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The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African *Bauhinia* species used traditionally to treat diarrhoea

Aroke S. Ahmed [a,*], Esameldin E. Elgorashi [a], Nivan Moodley [b], Lyndy J. McGaw [a], Vinasan Naidoo [c], Jacobus N. Eloff [a]

[a] Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[b] Biosciences, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, South Africa
[c] Biomedical Research Centre, University of Pretoria, South Africa

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ABSTRACT

Ethnopharmacological importance: Many *Bauhinia* species, including those indigenous to South Africa, are used in traditional medicine across the world for treating ailments such as gastrointestinal tract (GIT) disorders, diabetes, infectious diseases and inflammation.

Aims: Several relevant aspects of different fractions of leaf extracts of *Bauhinia bowkeri* (BAB), *Bauhinia galpinii* (BAG), *Bauhinia petersiana* (BAP), and *Bauhinia variegata* (BAV) used in South African traditional medicine to alleviate diarrhoea related symptoms were evaluated.

Materials and Methods: The antioxidative activities of the extracts were determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$) radical scavenging and ferric reducing antioxidant power (FRAP) methods. In vitro antimicrobial activities of the extracts were determined against bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*) and clinical isolates of the opportunistic fungal strains (*Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans*) using a serial dilution microplate method. The polyphenolic contents were quantified using standard methods, and anti-inflammatory activities of the crude extracts were determined using the cyclooxygenase and soybean 15-lipoxygenase enzyme inhibitory assays. The safety of the extracts was evaluated by determining the cytotoxicity against Vero cell lines.

Results: The acidified 70% acetone crude extract and their fractions had good antiradical potency against the DPPH and ABTS radicals. The methanol soluble portions of the butanol fractions were more potent (EC_{50} ranges from 0.64 ± 0.05 to 1.51 ± 0.07 and 0.88 ± 0.18 to 1.49 ± 0.09 μg/ml against DPPH and ABTS radical respectively) compared to the standard, trolox and ascorbic acid (EC_{50} ranges from 1.47 ± 0.24 to 1.70 ± 0.27 μg/ml) for both DPPH and ABTS. The crude extracts contained variable quantities of phenolic content. The crude extracts and their fractions had weak to good antimicrobial activities, inhibiting the growth of the organisms at concentrations ranging from 39 to 2500 μg/ml. The BAG crude extract and its fractions were the most active against the fungi (MICs ranging from 39 to 625 μg/ml) while the BAB extract and its fractions were the least active with the MICs ranging between 39 and 2500 μg/ml. *Aspergillus fumigatus* was the least susceptible fungus while *Cryptococcus neoformans* was the most susceptible.

Abbreviations: A, absorbance; A_{02}, Absorbance at time 0 min; A_{b2}, absorbance of blank; $ABTS^+$=2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid radical; $AlCl_3$, aluminium chloride; ANOVA, analysis of variance; ARM, antibiotic resistant microbe; A_{t2}, absorbance at time *T*; ATCC, American Type Culture Collection; BAB, *Bauhinia bowkeri*; BAG, *Bauhinia galpinii*; BAP, *Bauhinia petersiana*; BAV, *Bauhinia variegata*; BuOH, butanol; CE, catechin equivalent; CH_3COONa, Sodium ethyl acetate; CO_2, Carbon dioxide; COX, cyclooxygenase=; Cyn-3-glu, cyanidin-3-glucoside; DCM, dichloromethane; DNA, deoxyribose nucleic acid; DF, dilution factor; DMSO, dimethyl sulphoxide; DPM, disintegration min^{-1}; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; EC_{50}, effective concentration required to inhibit scavenge free radicals by 50%; ETOAc, ethyl acetate; FLL, flavonol; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; GIT, gastrointestinal tract; GT, gallotannin; HCl, hydrochloric acid; IBD, irritable bowel disease; IC_{50}, concentration required to reduce cyclooxygenase by 50%; INT, iodonitrotetrazolium violet; $K_2Fe_3(CN_6)$, potassium ferrocyanide; $K_2S_2O_4$, potassium persulphate; $K_3Fe_3(CN_6)$, potassium ferricyanide; LC_{50}, dose of extract necessary to induce cytotoxic effect by 50%; LE, leucoproanthocyanidin equivalent; LOX, lipoxygenase; LPO, lipid peroxidation; MEM, minimal essential medium; mg, milligram; MIC, minimum inhibitory concentration=; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyl tetrazolium bromide; $NaHCO_3$, Sodium hydrogen carbonate; NCCLS, National Committee for Clinical Laboratory Standards; NRF, National Research Fund; OH, hydroxyl; ORT, oral rehydration therapy; PBS, phosphate buffer solution; PG, prostaglandin; PVPP, polyvinylpolypyrrolidone; QE, quercetin equivalent; RNS, reactive nitrogen species; ROS, reactive oxygen species; SDS, sodium dodecyl sulphate solution; SE, standard error; TEAC, Trolox equivalent antioxidant capacity; TLC, thin layer chromatography; TF, total flavonoid; TP, total phenolic

