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Anti-inflammatory and mutagenic evaluation of medicinal plants used by Venda people against venereal and related diseases

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ABSTRACT

Ethnopharmacology relevance: Inflammation is a major risk factor for various human diseases including venereal diseases, often resulting in treatment complications. Plants have been traditionally used for treatment of many different diseases and have been successfully proven to be an alternative source in treatment of infectious diseases.

Aim of the study: This study was aimed at evaluating the anti-inflammatory activities and the mutagenic properties of 12 medicinal plants used by the Venda people against venereal and related diseases.

Materials and methods: The plants were evaluated for their anti-inflammatory activity against the cyclooxygenase (COX-1 and -2) enzymes and genotoxicity using the Ames test, with and without S9 (metabolic activation) against *Salmonella typhimurium* tester strain TA98.

Results: DCM and PE extracts of *Adansonia digitata* bark, *Bolusanthus speciosus* bark, *Pterocarpus angolensis* bark and *Pappea capensis* leaves and EtOH and water extracts of *Bolusanthus speciosus* stem and *Ekebergia capensis* bark showed the best anti-inflammatory activity in both COX-1 and -2 assays at 250 μg/ml. These were further evaluated at three other concentrations (31.25, 62.5, and 125 μg/ml) to determine IC_{50} values. Water extracts of *Ekebergia capensis* bark showed the best IC_{50} value towards COX-1. The Ames test revealed that all plant extracts were non-mutagenic towards *Salmonella typhimurium* strain TA98 except for *Elephantorrhiza burkei* and *Ekebergia capensis* that showed weak mutagenicity.

Conclusion: The active plants may offer a new source of chemicals for the effective treatment of anti-inflammatory conditions related to venereal diseases.

1. Introduction

Inflammation occurs as one of the first responses of living tissues to injury. The inflammatory response is essential for the removal of harmful stimuli and to initiate the healing process of the inflamed tissue (Vassileva and Piquette-Miller, 2010). However, if the cause of the inflammatory response is not eliminated it can become chronic and contribute to the perpetuation and progression of some diseases (Calder et al., 2009; Vassileva and Piquette-Miller, 2010). For example, in venereal diseases, untreated gonorrhoea and chlamydia result in life threatening conditions such as pelvic inflammation leading to chronic pelvic disease as well as infertility, ectopic pregnancy, neonatal ophthalmia and disseminated gonococcal infections (Tapsall, 2001; WHO, 2001). According to Pan et al. (2010) reduction/eliminating of chronic inflammation is a beneficial strategy to fight several human diseases.

Nonsteriodal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammatory joint diseases, however, they are associated with renal and gastrointestinal toxicity (Gilroy and Colville-Nash, 2000). This provides the impetus for the development of highly effective therapy from medicinal plants. A number of compounds derived from medicinal plants are already considered as effective and safer for the treatment of various diseases, including inflammation and pain (Tunón et al., 1995; Taylor and Van Staden, 2001).

Despite the therapeutic advantages, some plants are potentially toxic, carcinogenic and teratogenic (De Sá Ferreira and Vargas, 1999; Akinboro and Bakare, 2007). For example, numerous studies have shown that many plants used in traditional medicine have *in vitro* mutagenic or toxic and carcinogenic properties (Cardoso et al., 2006; Déciga-Campos et al., 2007; Mohd-Fuat et al., 2007).

According to Verschaeve and Van Staden (2008), it is important to screen medicinal plants for their mutagenic potency. This

Abbreviations: 2-AA, 2-Aminoanthracene; COX, Cyclooxygenase; DCM, Dichloromethane; DMSO, Dimethylsulfoxide; EtOH, 80% ethanol; GAE, Gallic acid equivalent; IC_{50}, Inhibition concentration; 4NQO, 4-Nitroquinoline-*N*-oxide; PE, Petroleum ether

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assessment can be used for potential chemotherapeutic drugs, safe use of plant-derived medicines and as a measure of safety for the continued long-term use of medicinal plants (Cavalcanti et al., 2006; Verschaeve et al., 2004). Because toxicity interferes with central functions of an organism, for example, neurotoxins affect the brain and the nervous system, while cytotoxins and metabolic poisons disturb the liver, kidneys, heart or the respiratory system (Wink and Van Wyk, 2008). Therefore, it is important to screen medicinal plants for their mutagenic potency.

Several bioassays to assess enzyme inhibition and mutagenicity have been developed to prove the efficacy and safety of crude plant extracts. In some cases this has led to the production of new drugs with few side effects (Demma et al., 2009; Houghton et al., 2007). This study was aimed at evaluating the inhibition of COX-1 and -2 and the mutagenic effect of 12 medicinal plants that are used against venereal diseases by the Venda people.

2. Material and methods

2.1. Sample collection

Plant materials were collected from Limpopo Province (Venda, Tshiendeulu mountain, Mandiwana village: 22°85′34.57″ S, 30°141′65.6″ E South Africa). The plant species used in this study have multiple traditional uses such as gonorrhoea, inflammation, headache, wounds and infertility. These plants have already been assessed for antibacterial, antifungal, antigonococcal, phenolic contents and their abilities to inhibit the HIV-1 RT enzyme (Mulaudzi et al., 2011). In Table 1 of Mulaudzi et al. (2011) the families; species and authorities; plant part used; voucher numbers; and traditional uses of the studied plants are outlined in detail.

