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Antibiotic-potentiation, antioxidant, cytotoxic, anti-inflammatory and anti-acetylcholinesterase potential of *Antidesma madagascariense* Lam. (Euphorbiaceae)

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ABSTRACT

Antidesma madagascariense Lam. (AM) is used in the treatment and management of a panoply of human diseases. Leaf decoction, acetone extracts and fractions of AM were evaluated for antimicrobial, antioxidant, anti-inflammatory, cytotoxicity and acetylcholinesterase (AChE) inhibitory activity. The antibiotic potentiating activity of crude acetone extract (AE) was assessed against ATCC bacterial strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 using standard antibiotics. Decoction extract showed significant inhibitory activity against *Acinetobacter* spp. (MIC: 0.25 mg/mL) which had higher inhibitory effect compared to the positive control Streptomycin (MIC: 1.00 mg/mL). Acetone and decoction extracts inhibited the growth of *Candida albicans* (MIC: 4.00 mg/mL). All fractions of AE showed broad spectrum activity against ATCC and clinical strains (MIC 0.03–4.00 mg/mL). The results of the combination profiles of the AE with Ciprofloxacin, Chloramphenicol and Streptomycin revealed synergistic interactions (FICI ≤ 0.50) against *P. aeruginosa*. Crude decoction, acetone extract and fractions were found to be potent reducing agent as well DPPH radical scavenger and ABTS cation scavenger. A significant correlation between DPPH, ABTS and total phenolic content ($p < 0.05$, $r = -0.75$ and -0.82 respectively) was recorded. Three fractions namely DCM:MeOH (85:15) (F5), DCM:MeOH (95:05) (F3) and DCM (F2) [IC_{50} 0.02–0.09 mg/mL] demonstrated significant anti-inflammatory activity as compared to the positive control, diclofenac sodium [IC_{50} 0.18 (0.10–0.31) mg/mL]. AE and its fractions showed AChE inhibitory activities at an IC_{50} of 35.92–492.6 μg/mL. Cytotoxicity study against Vero cells revealed that AE and hexane fraction were non-cytotoxic while decoction showed cytotoxic effect. Further studies are required to explore the potential of AM crude extracts and fractions as natural source of antimicrobial, antioxidant, anti-inflammatory agents as well as AChE inhibitors.

1. Introduction

Antidesma madagascariense Lam. (AM) belongs to the Euphorbiaceae family, its genus comprises of approximately 170–200 species (Buske et al., 2002; Mahomoodally et al., 2015). This specie also known as 'Bois bigaignon' is indigenous to the Mascarene Islands including Madagascar (Gurib-Fakim, 2007; Mahomoodally et al., 2015). AM has been traditionally used for the treatment and management of several ailments (Gurib-Fakim, 2007; Mahomoodally et al., 2015). The leaf and bark decoction acts as diuretic and astringent as well as effective against fever and diabetes (Gurib-Fakim, 2007; Mahomoodally et al., 2015). The leaf decoction is also used to wash skin infections and helps to relieve muscular and rheumatic pain (Gurib-Fakim, 2007; Mahomoodally et al., 2015). Infusion of the leaf is used against dysentery and albumin (Gurib-Fakim, 2007; Mahomoodally et al., 2015). Leaf decoction of AM in combination with that of *Toddalia asiatica* (L.) Lam. (Rutaceae) is used to cure jaundice (Gurib-Fakim, 2007; Mahomoodally et al., 2015).

Methanolic and aqueous extracts as well as the fractions of AM were found to scavenge DPPH, nitric oxide (NO) and superoxide anion radicals (Mahomoodally et al., 2012). These extracts and fractions also showed non-enzymatic antiglycation and anti-lipoxygenase activity (Mahomoodally et al., 2012). Narod et al. (2004) reported the antimicrobial activity of AM crude water extracts of the leaf and stem as well as their fractions against *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *A. niger* and *Candida albicans*. The extracts were also found to exhibit contractile properties on rat illeal smooth muscles (Narod et al., 2004). Another study showed that the methanol and aqueous extracts of AM inhibited α-glucosidase enzyme with IC_{50} values 10.40 ± 0.26 and 1.22 ± 0.05 μg/mL respectively

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which were significantly lower than the positive control acarbose (IC_{50}: 5115.73 ± 3.91 μg/mL) (Picot et al., 2014).

An infection may induce the process of inflammation which causes the release of biologically active mediators to attract neutrophils, leucocytes and monocytes to the infected area and these attack foreign debris and microorganisms through phacocytosis (Agyare et al., 2013). This may then lead to the production of oxygen-free radicals such as hydrogen peroxide, superoxide anion and hydroxyl anion and excess of these agents cause tissue damage in man or animal if they overwhelm the natural antioxidants of the host such as catalase, superoxide dismutase and glutathione (Agyare et al., 2013). Therefore, antioxidants hinder the activity of free radicals and prevent damage of cells and tissues. Alzheimer's disease (AD) is considered as a fatal and chronic neurodegenerative disease that develops slowly and worsens over time (Oztaskin et al., 2015). Moreover, strong experimental evidences have demonstrated that reactive oxygen species are associated with the pathogenesis of AD, as some cellular characteristics of this disease are either causes or effects of oxidative stress theory (Ali-Shtayeh et al., 2014). This disease is linked with tangles and plaques in the brain. About 35 million people worldwide are suffering from AD, and till now there has been no effective treatment developed against this disease. AD can be managed using acetylcholinesterase inhibitors which prevent the cholinergic degradation of acetylcholine (Oztaskin et al., 2015). Therefore, the aim of this study was to investigate the antimicrobial, antioxidant, anti-acetylcholinesterase and anti-inflammatory potential of AM extracts and fractions. The cytotoxicity of the crude extracts and hexane fraction were also evaluated.