* Corresponding author. Permanent address: Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria. Tel.: +2348034558023.
E-mail address: shahid_aroke@yahoo.com (A.S. Ahmed).

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The phenolic-rich crude extracts of BAB, BAG, and BAP had moderate to good dose-dependent cyclooxygenase-1 enzyme inhibitory activity with inhibitions between 22.8% and 71.4%. The extracts were however, inactive against cyclooxygenase-2. The extracts had some level of cytotoxicity towards Vero cell lines, reducing cell viability to less than 10% at concentrations more than 50 μg/ml.
Conclusion: The biological activities observed in *Bauhinia* species provide a scientific basis for the use of the plants in traditional medicines to treat diseases with multi-factorial pathogenesis such as diarrhoea, with each aspect of activity contributing to the ultimate therapeutic benefit of the plants. However, the use of the phenolic-rich extracts of these plants to treat diarrhoea or any other ailments in traditional medicine needs to be monitored closely because of potential toxic effects and selective inhibition of COX-1 with the associated GIT injury.

1. Introduction

Diarrhoea caused by infectious or non-infectious aetiologies presents clinical signs of reduced absorption and increased secretion of fluid and electrolytes in the small and/or large intestine resulting in profuse watery stool output (Baldi et al., 2009). It is one of the most dangerous gastrointestinal tract (GIT) disorders, as death can occur due to dehydration, loss of electrolyte and hypernatremia in humans and animals (Petri et al., 2008). In addition, diarrhoea associated with malnutrition could result in stunted growth, non-optimal immune functionality and increased susceptibility to infections. Physiologically, diarrhoea can be beneficial to the GIT as it provides an important mechanism of flushing away harmful luminal substances (Valeur et al., 2009). However, diarrhoea becomes pathological when the loss of fluids and electrolytes exceeds the body's ability to replace the losses.

Classification of the pathophysiology of diarrhoea is usually difficult due to numerous mechanisms involved and the heterogeneity of their effects on intestinal epithelial mucosa. Diarrhoea is often classified based on stool characteristics or pathological mechanisms as follows:

- Watery diarrhoea, typically referred to as secretory diarrhoea, results from increased chlorine secretion, decreased sodium absorption and increased mucosal permeability.
- Osmotic diarrhoea is also a watery form of diarrhoea caused by the ingestion of non-absorbable indigestible material (Baldi et al., 2009) or absence of brush border enzymes required for the digestion of dietary carbohydrates (Podewils et al., 2004).
- Inflammatory diarrhoea is manifested by the presence of mucus, blood, and leucocytes in the stool, indicating infectious process, allergic colitis or inflammatory bowel disease (IBD).

