

Biological activities of extracts and isolated compounds from *Bauhinia galpinii* (Fabaceae) and *Combretum vendae* (Combretaceae) as potential antidiarrhoeal agents

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Declaration

The research work described in the thesis was conducted in the Phytomedicine Programme in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria under the supervision of Professor JN. Eloff, Dr. N. Moodley, Prof. V. Naidoo and Dr. LJ. McGaw

The results presented herewith were generated from my own experiments, except where the work of others are quoted and referenced. There is no part of this work that has been submitted to any other University.

Aroke Shahid, Ahmed

Dedication

This work is dedicated to the memory of the following: My Father (Late Mr. Ahmed Aninya Aroke), my brothers (Late Salihu Aroke and Late Ibrahim Onimisi Ahmed), Late Olukemi Ore Udom (A friend and colleague who started her PhD, but could not finish the programme before death) and my dear sister (Late Mrs. Husseinatu Ohunene Abubakar).

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Abstract

Diarrhoea is one of the killer diseases resulting from the dehydration and loss of electrolytes through profuse and excessive excretion of loose stool. The pathoetiologies include infections, intestinal inflammation, imbalanced intestinal oxidative homeostasis and altered motility. Treatment with oral rehydration therapy (ORT) is a key intervention especially in secretory diarrhoea as supportive therapy. Symptomatic and non-symptomatic therapies directed at treating the intestinal tissues are available. However, these conventional treatments are still not sufficient in curing diarrhoea due to their associated hazards such as the development and spread of drug-resistant pathogens, changes in normal intestinal bacteria flora and potential chronic toxicity. Therapies targeted at intestinal tissue include antimotility and antisecretory agents have adverse effects such as addictiveness, constipation and fatal ischaemic colitis. Many ethnopharmacological and ethnobotanical therapies for treating diarrhoea exist among different cultures. The aims of this study were to evaluate the biological activities of plant extracts against some diarrhoeal pathophysiology.

A literature search in English of published articles and books that discussed ethnobotanical uses of medicinal plants in southern Africa was conducted. A list of 230 medicinal plants used in South African traditional medicines for treating diarrhoea and associated complications was created. The list included family, genus, species, biological activities and bioactive isolates as well as the remedies for diarrhoea. Twenty seven species were selected to evaluate for antimicrobial, antioxidant and anti-inflammatory activities. Safety of the plants was determined by determining the cytotoxicity of the crude extracts against Vero African green monkey kidney cell lines using a standard method. Motility effects of *Bauhinia galpinii* (BGE) and *Combretum vendae* (CVE) were determined by modulation of the contractility process of the isolated rat ileum induced by spasmogens.

Phenolic compositions of the crude extract were determined using various standard methods and finally bioactivity guided isolation of antimicrobial and antioxidant compounds from BGE and CVE were carried out using open column chromatography. Identification and characterization of the isolated compounds was achieved by NMR, EI-MS and UV spectroscopy.

The non-polar fractions had good antimicrobial activities with MIC ranged between 19 – 1250 µg/ml while the polar fraction had moderate antimicrobial activities with MIC ranged between 39 - >2500 µg/ml. In general the non-polar fractions had a higher antimicrobial activity.

The crude extracts contained wide range phenolic compounds with a total phenolic (74.91±1.26 to 467.04±15.82 mg GAE/g plant material), and total flavonoids (11.27±3.37 to 176±5.96 mg EQ/g plant material). The antioxidant activities were concentrated and potentiated in the polar fractions. The non-polar fractions had poor antioxidant activities with EC₅₀ values ranging from 0.21±0.03 to 303.65±3.84 µg/ml for DPPH radical scavenging and 0.43±0.03 to 1709±91.44 µg/ml for ABTS radical scavenging.

The crude extracts had selective COX-1 inhibitory activities ranging between 41.70 to 84.61% and had no COX-2 inhibitory activity. All the extracts tested had 15-LOX inhibitory capacity with LC₅₀ values ranging between 0.86±0.27 and 111.44±37.28 µg/ml. The cytotoxicity results indicated a wide variation in toxic potential of the crude extracts with LC₅₀ values ranging from 3.51 to 741.90 µg/ml.

The BGE extracts had dual activities as spasmolytic by stimulating the spontaneous contractility and also agonised contractions induced by spasmogens but it inhibited K⁺ induced contraction. CVE had spasmodic activities through a multiple mechanisms inhibiting contractions induced by spasmogens and K⁺ in a dose-dependent manner.

Several bioactive compounds were isolated from the *Combretum vendae* leaves, There were triterpenoids (ursol-12-en-28-oic acid, mixtures of corosolic acid and maslinic acid, and asiatic acid and arjunolic acid) as well as bibenzyls combretastatin B5-O-2'-β-D-glucopyranoside, combretastatin B1-O-2'-β-D-glucopyranoside and a flavonoid (apigenin)..

