

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

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BSc (Chemistry) (ABU), MSc (Chemistry) (UNILAG)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

in the

Phytomedicine Programme

Department of Paraclinical Sciences, Faculty of Veterinary Science



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January 2012

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).

Acknowledgements

This Ph.D. study would not have been possible without the support and encouragement of many people. I will thank my mum Mrs Omeneke Aminatu Ahmed who has been the pillar of my life. The love of my wife Mrs Rabiatu Isoyiza Ahmed and my children Enehu Mazidah, Onize Nusrah, Ometere Shamsiyah, Eneze Azeemah and Ava'ami AbdulAleem have been essential to my success. I thank you all for your understanding and endurance during my absence. I also wish to thank my siblings Mr. Aroke Haruna, Mr. Umar Omeiza Aroke and his wife Rukkayat, Mrs. Zulaiha Mubarak, Dr. Halidu Aroke Ahmed, Mrs. Khaltum Khamilu and Aisha Ahmed for their enormous support. I am also deeply indebted to my friends who stuck by me through the years: Dr. Muhammed Awwalu Usman, Engr. Yakubu Adajah, Mr. Yahaya Ohida Yusuf, Mr. Tijani Muhammed Isah, Abdulmumuni Enesi Umar, Dr. Muhammed Onujagbe Onoda, Mrs. Ukachi Ezenwa Igbo, Dr. Caroline Anyakorah, Dr. Oluwatoyin Taiwo and Dr. Chima Cartney Igwe among others. Financial support was a crucial element of my ability to devote so much time to this research. Major support that keeps me and my family afloat during the study was provided by a study leave with pay provide by Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. The University of Pretoria has provided support through University bursary, National Research Foundation (NRF) of South Africa provide fund for the research, Faculty of Veterinary Science also provide research fund through the research committee (RESCOM), and Department of Paraclinical Science also provide research fund.

I am extremely grateful to my supervisor, Prof. J. N. Eloff (overall supervisor) and my co-supervisors, Dr. Nivan Moodley (characterization of isolated compounds), Prof. Vinny Naidoo (isolated organ studies), and Dr. Lyndy J. McGaw (cellular toxicology) for allowing me to tap from the wealth of their knowledge and also giving me much of their time and energy through the duration of this study. I have a great respect and appreciated the instruction and assistance I received from each and every one of you. I sincere thank the Secretary to Phytochemical Programme, Tharien de Winnaar for all her assistance in coordinating the purchase of materials and other important aspects of this project.

I feel honoured to have opportunity to do some of the work at Bioscience, CSIR South Africa and lucky to meet with Dr. Vinesh Maharaj, Dr. Jacqueline Ndlebe, and Ms. Teresa Faleschini who are friends as well as crucial resources of information, techniques and instructions. I express my gratitude to CSIR for allowing me to use her NMR spectroscopic facilities.

At the UPBRC where I got my isolated organ part, the assistance of Dr. Tamsyn Pulleer, Mrs. Stephanie Keulder and Mrs. Ilse Janse van Rensburg were highly appreciated. The ability to collect plant material was essential to this project, and for that I want to recognize the help and outstanding collaboration of Ms Magda Nel of the Manie van der Schyff Botanical Garden and Ms Elsa van Wyk (Curator of the HGW Schweickert Herbarium of the University of Pretoria) for assistance in collection, identification and authentication of the plant samples. My appreciation also goes to Ms Lita Pauw of the Phytomedicine Programme for allowing me access her stored

plant samples. I also appreciated working in harmony with all other students of the Phytomedicine Programme, most especially Mr. Thanyani Ramandwa, Ms Bellonah Sakong, Ms Salaelo Raphatlelo, Ms Imelda Ledwaba and Mrs Edwina Muleya. I thank you all for your co-operation and immeasurable assistance.

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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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