml). The IC_{50} values for all other extracts were in excess of 95 μg/ml suggesting low *in vitro* toxicity for the majority of the species.

A phytochemical investigation of the non-volatile constituents of the leaf and stems was conducted using high performance liquid chromatography (HPLC). The HPLC profiles and UV spectra of the stem extracts, and the representative flavonoid patterns in the leaf extracts of the species indicate that a similarity exists in their chemical fingerprint.