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Antimicrobial activity of *Albizia gummifera* (*J.F.Gmel.*) *C.A.Sm* leaf extracts against four *Salmonella* serovars

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ABSTRACT

In the past few decades, non-typhoidal serovars of *Salmonella* have gained resistance to several classes of antibiotics including third generation Cephalosporins and Fluoroquinolones. There has also been an increase in the number of infections caused by antimicrobial resistant *Salmonella* serotypes including *Salmonella* Typhimurium DT 104 which is resistant to at least five antimicrobial agents. Therefore, the aim of this study was to investigate *Albizia gummifera* (Fabaceae), a native tree species of Africa that has been used extensively in African traditional medicine for the treatment of stomach infection, skin diseases and malaria for antimicrobial activity against four *Salmonella* serovars. Dichloromethane, methanol and acetone leaf extracts of *A. gummifera* were screened for antimicrobial activity against *Salmonella* Typhimurium, *S.* Enteritidis, *S.* Dublin, and *S.* Gallinarum using the microplate dilution method. The minimal inhibitory concentration (MIC) of the extracts ranged between 0.125 and 1 mg/ml, while minimal bactericidal concentrations (MBC) ranged between 0.25 and >2.00 mg/ml. The best activity was exhibited by the methanol extract against all the tested strains. The mutagenic and cytotoxic potential of the most active methanol extract were also determined. The extract was not mutagenic in the Ames tester strains TA98 and TA100 with and without metabolic activation. However, the methanol extract was cytotoxic to both Human hepatocellular carcinoma (C3A) and Madin-Darby Bovine kidney (MDBK) cell lines. The results obtained in this study validate in part the use of this plant in African traditional medicine for the treatment of stomach infection.

1. Introduction

Salmonella infections are of great importance in human and animal health. They cause significant morbidity and mortality worldwide. Infections caused by non-typhoidal *Salmonella* are either non-invasive, self-limiting enteric infections or invasive systemic infections that require effective antimicrobial therapy (Davies, 2004; Alcaine et al., 2007). The biggest challenge with administering effective treatment against *Salmonella* infection is antibiotic resistance among disease-causing strains (Parry, 2004). Antibiotic resistance of *Salmonella* has existed for decades (Threlfall, 2002). Studies conducted in the 1990s revealed a significant increase in resistance towards the first-line antibiotics such as Ampicillin, Chloramphenicol and Trimethoprim-sulfamethoxazole including clinically important antimicrobial agents such as third-generation Cephalosporins and Fluoroquinolones (Winokur et al., 2002; Shakespeare et al., 2005; NARMS, 2006; Crump and Mintz, 2010). The prevalence of multidrug resistant strains of *Salmonella* has increased in the recent years (CDC, 2006; Lauderdale et al., 2006). Of particular note has been the epidemic spread of multi-drug resistant *Salmonella* serotype Typhimurium definitive phage type 104 (DT104), which now appears to have an almost worldwide distribution (Grob et al., 1998; Threlfall, 2002).

Antimicrobial resistance has increasingly compromised the outcome of many bacterial infections that were once treatable with antimicrobial agents. The increasing prevalence of antibiotic resistant bacteria and bacterial infections caused by these bacteria has become a global concern. This is mainly due to the fact that infections caused by antimicrobial resistant bacteria are difficult to treat and often fail to respond to treatment. This may often result in prolonged and severe illness, thus increasing the risk of spreading antimicrobial resistance microorganisms to others, resulting in expensive health care systems and a great risk of death (McGowan, 2001; Barza, 2002).

The global emergence of drug resistant bacteria and the increasing prevalence of infections caused by these bacteria are limiting the effectiveness of the available antimicrobials (Hancock, 2005). The biggest challenge currently facing the world is a slow pace of antimicrobial drug development and limited investment in the discovery and

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development of new antibiotics (Spellberg et al., 2004; Norrby et al., 2005; Talbot et al., 2006). Moreover, the emergence of resistance to newly introduced antimicrobials indicates that even new families of antimicrobial agents will have a short life expectancy (Coates et al., 2002). Decreased effectiveness of currently available antibiotics and the drawbacks in the development of reliable antimicrobial drugs have led to the exploration of alternative therapies such as plant-based compounds to offer a solution to the challenge caused by antimicrobial resistance (Hancock, 2005; Hawkey, 2008). Numerous *in vitro* studies have demonstrated that plants possess antimicrobial properties (Mathabe et al., 2006; Fawole et al., 2009; Nielsen et al., 2012; Tekwu et al., 2012).

A. gummifera (Fabaceae) is a native tree species of Africa (Beentje, 1994; Orwa et al., 2009). The plant grows in dry or wet lowland or upland forest edges and also riverine forests. *A. gummifera* is used, in the African continent, for the treatment of various ailments. Pod extract of the tree is administered for the relief of stomach infections, paste made from the root is used to treat diseased skin, while a decoction made from its bark is administered to treat malaria (Ofulla et al., 1995, 1996). Extracts from different parts of *A. gummifera* possess antimicrobial (Mbosso et al., 2010; Mmushi et al., 2010; Unasho et al., 2009), antiplasmodial (Rukunga et al., 2007) and cytotoxic (Cao et al., 2007) activities. However, for conservation purposes, only leaves were investigated in this study. Therefore, the aim of this study was to investigate the leaf extracts of *A. gummifera* for anti-*Salmonella* activity and to screen the extract with promising antimicrobial activity for safety including genotoxic and cytotoxic potentials.