A number of microbes including bacteria, fungi (rarely), viruses and parasites have been identified as causative agents of infectious diarrhoea in humans and animals. The most common and economically important agents are diarrhoeagenic *Escherichia coli* (Gram-negative bacteria), *Staphylococcus aureus* (Gram-positive bacteria), *Pseudomonas aeruginosa* (Gram-negative bacteria), and *Candida albicans* (yeast). Other less common diarrhoeal pathogens include *Salmonella typhi, Shigella flexneri, Giardia intestinalis, Cryptosporidium parvum*, *Entamoeba histolytica* and various viruses like rotavirus, astrovirus, adenovirus and calicivirus (Brijesh et al., 2006). Some of the diarrhoeal pathogens execute their action through enterotoxin-mediated intestinal secretion of fluids and electrolytes (cholera toxins) or invasion of the intestinal enterocytes causing an exaggerated inflammatory response with resultant production and release of excessive inflammatory mediators that affect epithelial cell functionality. The immune system typically mobilises leucocytes to fight invading pathogens through activated macrophages and neutrophils releasing oxidative molecules (reactive oxygen species (ROS) or reactive nitrogen species (RNS)) and inflammatory mediators as microbicidal agents. Excessive generation of ROS/RNS causes peroxidation of the phospholipids of the intestinal mucosa epithelial lining with resultant release of cytotoxic aldehyde intermediates.

The primary intervention in diarrhoea management is the administration of glucose-electrolyte oral rehydration therapy (ORT) to correct dehydration, metabolic acidosis and prevent the possible complication of hypernatremia. Although ORT is effective in reducing the morbidity and mortality rate of diarrhoea, chronic watery excretion associated with profound vomiting may require additional pharmacological treatment to mitigate any complications. These include the use of an antimotility agent (loperamide), a pro-absorptive agent (racecadotril) or antisecretory (octreotide) and calcium blockers (otilonium and pinaverium) (Farhadi et al., 2001).

While antimicrobial therapy is not encouraged in diarrhoeal cases with no apparent systemic sign of infections, antimicrobial agents are generally required in cases such as febrile bloody diarrhoea, dysenteric shigellosis, protozoa infections, and cholera (Casburn-Jones and Farthing, 2004; Field, 2003; Gadewar and Fasano, 2005). Some of the antimicrobial drugs include tetracycline, doxycycline; fluoroquinolone (ciprofloxacin, norfloxacin fleroxacin, cinoxacin), azole (metronidazole, albendazole), amoxicillin, vancomycin, erythromycin and gentamicin.

Unfortunately, the overuse and abuse of antimicrobial drugs has led to the emergence of antibiotic resistant microbial (ARM) strains (Westh et al., 2004; Parekh, Chandra, 2007). A number of antibiotics have become less effective as a result of the emergence of antimicrobial resistance, often as a result of the selective pressure of antimicrobial usage. Among the more important emerging resistance problems are the oxacillin resistance in staphylococci, penicillin resistance in streptococci, vancomycin resistance in enterococci (and eventually staphylococci), resistance to extended-spectrum cephalosporins and fluoroquinolones in members of enterobacteriaceae, and carbapenem resistance in *Pseudomonas aeruginosa* (Oskay et al., 2009). The accumulation and spread of ARM has led to increased morbidity, mortality and health-care costs from infectious diseases as a result of treatment failure. While different methods have been put forward to overcome this resistance, there is increasing evidence to suggest that plant secondary metabolites may be beneficial in the management of these resistant strains (Gibbons, 2005). In addition to the management of diarrhoea through the enteric pathogenic organisms, plant metabolites may also offer other benefits such as attenuation of the inflammatory cascade and oxidative stress induced by the proliferation of the infectious micro-organisms within the GIT.

Plants form an integral part of South Africa's rich culture and biodiversity, with many species serving as a cornerstone of traditional medicine for symptomatic treatment and alleviation of various diseases. Many South Africans rely on medicinal plants for health care needs, including some patients who access orthodox drugs. The therapeutic activity of medicinal plant preparations is attributed to diverse phytochemicals such as phenolics, terpenoids or alkaloids.

Phenolics are phytochemicals characterised by the presence of an aromatic ring containing one (phenol) or more (polyphenols)