2.2. Preparation of plant extracts

Dried, ground plant materials were extracted sequentially with 20 ml/g of petroleum ether (PE), dichloromethane (DCM), 80% ethanol (EtOH) and water, each with sonication for 1 h. The temperature of the sonication baths being kept low by adding ice to the water bath. The extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum using a rotary evaporator. The concentrated extracts were then dried at room temperature under a stream of cold air.

2.3. Anti-inflammatory activity

The COX-1 and -2 bioassays were performed as described by Jäger et al. (1996) and Zschocke and Van Staden (2000), respectively and as detailed by Ndhlala et al. (2009) using enzymes (0.3 μg protein) isolated from sheep seminal vesicle microsomes (Sigma-Aldrich). Plant extracts were tested at a concentration of 10 mg/ml (organic extracts were resuspended in 80% ethanol and aqueous extracts in water) giving a final concentration of 250 μg/ml per test solution and aqueous extracts to give a final concentration of 2 mg/ml. Two, background sets of tubes were used in each assay, one in which the enzyme was inactivated with HCl before incubation and the other where the enzyme was not deactivated. The positive control indomethacin was used at 5 μM for COX-1 and 200 μM for COX-2. The reaction was initiated by adding ^{14}C-arachidonic acid (16 Ci/mol, 30 μM) before the mixtures were incubated in a water bath for 10 min at 37 °C. After the assay, the radioactive synthesized prostaglandins were eluted into scintillation vials and counted using a Beckman LS 6000LL scintillation counter (USA). Inhibition percentage was calculated using the equation below: The extracts which showed high percentage inhibition towards COX-1 and -2 were then further evaluated at 31.25, 62.5 and 125.0 mg/ml. Each assay was repeated twice in duplicate:

$$\text{COX inhibition (\%)} = \left\{1 - \left(\frac{\text{DPM}_{extract} - \text{DPM}_{background}}{\text{DPM}_{solventblank} - \text{DPM}_{background}}\right)\right\} \times 100$$

where $\text{DPM}_{\text{extract}}$ is the disintegrations per min for the plant extract, $\text{DPM}_{\text{background}}$ is the disintegrations per min in which the enzyme was inactivated and $\text{DPM}_{\text{blank}}$ is the disintegrations per min for the reaction mixture containing water. Results are presented as graphs (means ± standard errors) of two independent experiments. Graphs were plotted using SigmaPlot 2002 for Windows version 8.0 (SPSS inc., USA).

2.4. Mutagenicity test

Mutagenicity was tested using the *Salmonella* microsome assay as outlined by Maron and Ames (1983) and modified by Mortelmans and Zeiger (2000). All the water extracts and some organic extracts (active in the bioassays described above) were tested for their potential mutagenic properties using the plate-incorporation procedure with *Salmonella typhimurium* tester strain TA98, with and without enzyme (S9) bioactivation. Stock (100 μl) bacteria in 20 ml Oxoid nutrient No. 2 broth were incubated for 16 h at 37 °C with shaking until the cells reach a density of approximately 10^9 CFU/ml of microorganisms. The bacterial cultures (100 μl) were added to 100 μl of plant extract in 500 μl phosphate buffer or S9 mix and 2 ml of agar containing biotin-histidine (0.5 mM). The plant extract was poured on a minimal agar plate and incubated at 37 °C for 48 h. All the samples were tested in triplicate. Three dilutions (50, 500, 5000 μg/ml) were used per sample. 4-Nitroquinoline-*N*-oxide (4NQO) (2 μg/plate) was used as a positive control for the assay without metabolic activation while 2-aminoanthracene (2-AA) (2 μg/plate) was used where the assay was carried out with S9 metabolic activation. Sterile distilled water was used as a negative control in both assays. A test extract was classified as a 'mutagen' if the results satisfied two criteria (1) a dose dependent increase in the number of revertants was observed and (2) the number of his^+ (histidine) revertants was equal to two times greater than that of the negative control.

3. Results and discussion

3.1. Anti-inflammatory activity

The results of the inhibition of COX-1 and -2 enzymes by the 12 medicinal plants used by the Venda people are presented in Fig. 1. Four levels of activity were defined for the enzyme inhibition assays; with activity below 20% being considered insignificant, 20–40% low, 40–70% moderate and 70–100% high (Taylor and Van Staden, 2001).

All the solvent extracts tested showed moderate to high inhibition activity towards COX-1, except water extracts of *Adansonia digitata* bark, *Osyris lanceolata* leaves and *Pterocarpus angolensis* leaves and PE extracts of *Ximenia caffra* leaves. However, water extracts of *Acacia karroo* bark, *Elephantorrhiza burkei* roots, *Ekebergia capensis* leaves, *Peltophorum africanum* bark, *Pterocarpus angolensis* bark, *Pappea capensis* leaves, *Ximenia caffra* (leaves, roots) showed high activity (71.9–100%). Among the different solvent extracts tested, DCM extracts of most of the plant species displayed moderate to high activity towards COX-2, with *Ekebergia capensis* bark, *Osyris lanceolata* leaves and roots, *Ximenia caffra* leaves and roots being the only exception showing insignificant to low percentage inhibition. PE extracts of *Adansonia digitata* bark, *Bolusanthus speciosus* bark, *Grewia occidentalis*