2. Materials and methods

2.1. Plant collection and extraction

Leaves of *Albizia gummifera* were collected from Manie Van der Schijff Botanical Garden, University of Pretoria. A voucher specimen (specimen no. PRU 121455) was deposited at the H.G.W.J Schweickerdt Herbarium, Department of Plant Sciences, of the University of Pretoria. The collected plant material was cleaned and dried in an oven at 45 °C. After complete dryness, the plant material was ground into powder and stored in a glass jar until further use. The ground plant material was extracted sequentially with dichloromethane (DCM) and methanol (MeOH), and a separate extraction with acetone (Ac) was carried out. Ten milliliters of organic solvents (Merck) were added to 1 g of ground plant material, shaken for 2 h (Labotec model 20.2 shaker) and thereafter filtered through Whatman No.1 filter paper. The extract was concentrated under vacuum using a rotary evaporator (BÜCHI Rotavapor R-124, Labotec) at 35–40 °C. Dried crude extracts were stored at −20 °C until further use.

2.2. Antibacterial activity

2.2.1. Bacterial strains

Four non-typhoidal strains of *Salmonella enterica* namely *Salmonella* Typhimurium American Type Culture Collection (ATCC) 14028, *Salmonella* Enteritidis, *Salmonella* Dublin and *Salmonella* Gallinarum, were selected for this study. The bacterial strains were selected from host specific (*S.* Gallinarum), host restricted (*S.* Dublin), and generalist strains (*S.* Typhimurium and *S.* Enteritidis) classes of *Salmonella* serovars whose clinical manifestation is different in host species. Freeze dried wild-type isolates of *Salmonella* were obtained from the General Bacteriology and Feed and Food Analysis laboratories of Bacteriology section at Agricultural Research Council – Onderstepoort Veterinary Institute (ARC-OVI) collection of serotyped *Salmonella* isolates. The wild-type isolates were identified using biochemical tests and serotyping, which is phenotypic characterisation of *Salmonella* serotypes based on the immunologic reactivity of two surface antigens, namely somatic lipopolysaccharide (O) and flagellar (H) with specific antisera.

2.2.2. Minimal inhibitory concentration (MIC)

Minimal inhibitory concentrations (MICs) were determined using the microplate dilution method (Eloff, 1998). Methanol crude extracts were re-dissolved in 50% methanol while dichloromethane and acetone crude extracts were re-dissolved in 50% acetone to the concentration of 8 mg/ml. Hundred microliters of dissolved plant extract and gentamicin were serially diluted two-fold with distilled water in a 96 well plate and 100 μl of bacterial suspension standardised to McFarland standard No. 0.5 was added to each well. The sealed plates were incubated at 37 °C for 24 h. To indicate growth, 40 μl of *p*-iodonitrotetrazolium violet (INT) (Sigma-Aldrich) dissolved in water were added to the microplate wells and incubated at 37 °C for 30–45 min. The presence of bacterial growth was indicated by a pink/red color, and clear wells indicated the inhibition of bacterial growth by the plant extract. Gentamicin was used as a positive control (50 μg/ml) while the extract dissolving solvents (methanol, acetone) were used as negative controls. The assay was performed in quadruplicates and repeated three times.

2.2.3. Minimal bactericidal concentration (MBC)

Minimal bactericidal concentration (MBC), recorded as the lowest concentration of the crude plant extract that can kill 100% of the test organisms, was determined by adding 50 μl aliquots of the preparations which did not show any visible growth after incubation during the MIC assay and 50 μl of INT to 100 μl of Mueller Hinton broth. The plates were incubated at 37 °C for 24 h. The presence of bacterial growth was indicated by a pink/red color, and clear wells indicated the inhibition of bacterial growth by the plant extract. The lowest concentration indicating inhibition of growth was recorded as MBC.

2.3. Toxicity

2.3.1. Mutagenicity test

The mutagenic potential of *A. gummifera* was assessed using the plate-incorporation method performed with *Salmonella* Typhimurium tester strains TA 98 and TA 100 (Maron and Ames, 1983). Twenty milliliters of Fluka Nutrient Broth No 2 (Sigma) were inoculated with 100 μl of *Salmonella* Typhimurium bacterial stock and incubated at 37 °C for 16 h on a shaker. The crude plant extract was dissolved into DMSO and diluted to yield final concentrations of 0.25, 0.05, and 0.01 mg/ml. One hundred microliters of the overnight culture were added to a tube containing 100 μl plant extract, 500 μl phosphate buffer (for exposure without metabolic activation) or 500 μl of S9 mix (containing adequate amount of post-mitochondrial fraction for exposure with metabolic activation), 2 ml of top agar containing traces of biotin and histidine (Sigma). The mixture was poured over the surface of minimal agar plate and incubated at 37 °C for 48 h. Positive controls used for exposure with and without metabolic activation were aflatoxin B_1 and 4-nitroquinoline-1-oxide (4NQO) at a concentration of 2 μg/ml respectively, while 0.25% DMSO served as the negative control. The S9 mix was prepared by adding 2 ml of S9 (prepared from the liver of Aroclor-induced rats: Moltox™, Boone, North Carolina) to 19.75 ml of distilled water containing 1 ml of $MgCl_2$-KCl salts; 1 M glucose-6-phosphate; 0.1 M NADP; and 0.2 M phosphate buffer (pH = 7.4). Each sample was tested in triplicate for each concentration and repeated 3 times. After incubation, revertant (mutant) colonies were counted and compared to the number of colonies formed in unexposed cultures. An extract is considered mutagenic when the mean number of revertants is at least double that found in the negative control.