From *Bauhinia galpinii* the following bioactive compounds were isolated and characterized: β-3 ethoxy sitosterol, one new flavone (5, 7, 4' 5' tetrahydroxy-2'-methoxyflavone (isoetin 2'-methyl ether) or 5, 7, 2' 5' tetrahydroxy-4'-methoxyflavone (isoetin 4'-methyl ether)), 3, 5, 7, 3', 4'-pentahydroxyflavone and 3, 5, 7, 3', 4', 5'-hexahydroxyflavone, quercetin-3-O-β-galactopyranoside and myricetin-3-O-β-galactopyranoside

The extraction protocol used in this work potentiated the antimicrobial activities in the non-polar fractions while antioxidant activities were potentiated in the polar fractions. This indicated that using polar solvents as extractant for treating infectious diarrhoea may not be quite effective unless some other antidiarrhoeal mechanisms are involved. Therefore, mixture of organic solvent (ethanol) and water can be recommended for broad-based activity.

Bauhinia galpinii extracts had a dual- mechanism of action (prokinetic and relaxant) on gastro-intestinal motility, depending on the prevalent patho-physiological condition and *Combretum vendae* mediated spasmolytic effects on isolated rat ileum through multiple inhibitions of a wide range of contractile stimuli. Hence, the presence of multiple acting spasmolytic activities in the plant extract might be contributing towards its effectiveness in treating diarrhoea and abdominal spasm. The uses of these plants in traditional medicine need to be monitored closely because of the selective inhibition of COX-1 and its associated GIT injury, and the high toxicity potential of some of the extracts.

Further work evaluating the antidiarrhoea mechanisms, identification and isolation of bioactive compounds, sub-acute and acute toxicity of the plant extracts is recommended.

Key words: Antimicrobial, antioxidant, anti-inflammatory, diarrhoeal, antispasmodic, enteric nervous system, cytotoxicity.

List of Abbreviations

A

ABTS=2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

AMP=Antimicrobial peptides

B

BAB= *Bauhinia bowkeri*

BAG= *Bauhinia galpinii*

BAP= *Bauhinia petersiana*

BAV= *Bauhinia variegata*

BGE= *Bauhinia galpinii* extract

C

Ca²⁺= Calcium ion

Cl⁻= chloride ions

CNF-1= Cytotoxic necrotising factor 1

CNS= Central nervous system

COB= *Combretum bracteosum*

COP= *Combretum padoides*

COV= *Combretum vendae*

COX= Cyclooxygenase

COW= *Combretum woodii*

CVE= *Combretum vendae* extract

D

DAEC= diffusively adherent *Escherichia coli*

DNA

DPPH=2, 2-diphenyl-1-picrylhydrazyl

E

EAEC= Enteroaggregative *Escherichia coli*

EHEC= Enterohaemorrhagic *Escherichia coli*

EIEC= Enteroinvasive *Escherichia coli*

ENS= Enteric nervous system

EPEC= Enteropathogenic *Escherichia coli*

ETEC= Enterotoxigenic *Escherichia coli*

EUC=*Euclea crispa*

EUN= *Euclea natalensis*

F

FIC= *Ficus cratostoma*

FIG=*Ficus glumosa*

FRAP= Ferric reducing antioxidant capacity

G

GIT= Gastrointestinal tract

H

HIV/AIDS= Human immune deficiency virus/Acquired immune deficiency syndrome

HOCl= hypochlorite

HUB= Haemolytic uremic syndrome

I

IBS= Irritable bowel syndrome

IL= Interleukin

INC= *Indigofera cylindrica*

iNOS= inducible nitric oxide synthase

INT= p-iodonitrotetrazolium

L

LT= Heat labile enterotoxin

LTB= Leukotriene B

M

MDA= Malondialdehyde

MCP-1= Monocyte chemoattractant protein

MIC= Minimum inhibitory concentration

MPD= *Maytenus peduncularis*

MPR= *Maytenus procumbens*

MSE= *Maytenus senegalensis*

MUN= *Maytenus undata*

N

Na⁺= sodium ions

NAME= nitro

NH₂Cl= Ammonium chloride

NO= Nitric oxide

O

OH⁻ = Hydroxyl radical

ORT=Oral rehydration therapy

OZM= *Ozoroa mucronata*

OZP= *Ozoroa paniculosa*

P

PG= Prostaglandin

R

ROS= Reactive oxygen species

RNS= Reactive nitrogen species

S

SCB=*Schotia brachypetala*

SLE= *Searsia leptodictya*

SPD= *Searsia pendulina*

SPT= *Searsia pentheri*

ST= Heat stable enterotoxins

SYP= *Syzygium paniculatum*

T

TLC=Thin layer chromatography

TNF- α = Tumour necrosis factor- α

Trolox= 6-hydroxy-2, 5, 7, 8-tetrahydroxyl-chroman-2-carboxylic acid

U

UNICEF=United Nation Children Fund

W

WHO= World Health Organization

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