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Kakadu plum fruit extracts inhibit growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin components

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ABSTRACT

Rheumatoid arthritis is an autoimmune disease which can be triggered in genetically susceptible individuals by Proteus spp. infections. *Terminalia ferdinandiana* (Kakadu plum) fruit extracts were investigated by disc diffusion assay against reference and clinical strains of *Proteus mirabilis* and *Proteus vulgaris* and their MIC values were determined. Polar extracts displayed potent antibacterial activity against the bacterial triggers of rheumatoid arthritis, with MIC values as low as 32 μg/ml (methanolic extract against the *P. mirabilis* reference strain). The aqueous extract was also a potent inhibitor of Proteus growth (MIC values <300 μg/ml against all bacterial species). Whilst substantially less potent, the ethyl acetate and chloroform extracts also displayed moderate to good inhibition (as determined by MIC) against both *P. mirabilis* strains. All *T. ferdinandiana* fruit extracts were nontoxic in the *Artemia franciscana* bioassay. The most potent extract (methanolic extract) was analysed by HPLC-QTOF mass spectroscopy (with screening against 3 compound databases). Five stilbenes and 7 tannins were identified in the methanolic extract. The low toxicity of the *T. ferdinandiana* fruit extracts and their potent inhibitory bioactivity against some bacterial triggers of rheumatoid arthritis indicates their potential as medicinal agents in the treatment and prevention of this disease.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease which may afflict genetically susceptible individuals. There are currently no cures for RA and current treatment strategies aim to alleviate the symptoms (particularly pain and swelling) via the use of analgesics and anti-inflammatory agents, and/or to modify the disease process through the use of disease modifying anti-rheumatic drugs (DMARDs). None of

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Chemical compounds: Chebulic acid (PubChem CID: 72284); Combretastatin A1 (PubChem CID: 6078282); Corilagin (PubChem CID: 73568); Diethylstilbestrol (PubChem CID: 448537); Ellagic acid (PubChem CID: 5281855); Ethyl gallate (PubChem CID: 13250); Gallic acid (PubChem CID: 370); Gallocatechin (PubChem CID: 9882981); Piceid (PubChem CID: 5281718); Resveratrol (PubChem CID: 445154).

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these treatments is ideal as prolonged usage of these drugs can result in unwanted side effects and toxicity (Alataha, Kapral, & Smolen, 2003). There is a need to develop safer, more effective drugs for the treatment of RA which will not only alleviate the symptoms, but which may also cure or prevent the disease.

Eradication of the cause of an inflammatory disease is an attractive target for drug design as this would not only block/decrease the late phase inflammatory symptoms, but would also block the immune response and subsequent tissue damage associated with auto-immune inflammatory disorders. Whilst the causes of RA are not comprehensively understood, it is generally accepted that it is an autoimmune disorder which is triggered by specific microbial infections in genetically susceptible individuals (individuals with the MHC class 2 allele HLA-DR4) (Nepom et al., 1989). *Proteus mirabilis* infections have been proposed as a trigger of rheumatoid arthritis as elevated serum levels of *P. mirabilis* specific cross-reactive antibodies have frequently been reported in individuals suffering from RA (Blankenberg-Sprenkels et al., 1998; Chou, Uksila, & Toivanen, 1998; Rashid et al., 1999; Senior et al., 1995; Subair et al., 1995; Wanchu et al., 1997). *P. mirabilis* infections have also been frequently reported in urine samples from patients with RA (Senior et al., 1999). Furthermore, *P. mirabilis* antibodies from RA patients have cytopathic effects on joint tissue possessing *P. mirabilis* cross-reactive antibodies (Rashid & Ebringer, 2011) and sera from rabbits immunised with HLA-DR4 positive lymphocytes bind specifically to Proteus (Ebringer et al., 1985). Amino acid sequence homologies have been identified between the 'EQ/KRRAA' motif present in RA HLA-susceptibility antigens and the 'ESRRAL' amino acid sequence present in *P. mirabilis* haemolysins (Ebringer et al., 1992). Further sequence homology between the 'LRREI' sequence of type XI collagen (present in joint cartilage) and the 'IRRET' motif present in *P. mirabilis* urease enzyme has also been reported (Wilson et al., 1995).