2. Materials and methods

2.1. Extraction and fractionation

Fresh leaves of *A. madagascariense* were collected from Monvert Nature Park, Mauritius. The plant was identified by the Mauritius Herbarium, Agricultural Services (barcode number: MAU 26543). The leaves were dried at 40 °C in a drying cabinet for 4 to 5 days until constant mass was obtained. Dried powdered sample was extracted *via* maceration process using acetone (10:1 solvent to dry weight ratio). For decoction method, dried plant powder was extracted with distilled water at 100°C. The extract was dried under reduced pressure using a rotatory evaporator. Acetone crude extract was fractionated *via* flash chromatography method using hexane, dichloromethane and varying ratios of dichloromethane and methanol.

2.2. Antimicrobial activity

2.2.1. Microbial strains

The antimicrobial activity of AM crude extracts and fractions was evaluated against a panel of microorganisms sourced from American Type Culture Collection (ATCC) namely: *Escherichia coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *Propionibacterium acnes* (ATCC 11827 and 6919), *S. aureus* (ATCC 25923), *S. typhimurium* (ATCC 14028), *Vibrio parahaemolyticus* ATCC 17802, *C. albicans* (ATCC 10231) and clinical isolates such as *Acinetobacter* spp., *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* spp., *P. aeruginosa*, *Proteus mirabilis*, *Proteus* spp., methillin-resistant *S. aureus* (MRSA) and *S. aureus*. The clinical isolates were obtained from Victoria Hospital, Candos, Mauritius.

2.2.2. Broth microdilution susceptibility assay

A two-fold serial microdilution technique was used to determine the minimum inhibitory concentration values for the different extracts as described by Shai et al. (2008) with some modification. A 100 μL of each extract (16 mg/mL) was serially diluted two-fold in triplicate with sterile peptone water in 96-well microtitre plates. Fresh bacterial and fungal inoculums were prepared and adjusted to 0.5 McFarland which were further diluted 1:100 with fresh sterile peptone water broth to yield starting inoculums of approximately 10^6 CFU/mL. One hundred microliters of bacterial or fungal culture was added to each well of bacterial and fungal plates respectively. Chloramphenicol, ciprofloxacin, gentamicin and streptomycin were used as positive controls for bacteria while nystatin and amphotericin B were used against fungus. Negative control included sterile peptone water broth. The bacterial plates were incubated at 37 °C for 24 h while fungal plates were incubated for 48 h at 25 °C. After incubation, 40 μL of iodo-nitrotetrazolium chloride (0.2 mg/mL) was added to each well and the plates were further incubated for 20 min. Bacterial and fungal growth was denoted by red coloration. The well of lowest concentration in which no pinkish red coloration was observed was considered to be the MIC. The total activity (TA mL/g) value was calculated as the total mass extracted from 1 g of plant material divided by the MIC value (mg/mL) (Shai et al., 2008).

2.2.3. Antibiotic potentiating activity

Crude acetone extract was combined with commercial antibiotics namely ciprofloxacin (CIP), chloramphenicol (CHL), and streptomycin (STR) to determine any possible synergistic activity. Three different ratios of extract to antibiotic were prepared (30:70, 50:50 and 70:30) by mixing known volume of stock solution of the extract (16 mg/mL) with CIP (0.1 mg/mL), CHL (1 mg/mL) and STR (1 mg/mL) respectively as described by de Rapper et al., 2012. The assay was carried out *via* broth microdilution susceptibility method as described in Section 2.2.2. A 100 μL of each extract: antibiotic (30:70, 50:50 and 70:30) combinations were serially diluted two-fold with sterile peptone water, in triplicate in a 96-well microplate for each of the ATCC bacterial strains. The different antibiotics (CIP, CHL and STR) were used alone as positive controls and sterile peptone water as negative control. Inoculum (100 μL) was added to each well. The plates were then incubated overnight at 37 °C. After incubation, 40 μL of INT (0.2 mg/mL) was added to each well and the plates were further incubated for 20 min at 37 °C. The MICs were recorded and the results of the combined effects of the antibiotics and extracts were calculated and expressed in terms of a fractional inhibitory concentration (FIC) index which is denoted by the following formula:

$$FIC_{extract} = \text{MIC of extract in combination/MIC of extract alone.}$$

$$FIC_{antibiotic} = \text{MIC of antibiotic in combination/MIC of antibiotic alone.}$$

$$\text{FIC index} = FIC_{extract} + FIC_{antiobiotic,}$$

$FIC_{extract}$ is the fractional inhibitory concentration of the extract and $FIC_{antiobiotic}$ is the fractional inhibitory concentration of the antibiotic used. The results were considered as a synergy if the FIC index of the combination is ≤0.5, additive when it was 0.5 < FIC index < 1, subtractive when FIC index is greater than 1 and less than 4 and antagonism for FIC index > 4 (de Rapper et al., 2012; Duarte et al., 2012; Chaudhary et al., 2013).

2.3. Antioxidant activity

2.3.1. Radical scavenging activity using DPPH• method

The free radical scavenging capacity of extracts was spectrophotometrically assessed using DPPH (Hwang et al., 2001 cited in Parekh et al., 2012). Samples (400 μg/mL, 100 μL) were serially diluted using methanol (100 μL) in a 96-well microtiter plate. Methanolic solution of DPPH (100 μM, 200 μL) was added to each well and the plate was incubated at 37 °C for 30 min. The experiments were performed in triplicate and the absorbance was measured at 517 nm. Ascorbic acid and trolox were used as positive control and methanol as the