Many antibiotics are already known to inhibit Proteus spp. growth and/or have bactericidal effects towards Proteus spp. However, the development of super resistant bacterial strains has resulted in currently used antibiotic agents failing to end many bacterial infections. The search is ongoing for new antimicrobials, either by (a) the design and synthesis of new agents, or (b) re-searching the repertoire of natural resources for as yet unrecognised or poorly characterised antimicrobial agents. Recent studies have examined the anti-*P. mirabilis* activity of conventional antimicrobials such as carbapenems (Lee et al., 2011) and of complementary and alternative therapies including nano-metallic preparations (Cock et al., 2012) and traditional South African medicinal plants (Cock & van Vuuren, 2014). A re-examination of functional foods for the treatment of inflammation and rheumatic conditions is an attractive prospect as the antiseptic qualities of medicinal plants have been long recognised and recorded. Furthermore, there has recently been a revival of interest in herbal medications due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals.

Terminalia ferdinandiana is an endemic Australian plant which has been reported to have an extremely high antioxidant content (Netzel et al., 2007). Furthermore, it was reported that the fruit of this plant also has the highest ascorbic acid levels of any plant in the world, with levels reported as high as 6% of the recorded wet weight (Woods, 1995). This is approximately 900 times higher (g/g) than the ascorbic acid content in blueberries (which were used as a standard). *T. ferdinandiana* has previously been shown to have strong antibacterial activity against an extensive panel of bacteria (Cock & Mohanty, 2011). Solvent extracts of various polarities were tested against both Gram positive and Gram negative bacteria. The polar extracts proved to be more effective antibacterial agents, indicating that the antibacterial components were polar. Indeed, the polar extracts inhibited the growth of nearly every bacteria tested. Both Gram positive and Gram negative bacteria were susceptible, indicating that the inhibitory compounds readily crossed the Gram negative cell wall.

Recently, *T. ferdinandiana* leaf extracts were shown to have potent inhibitory activity against the bacterial triggers of several auto-immune inflammatory diseases including RA (Courtney et al., 2015). That study indicated that the inhibition of the bacterial triggers of RA by the leaf extracts may be due to their high tannin content. Despite this, and the extremely high antioxidant capacity of *T. ferdinandiana* fruit, the fruit extracts have not been rigorously evaluated for the ability to inhibit Proteus spp. growth, nor has the phytochemistry of these extracts been extensively examined. The current study was undertaken to test the ability of *T. ferdinandiana* fruit extracts to inhibit the growth of bacteria associated with RA aetiology and to determine if the fruit extracts have similar phytochemical compositions to the leaf extracts.

2. Materials and methods

2.1. T. ferdinandiana *fruit pulp samples*

T. ferdinandiana fruit pulp was a gift from David Boehme of Wild Harvest, Darwin, Northern Territory, Australia. The pulp was frozen for transport and stored at –10 °C until processed.

2.2. *Preparation of extracts*

T. ferdinandiana fruit pulp was thawed at room temperature and dried in a Sunbeam food dehydrator. The dried pulp material was subsequently ground to a coarse powder. A mass of 1 g of ground dried pulp was extracted extensively in 50 ml of methanol, deionised water, ethyl acetate, chloroform or hexane for 24 h at 4 °C with gentle shaking. All solvents were supplied by Ajax and were AR grade. The extracts were filtered through filter paper (Whatman No. 54). The solvent extracts were air dried at room temperature. The aqueous extract was lyophilised by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellets were dissolved in 10 ml deionised water (containing 0.5% dimethyl sulphoxide). The extract was passed through 0.22 μm filter (Sarstedt) and stored at 4 °C.

2.3. *Qualitative phytochemical studies*

Phytochemical analysis of the *Tasmannia stipitata* extracts for the presence of saponins, phenolic compounds, flavonoids, polysteroids, triterpenoids, cardiac glycosides,